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Higher dietary protein increases growth performance, anti-oxidative enzymes activity and transcription of heat shock protein 70 in the juvenile sea urchin (*Strongylocentrotus intermedius*) under a heat stress

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ABSTRACT

This study was conducted to investigate the effect of dietary protein concentration (12%, 18%, 24%, 30% and 36%) on the growth performance, activity of anti-oxidative enzymes and heat shock protein 70 (HSP70) transcription in the sea urchin (*Strongylocentrotus intermedius*) under a heat stress. After 112 days of feeding trial the sea urchins were heat stressed (26 °C) and the coelomic fluid and intestine sampled at time 0 and 15 min, 2 h and 6 h. The results showed that an increase in dietary protein (12%–24%), significantly increased ($p < 0.05$) the sea urchin weight gain rate (WGR). As dietary protein increased (from 18% to 36%), the gonadosomatic index (GI) of juvenile sea urchins also significantly increased ($p < 0.05$) from 18.0% to 22.6%. Superoxide dismutase (SOD) activity increased with dietary protein increase (12%–30%) and the enzyme activity was significantly higher ($p < 0.05$) in the coelomic fluid of sea urchins that were fed with 30% protein diets when compared to 12% and 36% protein diets at all time points after the heat stress. Catalase (CAT) activity showed a similar tendency with the increase in dietary protein concentration at time 0 and 15 min after the heat stress ($p < 0.05$). Transcription of HSP70 in the intestine also showed a similar trend to SOD and was highest in the animals that were fed with 30% protein diets ($p < 0.05$). Our results suggest that 24% protein diets could meet the requirements for growth performance but a 30% protein diet resulted in improved gonad development and anti-heat stress capacity in this sea urchin species.

1. Introduction

Recently, sea urchin roe has become a popular food in Japan, France, Chile, Barbados and China. However, due to over-fishing of sea urchins all over the world sea urchin roe is facing a decrease in production (Lawrence, Chang, Cao, Lawrence, & Watts, 2011; Phillips et al., 2010; Wei et al., 2016; Zhao, Liu, Zhou, Tian, & Chang, 2013). Sea urchin aquaculture could be a means by which to resolve this problem and meet increasing demands for roe in the future.

The sea urchin, *Strongylocentrotus intermedius*, was originally found in the coast from Hokkaido in Japan to the Far East Russia and in 1989 this species was introduced from Japan to China (Chang & Wang, 1997, pp. 7–14) and now is a commercially important species in the North China sea. In China, the annual production of *S. intermedius* roe (gonad) is 200 tons (Chang, Zhang, Zhao, & Song, 2012) and these species are traditionally fed on *Laminaria japonica* and *Laminaria religiosa* (Chang, Lawrence, Cao, & Lawrence, 2005). However, these two types of large algae are mainly available from December to July and are scarce during late Summer and Autumn (August to November) when juvenile sea urchins require abundant food of high-quality to sustain fast growth and allow adults to mature (Chang, Wang, & Wang, 1999).

Artificially formulated feeds are an alternative food source for adult sea urchins and artificial feeds have been shown to induce gonadal development and quality in the green sea urchin

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(*Strongylocentrotus droebachiensis*) (Daggett, Pearce, Tingley, Smc, & Chopin, 2005; Liyana-Pathirana, Shahidi, & Whittick, 2002; Pearce, Daggett, & Robinson, 2004), in the red sea urchin (*Strongylocentrotus franciscanus*) (McBride, Price, Tomb, Lawrence, & Lawrence, 2004), in the common sea urchin (*Evechinus chloroticus*) (Phillips et al., 2010) and also in *S. intermedius* (Chang et al., 2005; Lawrence, Chang, Cao, Lawrence, & Watts, 2011). Protein content is one of the most important components in aquaculture feeds (Heflin et al., 2012) and determination of optimal protein requirements is essential to formulate well-balanced and low-cost diets for sea urchins. Previous studies revealed that a 20% dietary protein content are optimal for gonad development in adult sea urchins (Hammer, Watts, Lawrence, Lawrence, Desmond, 2006a, 2004). However, information is scarce about diet formulation for juveniles.

The seasonal range of environmental temperature for *S. intermedius* is 5 °C–25 °C in the Sea of Japan (Lawrence, Cao, Chang, Lawrence, & Watts, 2009). However, during late summer and in the autumn, juvenile sea urchins (*S. intermedius*) are usually subject to heat stress due to the higher water temperatures (>26 °C) in the main culture areas in the North of China. Environmental stress could cause the production of reactive oxygen species (ROS) (Hegazi, Attia, & Ashour, 2010). Superoxide dismutase (SOD) and catalase (CAT) scavenge free radicals to avoid hyperoxidation of membrane phospholipids, exerting an important role in inhibiting the formation of ROS. Heat shock protein 70 (HSP70) is a class of molecular chaperones that are best known for their participation in the stress response and environmental acclimation of a diverse number of organisms (Deng, Hu, Zhan, Ma, & Tang, 2015; Huang et al., 2015; Metzger, Hemmer-Hansen, & Schulte, 2016). Thus, the present study was conducted to investigate the effects of dietary protein concentration on the growth performance, anti-oxidative enzyme activity and HSP70 gene expression in the sea urchin, *S. intermedius*. The aim was to ascertain optimal protein levels in formulated diets for juvenile sea urchins from the perspective of growth performance and anti-heat stress capacity.

2. Materials and methods

2.1. Experimental diets and feeding experiment

Ingredients and nutrient composition of the experimental diets are described in Table 1. Five semi-purified isolipidic (5.5% crude lipid) experimental diets were formulated to contain graded levels of protein from 12% to 36% of dry weight by supplementation of casein and gelatin (*w*:*w* = 4:1).

All ingredients were ground to a fine powder through a 320 μm mesh. Ingredients were first blended thoroughly by hand and subsequently using a mixing machine (Jinan Dingrun Machinery Company, Jinan, Shandong province, China). After that, an oil mixture of 1.5% fish oil and 1.5% soybean oil, was thoroughly mixed with all the ingredients. Finally, 25% water was incorporated to make a stiff dough. Pellets were made automatically using a pellet-making machine (Jinan Dingrun Machinery Company, Jinan, Shandong province, China) and dried for about 12 h in a ventilated oven at 40 °C. After drying, feeds were packed in double plastic bags and stored in nylon bags at room temperature until use.

The feeding experiment was conducted from October to January at the Key Laboratory of Mariculture, Ministry of Agriculture in Dalian, China. Sea urchins (initial body weight 1.53 ± 0.01 g) were obtained from the same Laboratory. Prior to the start of the experiment, sea urchins of a similar size were reared in holding tanks (180 cm × 100 cm × 80 cm) and fed with the lowest protein diet (Table 1) every two days for two weeks to adapt them to the experimental conditions and the formulated feeds. Then, sea urchins (n = 20, per cage) were distributed between 15 net cages (diameter = 10 cm, height = 25 cm) and each diet was randomly allocated to three cages. All cages were distributed randomly in a large holding tank (180 cm × 100 cm × 80 cm) with a total volume of 1440 L. The tank was in a flow-through system and the water flow was kept at a speed of 0.5 L/min. Feeds were supplied every 2 days and the feeding experiment lasted for 112 days. Water temperature ranged from 9 to 19 °C, salinity was 30 and sea water was aerated with dissolved oxygen (7 mg/L). The content of ammonia nitrogen and nitrite in the water were lower than 0.04 and 0.1 mg/L, respectively.

Table 1
Ingredients and proximate composition of the experimental diets (% dry diet).

Ingredients	Dietary treatments				
	P-12	P-18	P-24	P-30	P-36
Seaweed meal[a]	32.0	32.0	32.0	32.0	32.0
Soybean meal[b]	8.0	8.0	8.0	8.0	8.0
Kelp meal[c]	5.0	5.0	5.0	5.0	5.0
Wheat meal[d]	40.5	33.0	25.5	18.0	10.5
Casein[d]	0	6.0	12.0	18.0	24.0
Gelatin	0	1.5	3.0	4.5	6.0
Alginate sodium	5.0	5.0	5.0	5.0	5.0
Mineral premix[e]	2.5	2.5	2.5	2.5	2.5
Vitamin premix[f]	2.0	2.0	2.0	2.0	2.0
Fish oil	1.5	1.5	1.5	1.5	1.5
Soybean oil	1.5	1.5	1.5	1.5	1.5
Lecithin	2.0	2.0	2.0	2.0	2.0
Proximate composition					
Moisture	4.9	5.1	4.8	4.7	5.0
Crude protein	12.8	18.4	24.7	30.2	36.9
Crude lipid	5.8	5.7	5.6	5.5	5.4

[a] Seaweed meal: Mixture of *Sargassum thunbergii* and Ruppiaceae (*m*:*m* = 1:1), crude protein 14% dry matter, crude lipid 0.4% dry matter, Aotai Biotechnology, Dalian, China.
[b] Soybean meal: crude protein 49.4% dry matter, crude lipid 0.9% dry matter.
[c] Kelp meal (*Laminaria japonica*):crude protein 15% dry matter, crude lipid 0.5% dry matter.
[d] Wheat meal: crude protein 16.4% dry matter, crude lipid 1.0% dry matter; casein: 93% crude protein and 1% crude lipid, Alfa Aesar, Avocado Research Chemicals Ltd, UK.
[e] Mineral premix (mg or g/kgdiet): $CuSO_4 \cdot 5H_2O$, 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.
[f] Vitamin premix (mg or g/kgdiet): 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.

2.2. Heat stress procedure and sampling

At the end of the feeding experiment, all sea urchins (*S. intermedius*) were fasted for 24 h, counted and individually weighed. Intestine and coelomic fluid was sampled from three individuals per cage. Subsequently, the remaining animals were moved into a tank where water temperature was kept at 26 °C and three individuals from each treatment were sampled at 4 different time points 0 min, 15 min, 2 h and 6 h. The coelomic fluid was obtained from the cavity of three sea urchins in each cage using a 27-gauge needles and 1 mL syringes, coelomic cells were separated by centrifugation (3000 r/min, 5 min, 4 °C) and the upper fluid was frozen in liquid nitrogen and stored at −80 °C to determine the activity of SOD, CAT and the anti-oxidant capacity (AOC). The intestine of three sea urchins in each cage were sampled, pooled into 1.5 mL tube (RNAase-Free, Axygen, USA), frozen in liquid nitrogen and stored at −80 °C until determination of the expression of the heat shock protein 70 (HSP70).

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