Journal of
Marine Science and Engineering

MDPI

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Effects of Selenium Yeast Addition on the Growth, Intestinal Health, Immune Status and Body Composition of Juvenile Sea Cucumber *Apostichopus japonicus* before and after Aestivation

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Abstract: This study was performed to investigate the effects of selenium yeast (Se-yeast) on the growth, intestinal health, immune status and body composition of juvenile *Apostichopus japonicus* before and after aestivation. Five experimental diets were formulated with increasing addition of Se-yeast (0, 0.5, 1.0, 1.5 and 2.0 mg/kg), with the diet without Se-yeast as the control. Each diet was randomly assigned to three tanks of juvenile *A. japonicus* (initial body weight: 2.96 ± 0.04 g). The whole experiment lasted for 135 days, which included a 45-day feeding experiment before aestivation, a 60-day aestivation phage and a 30-day feeding experiment after aestivation. The results showed that weight gain rate (WGR) was significantly increased by the increasing addition of Se-yeast before aestivation. After aestivation, WGR was markedly elevated by 1.0 mg/kg Se-yeast but was inhibited by 1.5–2.0 mg/kg Se-yeast. The evisceration rate (ER) of *A. japonicus* was obviously inhibited by the relatively higher addition level of Se-yeast (1.5–2.0 mg/kg) before aestivation. After aestivation, the ER was significantly inhibited by Se-yeast at an addition of 1.5 mg/kg. Before aestivation, the highest activities of all digestive enzymes were obtained by Se-yeast addition at a level equal to or above 1.0 mg/kg Se-yeast. After aestivation, the activities of most digestive enzymes were maximized by Se-yeast at the addition level of no more than 1.0 mg/kg. Before aestivation, the activities of nitric oxide synthase and alkaline phosphatase significantly increased by the increasing addition of Se-yeast. After aestivation, immune related parameters exhibited the highest or comparable values when the addition level of Se-yeast was equal to or less than 1.0 mg/kg. Notably, the selenium content in the body wall of *A. japonicus* increased significantly as Se-yeast addition increased in the diets. These results showed that Se-yeast at the addition level of 1.5–2.0 mg/kg before aestivation and 1.0–1.5 mg/kg after aestivation was beneficial for the promotion of growth, intestinal health and immune status of juvenile *A. japonicus.*

Keywords: *A. japonicus*; sea cucumber; selenium yeast; growth performance; digestive enzymes; evisceration rate; aestivation

Citation: Zuo, R.; Wu, X.; Wang, Z.; Zhou, X.; Chang, Y.; Yang, Z.; Huang, Z.; Ding, J. Effects of Selenium Yeast Addition on the Growth, Intestinal Health, Immune Status and Body Composition of Juvenile Sea Cucumber *Apostichopus japonicus* before and after Aestivation. *J. Mar. Sci. Eng.* **2023**, *11*, 601. https://doi.org/10.3390/jmse11030601

Academic Editors: Dariusz Kucharczyk and Giuseppe Esposito

Received: 6 February 2023
Revised: 25 February 2023
Accepted: 7 March 2023
Published: 13 March 2023

1. Introduction

Sea cucumber (*Apostichopus japonicus*) is one of the most important marine culture species for its nutritional, medicinal and healthcare values in China [1]. In the past decades, wild populations of *A. japonicus* have declined seriously due to the overfishing and environmental destruction [2]. Aquaculture of *A. japonicus* has been attempted and gradually expanded in the Northern China, especially after overcoming the breeding and nursery technologies in the 1990s. Now, the annual output exceeds 180 thousand tons, which greatly soothes the reliance on the worldwide wild sea cucumber resources [3]. The indoor nursery of *A. japonicus* larvae and juveniles is usually challenged by a high water temperature during summer, when the incidence of disease outbreak and evisceration rate of *A. japonicus* is usually increased [4]. To deal with high temperature, sea cucumbers entered a hypometabolic state, which is called as aestivation [5]. During aestivation, sea cucumbers showed intestinal degeneration and cessation of feed intake. Therefore, the nutritional enrichment before and after aestivation is important for the sea cucumbers to build up their strength.

Selenium (Se), as an essential trace element, is involved in regulating growth performance, nonspecific immune response and antioxidant capacity of almost all organisms [6–12]. There are two existing forms of Se, among which organic Se is widely used as a feed additive due to its advantages of lower toxicity [13,14]. Selenium methionine (Se-met), biofermenting Selenium (Se-bio) and selenium yeast (Se-yeast) are the main forms of organic Se which have been previously used as a feed additive for *A. japonicus* [2,15–17]. It was found that dietary Se-met at an addition level of 0.4–0.6 mg/kg promoted the growth and immune parameters of juvenile *A. japonicus* [15]. Compared to Se-met, Se-bio showed better effects on accumulation efficiency and immune capacity of *A. japonicus* [17]. A recent study found that Se-yeast at an addition level of 0.5–1.0 mg/kg was beneficial for the promotion of growth and immune response of early juvenile (appropriately, 10,000 individuals/kg) *A. japonicus* [2]. However, to the best of our knowledge, the effects of Se-yeast addition on the growth, intestinal health, immune status and body composition of juvenile (appropriately 400 individuals/kg) *A. japonicus*, before and after aestivation, is unknown.

Thus, the effects of Se-yeast addition on the growth, intestinal health, immune status and body composition of juvenile *A. japonicus* were investigated before and after aestivation. The aim of this study was to ascertain the optimal addition level of Se-yeast for juvenile *A. japonicus* to better cope with high-temperature water.

2. Materials and Methods

2.1. Experimental Diets

The Se-yeast was obtained from Angel Yeast Co., Ltd. (Yichang, China). It was previously found that the optimal Se-yeast addition level was 0.5–1.0 mg/kg for the survival and growth performance in juvenile *A. japonicus* [2]. Thus, the actual Se-yeast addition level in this study was 0, 0.5, 1.0, 1.5 and 2.0 mg/kg, respectively. Table 1 shows the formulation and proximate analysis of experimental feeds. The fine ingredients of each feed were successively weighed and then mixed evenly in a plastic bag by hand. After that, about 30% water was added before the pellets were extruded through a pellet-making machine, which were then dried at 60 °C. Finally, the pellets were crushed and sieved into proper sizes.

Table 1. The ingredients and proximate analysis of experimental diets (g/kg).

Ingredient	Selenium Yeast Addition Level (mg/kg)				
	0	**0.5**	**1.0**	**1.5**	**2.0**
Sea mud [a]	500	500	500	500	500
Sargassum fusiforme powder [b]	400	400	400	400	400
Sargassum thunbergii powder [c]	60	60	60	60	60
Shell powder powder	30	30	30	30	30
Selenium-free yeast	10	9.75	9.5	8.7	9
Selenium yeast	-	0.25	0.5	0.75	1
Proximate composition					
Crude protein (%)	9.71	9.72	9.70	9.71	9.70
Crude lipid (%)	1.62	1.61	1.62	1.61	1.60

[a] Sea mud: crude protein 0.5% dry matter, Dalian Xin Yulong Marine Biological seed Technology Co., Ltd. (Dalian, Liaoning Province, China). [b] Sargassum fusiforme powder: crude protein 11% dry matter, crude lipid 1.6% dry matter, Dalian Xin Yulong Marine Biological seed Technology Co., Ltd. (Dalian, Liaoning Province, China). [c] Sargassum thunbergii powder: crude protein 16.8% dry matter, crude lipid 3% dry matter, Dalian Xin Yulong Marine Biological seed Technology Co., Ltd. (Dalian, Liaoning Province, China).

2.2. Feeding Experiment Program

Juvenile *A. japonicus* were provided by Dalian Xinyulong Marine Biological Seed Technology Co., Ltd. (Dalian, China), which were then acclimated for two weeks to adapt to the experimental procedures. Then, *A. japonicus* without obvious symptom of disease were randomly distributed into 15 tanks (500 L). Each tank was cultured with 60 individuals with an initial body weight of 2.96 ± 0.04 g. Each experimental diet was randomly assigned to three tanks of animals twice daily at 7:00 and 16:00. The whole experiment was divided into three stages. At the first stage, the water temperature was 19.0–22.0 °C, which lasted for 45 days. At the second stage, the water temperature was 23–25 °C, which was the aestivation stage and lasted for 60 days. At the third stage, the experimental water temperature was 19–22 °C, which lasted for 30 days. The detailed experiment program can be referred in Table 2. About 30% seawater was exchanged daily from each tank during the experiment, with water salinity, pH and dissolved oxygen maintained at 30 ± 1 ‰, 8.0 ± 0.1 and above 8.0 mg/L, respectively.

Table 2. Experimental procedures in this study.

Experimental Stages		Before Aestivation	Aestivation	After Aestivation
Feeding procedures	Feeding	+	-	+
	Water temperature	19.0–22.0 °C	23–25 °C	22–19 °C
	Lasting period	45 days	60 days	30 days
Sampling procedures	Digestive tract	+	-	+
	Body wall	+	+	+
	Coelomic fluid	+	+	+
Sample analysis	Digestive enzyme activities	+	-	+
	Proximate composition	+	+	+
	Immune and antioxidant capacity	+	+	+

2.3. Sampling Procedures

Before sampling, the feeding activities were stopped for 48 h at each stage. The body weight and the number of animals in each tank were recorded. Then, 15 individuals were chosen out of each tank, which were dissected for the coelomic fluid and digestive tract. The coelomic fluid was centrifugated (3500 rpm, 4 °C, 10 min) to obtain the supernatant, which was later analyzed for the immune parameters. The length and weight of intestine of *A. japonicus* were measured individually, pooled into sterile tubes, and then stored at −80 °C for analyzing digestive enzyme activities.

2.4. Analyzation of Digestive Enzymes and Immune Enzymes

The procedures of supernatant preparation have been previously described by Ning et al. [2]. Briefly, the digestive tract from each tank was homogenated with cold 0.9% NaCl. Then, the homogenate was centrifuged (4 °C, 2500 rpm, 10 min) to obtain the supernatant. Commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used for the determination of the activities of digestive enzymes (protease, amylase, cellulase and lipase) and the total nitric oxide synthase (T-NOS), alkaline phosphatase (AKP), superoxide dismutase (SOD) and catalase (CAT).

2.5. Analyzation of Body Compositon

The proximate body composition (crude protein, crude lipid, carbohydrates and moisture) was analyzed according to the methods described by AOAC [18]. The moisture was determined by drying the samples to constant weight at 105 °C. Crude protein and lipid were determined by using the Kjeldahl method and Soxhlet method, respectively. The detailed procedures of Se determination have been recorded in Ning et al. [2]. Briefly, an appropriate amount of dry sample was cold digested overnight by adding 10 mL of the mixed acid (perchloric acid: nitric acid, 1:9), and it was then heat digested by adding 2 mL nitric acid and 5 mL HCl successively. After that, the solution was transferred and mixed with 2.5 mL K_3 $Fe(CN)_6$. At last, Se contents were assayed based on hydride atomic fluorescence spectrometry methods [19].

2.6. Calculations and Statistical Analysis

Survival rate (SR, %) = $N_f/N_i \times 100$
Weight growth rate (WGR, %) = $(FBW - IBW)/IBW \times 100$
Relative digestive tract length (RDL, %) = $DL/BL \times 100$
Relative digestive tract weight (RDW, %) = $DW/FBW \times 100$
Visceral somatic index (VSI, %) = $VW/FBW \times 100$
Evisceration rate (ER, %) = $N_e/N_i \times 100$

where N_i, N_f, *IBW* and *FBW* are the initial number, final number, initial body weight and final body weight of *A. japonicus* from each tank, respectively; *DL* and *BL* are the digestive tract length and whole body length of *A. japonicus* from each tank, respectively; *DW*, *VW* and *BW* are the digestive tract weight, viscera weight and body weight of *A. japonicus* from each tank, respectively; and N_e was the number of *A. japonicus* with evisceration of each tank.

The experimental data were analyzed by using software SPSS 22.0 (Redmond, WA, USA). One-way analysis of variance (ANOVA) was used to detect whether there is a significance among dietary groups ($p < 0.05$). Then, Duncan multiple comparisons was chosen to compare the differences in means between dietary groups. All data are presented in the form of means ± standard error (SE).

3. Results

3.1. Growth Performance

Before aestivation, the WGR of *A. japonicus* significantly increased with the increase in Se-yeast addition level in the diets ($p < 0.05$). As the addition level increased from 0 to 0.5 mg/kg, the VSI of *A. japonicus* significantly increased ($p < 0.05$), and then decreased as the addition level increased from 0.5 to 2.0 mg/kg ($p < 0.05$). The ER of *A. japonicus* was obviously inhibited by Se-yeast at relatively higher addition levels (1.5–2.0 mg/kg) (Table 3).

Table 3. Effects of selenium yeast addition on the growth and physiological performance of juvenile sea cucumber (*Apostichopus japonicus*) before and after aestivation (means ± SE, n = 3).

Indices	Selenium Yeast Addition Level (mg/kg)				
	0	**0.5**	**1.0**	**1.5**	**2.0**
Before aestivation					
IBW (g)	2.97 ± 0.07	3.04 ± 0.02	2.86 ± 0.01	2.88 ± 0.09	3.04 ± 0.03
FBW (g)	4.67 ± 0.04 ^{e}	5.76 ± 0.04 ^{b}	4.99 ± 0.05 ^{d}	5.34 ± 0.04 ^{c}	5.94 ± 0.05 ^{a}
BL (cm)	3.68 ± 0.05 ^{c}	4.35 ± 0.11 ^{a}	3.81 ± 0.06 ^{c}	3.7 ± 0.04 ^{c}	4.10 ± 0.10 ^{b}
BW (cm)	1.17 ± 0.05 ^{c}	1.27 ± 0.03 ^{bc}	1.23 ± 0.02 ^{bc}	1.54 ± 0.04 ^{a}	1.34 ± 0.04 ^{b}
DL (cm)	11.91 ± 0.33 ^{c}	14.56 ± 0.57 ^{b}	17.65 ± 0.08 ^{a}	12.94 ± 0.72 ^{bc}	14.38 ± 0.65 ^{b}
DW (g)	0.24 ± 0.02 ^{b}	0.25 ± 0.01 ^{b}	0.25 ± 0.01 ^{b}	0.29 ± 0.01 ^{b}	0.34 ± 0.02 ^{a}
WGR (%)	57.90 ± 1.0 ^{d}	83.3 ± 1.6 ^{b}	74.3 ± 0.6 ^{c}	85.6 ± 0.5 ^{b}	94.3 ± 1.4 ^{a}
SR (%)	96.43 ± 1.11	98.85 ± 1.15	97.41 ± 1.37	100.00	97.04 ± 1.61
RDL (%)	3.24 ± 0.13 ^{b}	3.27 ± 0.19 ^{b}	4.58 ± 0.13 ^{a}	3.53 ± 0.20 ^{b}	3.5 ± 0.10 ^{b}
RDW (%)	0.05 ± 0.00 ^{b}	0.06 ± 0.00 ^{a}	0.05 ± 0.00 ^{b}	0.05 ± 0.00 ^{b}	0.05 ± 0.00 ^{b}
ER (%)	5.93 ± 0.49 ^{b}	8.93 ± 0.47 ^{a}	9.63 ± 0.35 ^{a}	0 ^{c}	0 ^{c}
VSI (%)	45.44 ± 0.10 ^{c}	69.79 ± 2.58 ^{a}	52.93 ± 1.80 ^{b}	58.01 ± 0.38 ^{b}	47.11 ± 0.62 ^{c}
VBI (%)	32.49 ± 1.25 ^{b}	40.92 ± 0.25 ^{a}	32.28 ± 1.28 ^{b}	33.84 ± 0.42 ^{b}	31.85 ± 0.42 ^{b}

Table 3. *Cont.*

Indices	Selenium Yeast Addition Level (mg/kg)				
	0	0.5	1.0	1.5	2.0
After aestivation					
IBW (g)	1.89 ± 0.24	2.09 ± 0.05	1.65 ± 0.23	1.55 ± 0.04	1.77 ± 0.28
FBW (g)	3.38 ± 0.41 ^{ab}	4.13 ± 0.13 ^{a}	3.40 ± 0.01 ^{ab}	3.07 ± 0.21 ^{b}	3.66 ± 0.15 ^{ab}
BL (cm)	3.36 ± 0.11 ^{b}	4.73 ± 0.43 ^{a}	3.81 ± 0.22 ^{ab}	3.72 ± 0.49 ^{ab}	3.79 ± 0.10 ^{ab}
BW (cm)	1.49 ± 0.01	1.52 ± 0.11	1.53 ± 0.09	1.55 ± 0.11	2.06 ± 0.61
DL (cm)	10.58 ± 0.75	10.43 ± 1.40	10.99 ± 0.17	11.20 ± 1.92	10.01 ± 1.28
DW (g)	0.22 ± 0.02	0.32 ± 0.06	0.29 ± 0.02	0.35 ± 0.19	0.39 ± 0.02
WGR (%)	89.0 ± 2.3 ^{c}	104.1 ± 6.2 ^{bc}	130.6 ± 1.6 ^{a}	103.2 ± 3.1 ^{bc}	121.5 ± 5.2 ^{ab}
SR (%)	100 ± 0 ^{a}	100 ± 0 ^{a}	100 ± 0 ^{a}	100 ± 0 ^{a}	94.66 ± 2.68 ^{b}
RDL (%)	3.14 ± 0.12 ^{a}	2.21 ± 0.23 ^{b}	2.9 ± 0.18 ^{a}	2.98 ± 0.17 ^{a}	2.63 ± 0.28 ^{ab}
RDW (%)	0.07 ± 0.00	0.08 ± 0.02	0.10 ± 0.01	0.12 ± 0.07	0.11 ± 0.01
ER (%)	8.46 ± 1.00 ^{b}	8.10 ± 0.96 ^{b}	9.09 ± 0.05 ^{ab}	3.30 ± 0 ^{c}	11.25 ± 0.72 ^{a}
VSI (%)	39.3 ± 4.14	40.26 ± 3.96	46.26 ± 11.88	43.09 ± 5.85	44.81 ± 4.21

At each experimental stage, mean values of each index with different superscript letters are significantly different between dietary groups ($p < 0.05$). Abbreviations: IBW, initial body weight; FBW, final body weight; BL, body length; BW, body width; DL, digestive tract length; DW, digestive tract weight; WGR, weight gain rate; SR, survival rate; RDL, relative digestive tract length; RDW, relative digestive tract weight; ER, evisceration rate; VSI: Viscera somatic index; VBI, Visceral body weight index.

After aestivation, WGR of *A. japonicus* showed a "first increasing and then decreasing tendency" with the increase in Se-yeast addition. The highest WGR was observed in the group with 1.0% Se-yeast, which was significantly higher than that in the control group ($p < 0.05$). As the Se-yeast addition level increased, the VSI of *A. japonicus* showed an obviously increasing tendency ($p > 0.05$). The ER of *A. japonicus* showed an opposite changing tendency with WGR as Se-yeast addition level increased, with the lowest value observed in the group with the addition of 1.5 mg/kg Se-yeast (Table 3).

3.2. Digestive Enzyme Activities

Before aestivation, as the Se-yeast addition level increased, pepsin and amylase activities first increased and then decreased. The highest pepsin and amylase activities were observed in the dietary group with the addition of 1.0 mg/kg Se-yeast ($p < 0.05$). While the activities of cellulase and lipase increased significantly as Se-yeast addition elevated in the diets ($p < 0.05$) (Table 4).

Table 4. Effects of selenium yeast addition on digestive enzyme activities of juvenile sea cucumber (*Apostichopus japonicus*) before and after aestivation (means ± SE, n = 3).

Indices	Selenium Yeast Addition Level (mg/kg)				
	0	0.5	1.0	1.5	2.0
Before aestivation					
Pepsin	1.53 ± 0.01 ^{c}	1.57 ± 0.02 ^{bc}	1.67 ± 0.02 ^{a}	1.63 ± 0.02 ^{ab}	1.56 ± 0.03 ^{bc}
Amylase	1.47 ± 0.02 ^{ab}	1.49 ± 0.00 ^{ab}	1.51 ± 0.02 ^{a}	1.50 ± 0.02 ^{a}	1.42 ± 0.03 ^{b}
Cellulase	7.98 ± 0.05 ^{e}	10.16 ± 0.11 ^{d}	12.17 ± 0.13 ^{c}	13.34 ± 0.09 ^{b}	15.09 ± 0.08 ^{a}
Lipase	4.44 ± 0.06 ^{e}	5.04 ± 0.02 ^{d}	5.35 ± 0.02 ^{c}	5.52 ± 0.01 ^{b}	9.62 ± 0.06 ^{a}
After aestivation					
Pepsin	1.41 ± 0.19 ^{b}	1.59 ± 0.44 ^{b}	2.50 ± 0.14 ^{a}	2.29 ± 0.32 ^{ab}	2.08 ± 0.09 ^{ab}
Amylase	1.33 ± 0.10	2.24 ± 0.04	1.85 ± 0.35	1.76 ± 0.29	1.87 ± 0.38
Cellulase	9.30 ± 2.43	11.64 ± 1.76	12.13 ± 2.12	13.53 ± 3.38	13.42 ± 1.78
Lipase	5.24 ± 1.13 ^{c}	10.22 ± 1.65 ^{abc}	13.10 ± 0.71 ^{a}	11.95 ± 1.62 ^{ab}	6.76 ± 0.72 ^{bc}

At each experimental stage, mean values of each index with different superscript letters are significantly different between dietary groups ($p < 0.05$). The unit of pepsin, amylase and cellulase was U/mg prot; the unit of lipase was U/g prot.

After aestivation, pepsin and lipase activities showed a "first increasing and then decreasing tendency" with the increase in Se-yeast addition. The highest activities of pepsin and lipase were detected in the 1.0 mg/kg Se-yeast group. The cellulase activity showed an increasing tendency with the increase in Se-yeast addition ($p > 0.05$) (Table 4).

3.3. Immune Reponse

Before aestivation, NOS and AKP activities significantly increased as the Se-yeast addition level increased in the diets. The highest SOD and CAT activities were obtained by Se-yeast at an addition of 1.0 mg/kg Se-yeast, which was significantly higher than those at other addition levels except for the 1.5 mg/kg ($p > 0.05$) (Table 5).

Table 5. Effects of selenium yeast supplementation on the immunity enzyme activities and antioxidant capacity of juvenile sea cucumber (*Apostichopus japonicus*) before and after aestivation (means ± SE, n = 3).

Indices	Selenium Yeast Addition Level (mg/kg)				
	0	0.5	1.0	1.5	2.0
Before aestivation					
T-NOS	4.81 ± 0.02 ^{e}	6.19 ± 0.03 ^{d}	8.27 ± 0.07 ^{c}	9.72 ± 0.31 ^{b}	11.19 ± 0.73 ^{a}
AKP	0.61 ± 0.01 ^{c}	0.68 ± 0.03 ^{c}	0.89 ± 0.02 ^{b}	0.93 ± 0.06 ^{b}	1.28 ± 0.06 ^{a}
SOD	135.09 ± 0.97 ^{c}	142.97 ± 0.42 ^{b}	148.22 ± 0.72 ^{a}	144.21 ± 0.24 ^{ab}	142.18 ± 2.83 ^{b}
CAT	0.36 ± 0.01 ^{c}	0.40 ± 0.00 ^{b}	0.43 ± 0.01 ^{a}	0.42 ± 0.01 ^{ab}	0.36 ± 0.01 ^{c}
GSH-Px	14.82 ± 0.86 ^{a}	10.58 ± 0.07 ^{bc}	12.66 ± 0.58 ^{ab}	13.17 ± 1.08 ^{a}	9.06 ± 0.14 ^{c}
MDA	1.06 ± 0.06 ^{a}	1.06 ± 0.05 ^{a}	0.67 ± 0.01 ^{b}	0.56 ± 0.02 ^{b}	0.43 ± 0.05 ^{c}
Aestivation					
T-NOS	1.59 ± 0.184 ^{a}	1.08 ± 0.028 ^{b}	1.57 ± 0.039 ^{a}	1.14 ± 0.078 ^{b}	1.16 ± 0.028 ^{b}
AKP	0.74 ± 0.17 ^{c}	1.04 ± 0.06 ^{bc}	1.45 ± 0.12 ^{a}	1.26 ± 0.08 ^{ab}	0.34 ± 0.02 ^{d}
SOD	132.91 ± 0.85 ^{ab}	121.01 ± 9.38 ^{b}	121.80 ± 1.14 ^{b}	143.42 ± 2.21 ^{a}	124.63 ± 4.61 ^{b}
GSH-Px	13.05 ± 0.83 ^{b}	16.12 ± 3.47 ^{ab}	17.12 ± 2.58 ^{ab}	21.30 ± 0.33 ^{a}	16.64 ± 1.63 ^{ab}
CAT	0.31 ± 0.05	0.39 ± 0.02	0.36 ± 0.06	0.34 ± 0.03	0.34 ± 0.02
MDA	0.32 ± 0.07 ^{c}	0.38 ± 0.17 ^{bc}	0.58 ± 0.10 ^{bc}	0.74 ± 0.14 ^{b}	2.09 ± 0.10 ^{a}
After aestivation					
T-NOS	4.00 ± 0.40	4.82 ± 0.75	4.11 ± 0.44	3.55 ± 0.77	4.96 ± 0.37
AKP	1.08 ± 0.21 ^{a}	0.63 ± 0.13 ^{b}	0.61 ± 0.05 ^{b}	0.54 ± 0.08 ^{b}	0.59 ± 0.15 ^{b}
SOD	130.34 ± 0.75 ^{b}	130.37 ± 0.34 ^{b}	139.77 ± 5.80 ^{ab}	135.27 ± 0.77 ^{ab}	141.82 ± 0.19 ^{a}
GSH-Px	19.76 ± 0.71 ^{a}	17.23 ± 1.28 ^{ab}	15.13 ± 1.09 ^{bc}	11.59 ± 2.40 ^{cd}	10.38 ± 0.59 ^{d}
CAT	0.28 ± 0.03 ^{c}	0.43 ± 0.02 ^{a}	0.43 ± 0.02 ^{a}	0.36 ± 0.00 ^{b}	0.31 ± 0.01 ^{bc}
MDA	0.64 ± 0.08	0.83 ± 0.23	0.79 ± 0.29	0.68 ± 0.14	0.86 ± 0.21

At each experimental stage, mean values of each index with different superscript letters are significantly different between dietary groups ($p < 0.05$). Abbreviations: SOD (U/mL), superoxide dismutase; MDA (nmol/mL), malonaldehyde; CAT (U/mL), catalase; T-NOS (U/mL), total nitricoxide synthase; AKP (U/100 mL), alkaline phosphatase; GSH-PX (U/mL), glutathione peroxidase.

During aestivation, activities of AKP, SOD and GSH-Px showed "first increasing and then decreasing" changing tendencies as dietary Se-yeast addition increased, with the highest values achieved by 1.0–1.5 mg/kg addition level ($p < 0.05$). No significance was detected in CAT activity between different groups ($p > 0.05$) (Table 5).

After aestivation, AKP and GSH-Px activities significantly decreased with the increase in Se-yeast level ($p < 0.05$). On the contrary, SOD activity increased significantly as dietary Se-yeast increased ($p < 0.05$). As the Se-yeast addition level increased, the CAT activity first increased and then decreased significantly ($p < 0.05$). The highest CAT activity was achieved by Se-yeast at a moderate addition level (0.5–1.0 mg/kg) (Table 5).

3.4. Proximate Compositions

Before and after aestivation, crude protein and moisture showed no significant differences in the body wall of *A. japonicus* ($p > 0.05$). At three sampling timepoints, crude lipid showed a "first increasing and then decreasing" changing tendency as the addition level increased, with *A. japonicus* fed diets with 1.0 mg/kg Se-yeast exhibiting the highest lipid level ($p < 0.05$). During aestivation, crude protein increased significantly with the increasing addition of Se-yeast ($p < 0.05$). *A. japonicus* fed diets with relatively higher addition level (1.5–2.0 mg/kg) of Se-yeast showed significantly higher content of crude protein than those without or with 1.0 mg/kg Se-yeast ($p < 0.05$). The content of carbohydrates significantly increased with the increase in Se-yeast after aestivation ($p < 0.05$) (Table 6).

Table 6. Effects of selenium yeast addition on the proximate composition in the body wall of juvenile sea cucumber (*Apostichopus japonicus*) before and after aestivation (means ± SE, n = 3).

Indices	Selenium Yeast Addition Level (mg/kg)				
	0	0.5	1.0	1.5	2.0
Before aestivation					
Crude protein	44.25 ± 1.62	41.95 ± 0.23	42.82 ± 0.79	44.77 ± 1.79	43.38 ± 1.36
Moisture	91.75 ± 0.29	92.02 ± 0.20	92.20 ± 0.26	91.93 ± 0.17	91.86 ± 0.27
Crude lipid	0.09 ± 0.01 [c]	0.10 ± 0.01 [bc]	0.12 ± 0.00 [a]	0.11 ± 0.00 [ab]	0.10 ± 0.00 [bc]
Carbohydrate	11.50 ± 1.17 [a]	8.41 ± 0.23 [b]	11.14 ± 0.50 [a]	8.76 ± 0.24 [b]	11.43 ± 0.09 [a]
Aestivation					
Crude protein	45.07 ± 1.16 [b]	46.67 ± 1.23 [ab]	45.20 ± 0.20 [b]	48.75 ± 0.47 [a]	49.34 ± 0.76 [a]
Moisture	90.62 ± 0.08	90.57 ± 0.13	90.40 ± 0.23	90.31 ± 0.07	90.34 ± 0.08
Crude lipid	0.11 ± 0.00 [a]	0.11 ± 0.00 [ab]	0.12 ± 0.00 [a]	0.10 ± 0.01 [ab]	0.09 ± 0.01 [b]
Carbohydrate	7.34 ± 1.13	7.16 ± 0.48	8.28 ± 0.03	8.94 ± 0.55	8.30 ± 0.80
After aestivation					
Crude protein	45.56 ± 1.21	43.19 ± 1.15	45.08 ± 1.50	45.29 ± 0.70	43.03 ± 0.03
Moisture	92.64 ± 0.08	92.80 ± 0.11	92.81 ± 0.52	92.56 ± 0.18	92.72 ± 0.09
Crude lipid	0.09 ± 0.01 [b]	0.11 ± 0.01 [a]	0.12 ± 0.00 [a]	0.12 ± 0.01 [a]	0.11 ± 0.00 [a]
Carbohydrate	11.56 ± 0.04 [b]	10.51 ± 0.27 [c]	12.03 ± 0.23 [ab]	12.29 ± 0.28 [ab]	12.35 ± 0.23 [a]

At each experimental stage, mean values of each index with different superscript letters are significantly different between dietary groups ($p < 0.05$).

3.5. Selenium Accumulation

At each stage, *A. japonicus* showed an obviously increasing retention of Se by the increasing addition level of Se-yeast in the diets ($p < 0.05$). Compared to the contents before aestivation, the Se content decreased during aestivation, and then it bounced to even higher values after aestivation (Figure 1).

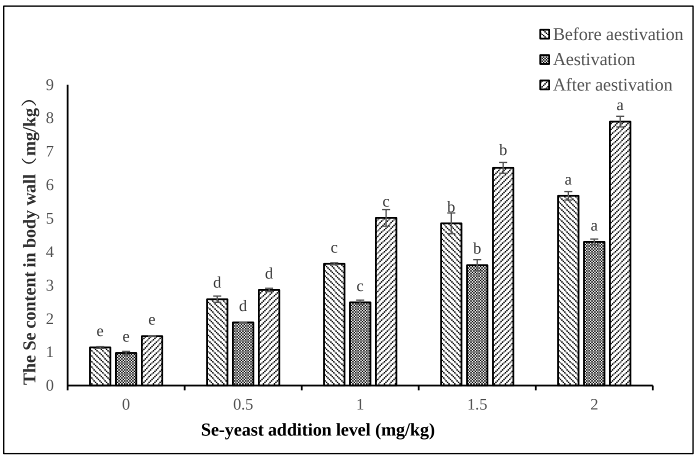

Figure 1. Effects of selenium yeast supplementation in the body wall of juvenile sea cucumber (*Apostichopus japonicus*). At each experimental stage, mean value bars bearing with different letters are significantly different between dietary groups ($p < 0.05$).

4. Discussion

When sea water temperature rises above 22 °C, *A. japonicus* can enter the state of aestivation. During aestivation, *A. japonicus* shows a complete cessation of feeding due to the degeneration of the digestive tract. When water temperature is below about 18 °C, *A. japonicus* is more active and gradually returns to normal food intake.

In this experiment, the WGR of experimental animals before aestivation increased significantly with the increase in Se-yeast addition. A recent study by our research team found that the WGR of early juvenile *A. japonicus* with the initial body weight of about 0.1 g was increased by 0.5–1.0 mg/kg Se-yeast but was inhibited by an overdose (2 mg/kg) addition of Se-yeast [2]. The discrepancy could be due to the different initial body weight of experimental animals, with larger individuals less sensitive to the equal dosage of Se-yeast. After aestivation, Se-yeast showed inhibitory effects on *A. japonicus* as the addition level was equal to or higher than 1.5 mg/kg. It was commonly accepted that Se showed highly toxic effects on animals when the addition level exceeded the optimal dosage [20,21]. Indeed, the detrimental effects of Se have been reported on tilapia (*Oreochromis niloticus*) if the addition level of Se was higher than 2 mg/kg [22]. The digestive enzyme activities of grass carp (*Ctenopharyngodon idella*) reached the maximum values by Se addition at the addition level of 0.6 mg/kg [23]. However, juvenile pacu (*Piaractus mesopotamicus*) showed no significant differences in the activities of digestive enzymes when the addition of Se-yeast ranged from 0.72 to 2.51 mg/kg [24]. The inconsistence could be related to the differences in animal species, initial body weight and feed formulation. The results of the present study showed that the activities of digestive enzymes of *A. japonicus* were promoted by Se-yeast at a moderate or relatively higher addition level (1.0–2.0 mg/kg) before aestivation, while the ideal activities of digestive enzymes were achieved by Se-yeast at the addition level of no more than 1.0 mg/kg. Thus, the optimal addition level of Se-yeast was estimated to be no more than 2.0 mg/kg before aestivation, and 1.0 mg/kg after aestivation for juvenile *A. japonicus* from the perspective of digestive enzyme activities. It could be the previous accumulation of Se in the body wall before aestivation that accounted for the relatively lower requirement of Se after aestivation.

In this study, Se-yeast at moderate or relatively higher addition level (1.0–2.0 mg/kg) significantly increased the immune status of sea cucumbers before aestivation. This was consistent with the findings of Durigon et al. [8], who found that dietary Se ($Na_2 SeO_3$) at an addition level of 0.86 mg/kg enhanced the antioxidant capacity of juvenile tilapia (*Oreochromis niloticus*). However, the optimal addition level of Se-yeast was lower than the findings of Chen et al. [25] and Zeng et al. [19], who found that the highest immune and antioxidant capacity were achieved by 3–12 mg/kg Se-yeast and 5 mg/kg Se-met in the diets. It was previously found that the optimal Se-yeast addition level was 0.5–1.0 mg/kg for the survival and growth performance in juvenile *A. japonicus* [2]. Thus, the highest addition level of Se-yeast was only set at 2.0 mg/kg in this study. Thus, further studies are needed to investigate the effects of Se-yeast at the addition of more than 2.0 mg/kg on the immune and antioxidant status of *A. japonicus*. Furthermore, the ER of was significantly inhibited by relatively higher addition level of Se-yeast (1.5–2.0 mg/kg) before aestivation. This indicated that the good immune status was beneficial for the intestinal health.

The body wall is the main edible part of *A. japonicus*, with abundant nutrients and active metabolites [26,27]. In this study, Se-yeast addition did not significantly affect the crude protein before or after aestivation of *A. japonicus*. During aestivation, the protein content of *A. japonicus* showed an increasing tendency as the addition of Se-yeast increased in the diets. This could be due to the effects of Se on the hindered degradation of protein in the body wall of *A. japonicus*. In this study, the contents of crude lipid in the body wall of *A. japonicus* were markedly increased by the moderate addition level (1.0 mg/kg) of Se-yeast. Zhao et al. [28] found that the lipid deposition in the pigs (*Susscrofa domestica*) were promoted by an addition level of 3 mg/kg Se in the diets. In this study, the increasing addition of Se-yeast increased the contents of carbohydrates in the body wall of *A. japonicus* during or after aestivation. It is possible that the addition of Se-yeast increased the contents of polysaccharide, which is one of the most important nutrients of *A. japonicus.* However, the contents of polysaccharide were not assayed in this study. Thus, further research is needed to clarify this confusion in the future. Se addition has been verified to improve the nutritional value of edible part in several aquatic animals including *A. japonicus* [2], meagre (*Argyrosomus regius*) [12], common carp (*Cyprinus carpio*) [14] and grouper (*Epinephelus malabaricus*) [29]. In accordance, *A. japonicus* of this study showed an obviously retention of Se in the body wall as the addition level of Se-yeast increased. This further verified that application of Se-yeast was an effective strategy to enrich *A. japonicus* with Se elements. During evisceration, the Se content decreased to some extent in the body wall of *A. japonicus*. This indicated that some amount of Se was used for dealing with oxidative stress induced by high temperature.

5. Conclusions

In summary, Se-yeast at the addition level of 1.5–2.0 mg/kg before aestivation and 1.0–1.5 mg/kg after aestivation was beneficial for the promotion of growth, intestinal health and immune status of juvenile *A. japonicus.* Notably, the selenium content in the body wall of *A. japonicus* increased significantly as Se-yeast addition increased in the diets. These results will contribute to providing clues for formulating functional feeds for juvenile *A. japonicus* to better deal with challenges during aestivation.

Author Contributions: R.Z. and X.W. performed the whole experiment. R.Z. and Z.W. drafted the manuscript. X.Z., Z.Y. and Z.H. analyzed the nutrient profile of body wall. J.D. and Y.C. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by High-Level Talent Support Grant for Innovation in Dalian (2020RD03, 2022RJ14).

Institutional Review Board Statement: The animal study protocol was approved by the Animal Care Committee of Dalian Ocean University.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data are available from the corresponding author upon request.

Acknowledgments: We are grateful to all the laboratory members for their technical advice and helpful discussions.

Conflicts of Interest: There is no conflict of interest to declare.

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