

Arachidonic Acid Content in the Feed on the Growth Performance, Antioxidant Capacity and Fatty Acid Generation of Sea Cucumber

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Abstract

In order to explore the effects of arachidonic acid (ARA) on the growth performance, antioxidant capacity and fatty acid metabolism of *Apostichopus japonicus*, the initial weight of (7.78 ± 0.06) g was used as the research object. Using fishmeal and fermented soybean meal as the main protein source, wheat flour as the main sugar source to make the basic feed, by adding different proportions of ARA-purified oil to the basic feed, the ARA content was 0.02% (control group), 0.17%, The experimental feeds of 6 groups of nitrogen and other lipids of 0.36%, 0.51%, 0.59% and 0.98% (dry weight of feed) were subjected to a 56-day culture experiment in an indoor circulating aquaculture system. The results showed that with the increase of ARA content in feed, the weight gain rate (WGR) of the sea cucumber increased first and then decreased. The WGR of 0.36% and 0.51% ARA feed group was significantly higher than other treatment groups. ($P < 0.05$), the specific growth rate (SGR) and feed efficiency (FE) of sea cucumber have the same trend as WGR; the crude fat content of sea cucumber body decreased first with the increase of feed ARA content. The increase trend was the lowest in the 0.51% ARA feed group, and significantly lower than the control group and the 0.98% ARA feed group ($P < 0.05$). Meanwhile, with the increase of ARA content in the feed, the ARA and n in the wall of the sea cucumber -6 polyunsaturated fatty acids (n-6 PUFA) content increased significantly, while eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) And n-3 polyunsaturated fatty acids (n-3 PUFA) content decreased significantly ($P < 0.05$); antioxidant capacity, 0.36% and 0.51% ARA feed group in the intestinal tract superoxide Superoxide dismutase (SOD), catalase (CAT) and total The activity of total antioxidant capacity enzyme (T-AOC) was significantly higher than that of the control group and 0.98% ARA diet group ($P < 0.05$), while the content of malondialdehyde (MDA) in the intestine showed opposite trend. The activity of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) in the intestinal tract of sea cucumber decreased significantly with the increase of ARA content ($P < 0.05$). The activity of carnitine palmitoyltransferase-1 (CPT-1) in the intestinal tract of the sea cucumber increased first and then decreased with the increase of ARA content ($P > 0.05$). Studies have shown that under the conditions of this experiment, the addition of proper amount of ARA (0.36%-0.51%) in the feed can promote the growth and

antioxidant capacity of sea cucumber, and the results show that the content of feed ARA will be in the intestinal tract of sea cucumber. Fatty acid metabolism has a certain effect.

Keywords

Sea cucumber; Arachidonic acid; Growth; Antioxidant capacity; Fatty acid metabolism.

Introduction

In recent years, n-6 highly unsaturated fatty acid (n-6 HUFA), especially arachidonic acid (20: 4n-6, arachidonic acid, ARA) has been used in marine animals. More and more attention. ARA has been shown to be a precursor to a variety of biologically active compounds, collectively referred to as eicosanoids, primarily including prostaglandins (PGs), thromboxane (TX), and leukotrienes (LTs).

These bioactive substances play an important role in animals and can regulate a series of important physiological metabolisms. At the same time, ARA not only plays an important regulatory role in the growth, survival, anti-stress and immunity of marine animals, but also has a certain regulatory effect on lipid metabolism and sex steroid synthesis (Larson et al., 2013).

Apostichopus japonicus is a typical temperate species and is an important marine aquaculture economic variety in China. At present, research on the nutrition of ginseng feed lipids is still in its infancy. Research reports on fatty acid nutrition are rare, and related reports are mainly focused on n-3 highly unsaturated fatty acid (HUFA).

Studies have shown that the appropriate addition amount of crude fat in the feed of ginseng is 3%-5%; it is determined through breeding experiments that the feed with n-3HUFA level of 0.22%-0.38% can make the ginseng have the best growth. Performance and higher nutritional value.

At the same time, it was reported that when 0.60% DHA was added to the feed, the growth rate of sea cucumber was the fastest (Slater and Carton, 2010). While ARA, an essential fatty acid for marine fish, has also been shown to have an effect on the growth of

other marine animals, no relevant reports have been reported in sea cucumbers (Wei et al., 2015).

Therefore, this study studied the effects of ARA content on the growth, antioxidant and fatty acid metabolism of sea cucumber, in order to explore the appropriate addition level of ARA in sea cucumber feed, and preliminarily explained the effect of ARA on the fatty acid metabolism of sea cucumber.

The database of nutritional parameters of sea cucumber is well improved, and it also provides a theoretical basis for studying the fatty acid metabolism of sea cucumber.

Materials and Methods

Experimental Feed

The basic feed uses fishmeal and fermented soybean meal as the main protein source, and wheat flour is the main sugar source.

By adding 0, 0.5%, 1.0%, 1.5%, 2.0% and 2.5% of ARA-purified oil to the basal feed (the ARA content is about 48.70% of the total fatty acids), leveling with stearic acid triglyceride, prepared into 6 groups of nitrogen and other lipid feeds (Table 1).

After gas chromatography analysis, the contents of ARA in each feed were: 0.02% (control group), 0.17%, 0.36%, 0.51%, 0.59% and 0.98% (dry weight of feed). The fatty acid composition of feed was shown in Table 2.

Firstly, all the raw materials are crushed and sieved, then the various raw materials are uniformly mixed, and then the ARA-purified oil is thoroughly mixed with the mixed raw materials, and then an appropriate amount of water is added to prepare a dough, which is then granulated by an automatic granulator.

The prepared feed is placed in an oven at about

45 ° C for drying, and then stored in a cool dry place for later use.

Aquaculture Management

The experimental site is Dongying Experimental Base of Shandong Institute of Marine Resources and

Environment. The breeding cycle is 56 days. The farming method is recirculating aquaculture.

The experimental sea cucumber is the same batch of seedlings that were bred in the same year (Sicuro and Levine, 2011).

Table 1: Formulation and nutrient compositions of the experimental diets.

Ingredient	Dietary arachidonic acid level					
	0.02	0.17	0.36	0.51	0.59	0.98
Fish meal	8.00	8.00	8.00	8.00	8.00	8.00
Fermented soybean meal	17.00	17.00	17.00	17.00	17.00	17.00
Wheat meal	14.00	14.00	14.00	14.00	14.00	14.00
Algae powder	20.00	20.00	20.00	20.00	20.00	20.00
Sea mud	35.50	35.50	35.50	35.50	35.50	35.50
Premix	2.00	2.00	2.00	2.00	2.00	2.00
Soy lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Ara-enrich oil	0	0.50	1.00	1.50	2.00	2.50
Tristearin	2.50	2.00	1.50	1.00	0.50	0
Rotal	100.00	100.00	100.00	100.00	100.00	100.00
Proximate analysis						
Crude protein	19.90	20.18	19.70	19.94	19.87	19.69
Ether extract	4.93	4.95	4.97	4.98	5.01	5.02
Ash	45.92	45.90	45.71	45.60	45.63	45.38
Ara	0.02	0.17	0.36	0.51	0.59	0.98.

Note: *Premix includes 1% mineral premix and 1% vitamin premix which are purchased from Shandong Fishery Feed Research Center. Mineral premix (mg/kg diet): Zn 35.00 mg, Mn 21.00 mg, Cu 8.30 mg, Fe 23.00 mg, Co 1.20 mg, I 1.00 mg, Se 0.30 mg. Vitamin premix(mg/kg or I U/kg diet): Vitamin A 7500.00 IU, vitamin D 1500.00 IU, vitamin E 60.00 mg, vitamin K3 18.00 mg, vitamin B1 12.00 mg; vitaminB2 12.00 mg, vitamin B12 0.10 mg, pantothenate acid 48.00 mg, niacin 90.00 mg, folic acid 3.70 mg, D-biotin 0.20 mg, pyridoxine 60.00 mg, vitamin C 310.00 mg. b ARA-enriched oil: ARA content, 48%. Cabio Biotech (Wuhan) Co. Ltd.

Table 2: The key fatty acid compositions of the experimental diets.

Fatty acid	ARA-enriched oil	Dietary arachidonic acid level					
		0.02	0.17	0.36	0.51	0.59	0.98
C _{18:2n-6}	6.80	12.69	14.18	15.98	17.27	16.28	20.17
C _{20:4n-6}	48.70	0.36	4.07	8.69	12.98	17.12	24.33
C _{18:3n-3}	-	1.35	1.37	1.52	1.51	1.39	1.68
C _{20:5n-3}	-	0.99	1.01	1.04	1.11	1.01	1.20
C _{22:5n-3}	-	0.26	0.28	0.32	0.42	0.34	0.50
C _{22:6n-3}	-	0.97	1.01	1.03	1.03	0.95	1.12

Firstly, all the experimental parameters were placed in the culture tank, and the basic feed was kept

for 15 days to adapt to the experimental feed and breeding conditions. After the end of the holding

period, the healthy and uniform size of the sea cucumber [initial weight (10.78±0.06) g] was randomly divided into 18 culture barrels (75 cm in diameter and 80 cm in depth), and 40 in each breeding barrel (Olivera-Castillo et al., 2011). Head sea cucumber, 3 replicates per experimental group, 2 buckets of sea cucumbers covered with corrugated plates in each bucket, controlling the water depth to 50 cm.

The experiment is fed every day at the specified time (07:00), and the feeding amount is 2% of the body weight of the sea cucumber. The specific feeding amount should be adjusted according to the feeding condition of the sea cucumber. During the experiment, the water temperature was controlled at 17-19 °C, the salinity was 24-26, the dissolved oxygen was >7 mg/L, the ammonia nitrogen and nitrite nitrogen were both <0.05 mg/L, and the water was changed once every 2 days.

Sample Collection and Analysis

At the end of the culture experiment, the experimental sea cucumbers were fasted for 48 h, and then the sea cucumbers in each culture tank were counted and weighed. After that, 10 pieces of sea cucumber were randomly taken from each breeding bucket, placed on an ice tray for anatomical sampling, body cavity fluid, intestinal tract and body wall were taken respectively, and intestinal and body wall samples were stored in -80 °C ultra-low temperature refrigerator in time (Lee et al., 2014). At the same time, the body cavity fluid was centrifuged (3000 r/min, 4 °C, 10 min), and the supernatant was dispensed into a centrifuge tube, and then stored in a -80 °C ultra-low temperature freezer.

Sample Routine and Biochemical Analysis

The moisture of the experimental feed and the body wall of the sea cucumber was determined by the drying constant method at 105 °C (GB/T 6435-2006); the crude protein was determined by the Kjeldahl method (GB/T 6432-2006); Ultrasound-assisted Soxhlet extraction method, crude fat of tissue samples was determined by Soxhlet extraction method (GB/T

6433-2006); crude ash was determined by muffle furnace 550 °C weight loss method (GB/T 6438-2007).

The method for determining the fatty acid content of the body and the sea cucumber is slightly modified. Take about 100 mg of freeze-dried and ground sample, place it in a 15 mL headspace sample glass bottle, add 3 mL of 1N KOH-methanol solution, heat in a 75 °C water bath for 20 min, cool to room temperature, then add 2 mL of 2NHCL-methanol solution was heated in a 75 °C water bath for 20 min (Pangestuti et al., 2016).

After cooling, 1.5 mL of n-hexane (chromatographic grade) was added, shake-extracted, and allowed to stand for stratification. Carefully pipet the mixture of the upper n-hexane and fatty acid methyl ester, and inject 1 µL into the gas chromatograph using a micro-injector, using a flame ionization detector. Finally, the fatty acid species in the sample were determined based on the peak time of the standard fatty acid and determined by peak area normalization (Woo et al., 2013).

Total antioxidant capacity enzyme (T-AOC), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), fatty acid in the intestine Synthetic enzyme (FAS), carnitine palmitoyl transferase-1 (CPT-1) and acetyl-CoA carboxylase (ACC) were all constructed using Nanjing Bioengineering The corresponding kits produced by the institute were measured.

Calculation Formula and Statistical Analysis

Weight gain rate (WGR, %)=100×(the weight of the ginseng at the end of the weight of the thorns)/specific growth rate (SGR, %/d)=100×[ln (the weight of the sea cucumber) / ln (the initial weight of the sea cucumber)] / Survival rate (SR, %) = 100 × the number of thorns / the initial feed efficiency (Fe, %) = 100×(the weight of the sea cucumber) was measured by mean ± standard error ($\bar{x} \pm SE$ ANOVA), and multiple comparisons were performed using Tukey's method, which showed a significant difference when $P < 0.05$.

Results and Analysis

Effect of ARA Content in Feed on the Growth Performance of Sea Cucumber

Figures shows the effect of ARA content in feed on the growth performance of sea cucumber. The results showed that there was no significant difference in the survival rate of sea cucumber ($P>0.05$), which was between 87.50% and 94.17%. When the ARA content in the feed increased, the WGR of sea cucumber increased first and then decreased. Trends, 0.36% and 0.51% ARA feed group WGR was significantly higher than other treatment groups ($P<0.05$), and there was no significant difference between the two groups ($P>0.05$).

The SGR of sea cucumber has the same trend as

WGR. When the ARA content increased from 0.02% to 0.51%, the feed efficiency (FE) of sea cucumber increased from 57.93% to 111.64%, which was the highest in the 0.51% ARA feed group, and significantly higher than other groups ($P<0.05$) (Table 3).

With the further increase of ARA content, FE showed a significant downward trend ($P<0.05$). By quadratic regression analysis of WGR(Y) and ARA content (X) of sea cucumber, the quadratic curve equation $Y = -71.831X^2 + 73.387X + 30.194$ ($R^2 = 0.6968$) was obtained, and the highest WGR of sea cucumber was obtained. The corresponding ARA content was 0.51% (Figure 1).

Table 3: Effects of different dietary arachidonic acid levels on growth performance of *Apostichopus japonicus*.

Parameter	Dietary arachidonic acid level (%)					
	0.02	0.17	0.36	0.51	0.59	0.98
Initial body Weigh	10.78±0.07	10.78±0.13	10.79±0.08	10.79±0.09	10.81±0.05	10.79±0.05
Final body Weigh	14.03±0.07 ^c	15.24±0.21 ^b	16.11±0.17 ^a	16.76±0.20 ^a	15.06±0.17 ^b	14.50±0.16 ^{bc}
Survival	89.17±2.20	89.17±0.83	94.17±3.63	93.33±1.67	87.50±1.44	90.83±1.44
WGR	30.16±1.45 ^c	41.37±0.66 ^b	49.40±2.42 ^a	55.39±2.15 ^a	39.39±1.55 ^b	34.37±0.98 ^{bc}
SGR	0.47±0.02 ^c	0.62±0.01 ^b	0.72±0.03 ^a	0.79±0.02 ^a	0.59±0.02 ^b	0.53±0.02 ^{bc}
FE	57.93±2.56 ^d	79.58±2.42 ^c	100.48±7.03 ^b	111.64±5.67 ^a	74.42±2.08 ^c	67.39±2.33 ^d

Note: Values with different superscripts in the same row are significantly different ($P<0.05$).

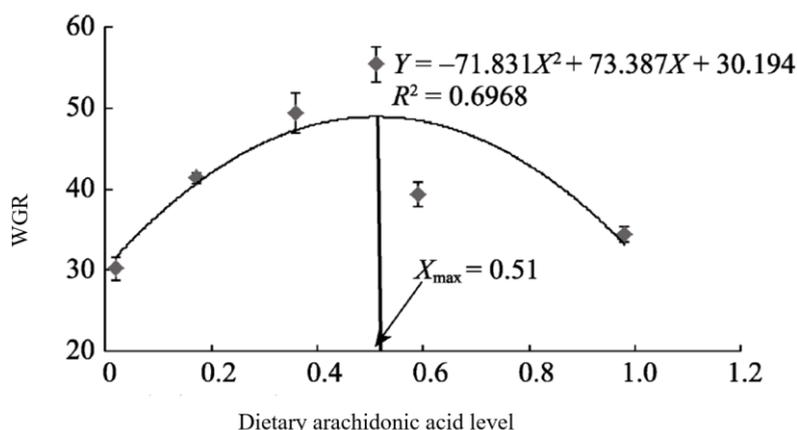


Figure 1: Regression analysis between dietary ARA level and weight gain rate (WGR) of *Apostichopus japonicus*.

Effect of ARA Content in Feed on Chemical Composition of Sea Cucumber Wall

It can be seen from Table 4 that the ARA content in the feed has no significant effect on the water, crude

protein and ash content of the body wall of the sea cucumber ($P>0.05$), and the crude protein content in the body wall of the sea cucumber is 46.15%-46.89% (dry). Heavy, the ash content was 31.06%-32.46% (Baharara et al., 2016).

With the increase of ARA content in the feed, the crude fat content in the body wall of the sea cucumber decreased first and then increased, and reached the lowest value in the 0.51% ARA feed group. 3.71%), and significantly lower than the control group, 0.17% and 0.98% ARA feed group ($P < 0.05$), no significant difference with 0.36% and 0.59% ARA feed group ($P > 0.05$) (Table 4).

Effect of ARA Content in Feed on Fatty Acid Composition of Body Wall of Sea Cucumber

Statistics also shows the effect of ARA on the fatty acid composition of the body wall of sea cucumber. The results showed that with the increase of

ARA content in the feed, the contents of ARA and n-6 PUFA in the wall of the sea cucumber showed a significant increase, while the contents of EPA, DHA and n-3 PUFA decreased significantly ($P < 0.05$). When the feed ARA content increased, the PUFA content in the wall of the sea cucumber increased significantly from 35.08% to 38.00%, while the $\Sigma n-3/\Sigma n-6$ decreased significantly from 0.48 to 0.31 ($P < 0.05$). In addition, with the increase of ARA addition, MUFA and C18:2n-6 in the wall of sea cucumber showed a decreasing trend ($P < 0.05$).

Table 4: Effects of different dietary arachidonic acid levels on body wall chemical composition of *Apostichopus japonicus*.

Parameter	Dietary arachidonic acid level (%)					
	0.02	0.17	0.36	0.51	0.59	0.98
Moisture	90.69±0.17	90.94±0.06	91.02±0.10	91.07±0.23	90.88±0.21	90.58±0.40
Crude protein	46.67±0.52	46.83±0.62	46.89±0.68	46.65±0.63	46.15±0.92	46.59±1.24
Ether extract	4.66±0.10 ^{ab}	4.61±0.15 ^{ab}	3.99±0.15 ^{bc}	3.17±0.14 ^c	4.29±0.26 ^{abc}	4.88±0.11 ^a
ASH	31.37±0.67	31.77±0.30	32.46±0.22	32.34±0.41	32.04±0.48	31.06±0.73

Note: Values with different superscripts in the same row are significantly different ($P < 0.05$).

When the content of ARA in the feed increased from 0.02% to 0.98%, the contents of MUFA and C18:2n-6 in the wall of the sea cucumber decreased from 17.09% to 15.26% and from 8.23% to 4.07% ($P < 0.05$). There was no significant difference in the content of SFA (C14:0, C16:0, C18:0 and C20:0) in the wall of the sea cucumber between different experimental groups ($P > 0.05$).

Effect of ARA Content in Feed on Antioxidant Capacity of Sea Cucumber in Sea Cucumber

Figures in Table 5 shows that with the increase of ARA content in the feed, the activity of superoxide dismutase (SOD) in the intestinal tract of the sea cucumber increased first and then decreased, reaching the maximum in the 0.51% ARA feed group, and significantly higher than the control group, 0.17% and 0.98% ARA feed group ($P < 0.05$), no significant difference with 0.36% and 0.59% ARA feed group

($P > 0.05$). Total antioxidant capacity in the intestinal tract of sea cucumber (total Antioxidant capacity enzyme, T-AOC) activity and catalase (CAT) activity showed a similar trend with SOD (Janakiram et al., 2015).

Meanwhile, when the ARA content in the feed increased from 0.02% to 0.51%, the MDA content in the intestinal tract of the sea cucumber was not significant changes ($P > 0.05$), and when the ARA content was further increased to 0.98%, the content of malondialdehyde (MDA) in the intestine increased significantly, and reached a maximum in the 0.98% ARA feed group, which was significantly higher (Qi et al., 2013). There were no significant differences between the first 4 treatment groups (0.02%, 0.17%, 0.36%, and 0.51% ARA feed groups) ($P < 0.05$), and 0.59% ARA feed group ($P > 0.05$).

Table 5: Effects of different dietary arachidonic acid levels on antioxidant capacity in intestinal tract of *Apostichopus japonicus*.

Parameter	Dietary arachidonic acid level (%)					
	0.02	0.17	0.36	0.51	0.59	0.98
SOD	46.64±0.56 ^c	50.46±1.55 ^{bc}	55.18±0.70 ^a	55.23±1.17 ^a	51.55±0.93 ^{ab}	49.44±0.47 ^{bc}
MDA	3.28±0.07 ^b	3.49±0.09 ^b	3.37±0.09 ^b	3.22±0.07 ^b	5.43±0.16 ^a	5.56±0.23 ^a
T-AOC	1.14±0.04 ^d	1.34±0.08 ^{cd}	1.77±0.04 ^{ab}	1.86±0.05 ^a	1.58±0.09 ^{abc}	1.52±0.06 ^{bc}
CAT	89.34±1.91 ^{cd}	98.35±3.35 ^{bcd}	109.52±3.56 ^{ab}	115.60±4.75 ^a	103.16±3.76 ^{abc}	85.94±3.71 ^d

Note: Values with different superscripts in the same row are significantly different ($P < 0.05$).

Effect of ARA Content in Feed on the Activities of FAS, ACC and CPT-1 in the Intestinal Tract of Sea Cucumber

It can be seen from Table 6 that when the ARA

content in the feed is increased from 0.02% to 0.59%, the fatty acid synthase (FAS) activity in the intestinal tract of the sea cucumber has no significant difference among the treatment groups ($P > 0.05$).

Table 6: Effects of different dietary arachidonic acid levels on activities of FAS, ACC and CPT-1 in intestinal tract of *Apostichopus japonicus*.

Parameter	Dietary arachidonic acid level (%)					
	0.02	0.17	0.36	0.51	0.59	0.98
Fatty acid synthase, FAS	4.32±0.05 ^a	4.38±0.05 ^a	4.64±0.11 ^a	4.31±0.09 ^a	4.53±0.08 ^a	2.94±0.07 ^b
Acetyl-CoA carboxylase, ACC	25.34±0.07 ^a	24.58±0.31 ^{ab}	25.37±0.61 ^a	25.51±0.69 ^a	25.45±0.51 ^a	23.15±0.25 ^b
Palmitoyltransferase-1, CPT-1	22.23±0.11 ^b	22.72±0.33 ^b	25.32±0.37 ^a	25.00±0.19 ^a	24.61±0.23 ^a	23.01±0.20 ^b

Note: Values with different superscripts in the same row are significantly different ($P < 0.05$).

When the ARA content in the feed was further increased to 0.98%, the intestinal FAS activity decreased significantly, which was significantly lower than that of other treatment groups ($P < 0.05$). The activity of acetyl-CoA carboxylase (ACC) in the intestinal tract of sea cucumber's change of ARA content showed a similar trend with FAS.

In addition, the activity of carnitine palmitoyltransferase-1 (CPT-1) in the intestinal tract of the sea cucumber increased first and then decreased with the increase of ARA content in the feed (Olivera Castillo et al., 2011). The activities of CPT-1 in the 0.36%, 0.51% and 0.59% ARA feed groups were significantly higher than those in the control group,

0.17% and 0.98% ARA diet group ($P < 0.05$), and there was no significant difference between the three groups. There was also no significant difference in CPT-1 activity between the 0.17% and 0.98% ARA feed groups ($P > 0.05$).

Discussion

Effect of Ara Content in Feed on the Growth Performance of Sea Cucumber

In recent years, with the deepening of ARA research, numerous studies have shown that ARA plays an important role in the growth and survival of marine animals. When the ARA content in the feed is

0.5%-1.0% (dry matter), the turbot (*Scophthalmus maximus* L.) juveniles have better growth efficiency and survival rate; in the study of (*Lateolabrax japonicus*), based on Growth performance analysis, the optimal requirement for large-scale for ARA is 0.37%.

Similarly, in the study of gingival (*Paralichthys olivaceus*) and large yellow croaker (*Larmichthys crocea*), it is shown that the appropriate amount of ARA has a certain promotion on its growth and survival. effect. At the same time, studies have reported that when the ARA content in the feed is too high, it will inhibit the growth of fish. Xu added excessive ARA (0.56% - 2.12%, dry matter) to the juvenile fish feed, which severely inhibited its growth and caused related inflammation; in the study of juvenile fish of *Xenocypris davidi* (Pan et al., 2014).

It showed that when the 4% ARA level feed was used to feed the yellowtail fish, it had a negative impact on its growth. The results of this study showed that the weight gain rate and feed efficiency of sea cucumber showed a certain change trend with the change of feed ARA content, and the weight gain rate was the evaluation index. The optimum addition amount of ARA in sea cucumber feed was 0.51% (feed dry) Substance), which indicates that an appropriate amount of ARA has a certain promoting effect on the growth performance of sea cucumber.

At the same time, the study also showed that when the ARA content in the feed was higher than 0.51% (feed dry matter), the weight gain rate and feed efficiency of sea cucumber were inhibited, which is consistent with the research results in marine fish (Ochiewo et al., 2010).

The main reasons for this growth inhibition are as follows: Firstly, because of the competitive effects of ARA and EPA metabolites, when the ARA content in the feed is high, the biotransformation of EPA in the body is inhibited, which affects the growth of the animal; Second, higher levels of ARA can cause inflammation in the body, and the body's immune system is affected, which inhibits growth. In addition, studies have confirmed that ARA has no significant effect on the growth performance of some marine

animals. The reason for this difference may be caused by different culture types, breeding environments and farming methods (Seo et al., 2011).

Related studies have demonstrated that ARA is a precursor to a variety of biologically active compounds, collectively known as eicosanoids, which are released from phospholipids in response to hormone stimulation and inflammatory responses (Ahmed, 2015).

These bioactive substances play an important role in animals and can regulate a range of important physiological metabolisms, including lipid protein metabolism, cardiovascular system, leukocyte function, platelet activation, reproductive function and nervous system control.

This shows that ARA can play a certain role in regulating the growth and survival of marine animals, mainly because of the presence of its derivatives. It also explains why ARA enhances the anti-stress ability of organisms. It has been reported that adding appropriate amount of ARA to feed can significantly improve the survival rate of juveniles of seawater. However, this study showed that the survival rate of sea cucumber did not change with the change of ARA content, and remained between 87.50% and 94.17%. There was no significant difference between different treatment groups ($P>0.05$), which is related to fish (La Ming-Ping et al., 2012).

The results in the study are similar. The reason for this difference may be because the autoimmune system of the larval stage is weak, so higher levels of ARA are needed to enhance its anti-stress ability; and when development is completed, because of its strong anti-stress ability, Therefore, ARA has a small effect. Combined with the results of this experiment, it can be speculated that sea cucumber itself may have certain anti-stress properties, so different levels of ARA feed have no significant effect on its survival rate, and its related regulation mechanism needs further study.

Effect of ARA Content in Feed on Biochemical Composition and Fatty Acid Composition of Body Wall of Sea Cucumber

Numerous studies have shown that the ARA

content in the feed affects the conventional biochemical composition of the animal, which may be due to the different effects of different levels of ARA on the related metabolic enzyme activities in the body. The results of this study showed that with the increase of feed ARA content, the crude fat content of sea cucumber body decreased first and then increased, and the lowest content in 0.51% ARA feed group (fat: 8.71% dry weight), while ARA. When the content is 0.02% or 0.98%, the crude fat content is significantly higher than that of the 0.51% ARA feed group, which indicates that the proper amount of ARA may promote the activation of fat cells in the sea cucumber, promote fat metabolism, and thus reduce the body fat level of sea cucumber (Geng et al., 2015).

The high content of ARA leads to the imbalance of fatty acids in the feed or the high organic acid in the feed, which makes the body not ingest and absorb fatty acids well, thus causing deposition in the body wall, resulting in higher body wall fat content. Studies in juvenile turbot have shown that high levels of ARA in feeds result in a significant decrease in crude fat content in fish and muscle, probably because ARA inhibits very low density lipoprotein B-100 secretion.

Lipoprotein cholesterol is assembled to regulate liver fat transport, and high levels of ARA can inhibit FAS gene expression, which in turn affects fat deposition in fish and muscle. The reason for this difference may be due to the large difference in the actual content of ARA in the feed, and the different needs and utilization of ARA by different experimental subjects.

Studies in marine fish have shown that feed fatty acid composition can significantly affect fish and tissue fatty acid composition. In this study, with the increase of ARA content in the feed, the ARA content in the body wall also increased significantly ($P < 0.05$), and there was a positive correlation between the two. It is also worth noting that the wall of the sea cucumber in different treatment groups.

The ARA content is the highest content of fatty acids in this group, which also implies that ARA has a strong enrichment ability in the wall of the sea

cucumber. The EPA content in the body wall is inversely related to the ARA content of the feed (Liu et al., 2016). This may be due to the competitive interaction between ARA and EPA metabolites, which inhibits the biotransformation of EPA in the body, which is consistent in other studies. The results showed that although the content of $C_{16:0}$, $C_{18:0}$ and saturated fatty acid (SFA) in the feed showed a downward trend, the content of the three in the wall of the sea cucumber was basically constant.

The content of $C_{18:2n-6}$ is consistent with the trend of ARA content in the body wall, which may be because the sea cucumber itself has a potential biosynthesis pathway of high unsaturated fatty acids. Studies in sea cucumber also showed that with the increase of the ratio of cornstarch and soybean meal in the feed of *S. cerevisiae* (12% dry matter of ARA), the ARA content in the body wall did not decrease significantly, so it is speculated that the sea cucumber may exist in the body. A potential pathway for the synthesis of n-6 HUFA, which is capable of extending and desaturation of $C_{18:2n-6}$ to produce ARA, and the team also speculated that the sea cucumber may also have the potential for ALA to synthesize EPA.

The synthesis of polyunsaturated fatty acids involves a variety of enzymes, of which $\Delta 6$ fatty acid desaturase ($\Delta 6FAD$) and fatty acid elongase (ELOVL5) are the two key enzymes in fatty acid synthesis. The $\Delta 6FAD$ gene of sea cucumber was obtained by homologous cloning and RACE technology, and the expression of this gene in different tissues of sea cucumber was determined. The results showed that $\Delta 6FAD$ was expressed in the intestine, testis, body wall and respiratory tree of sea cucumber. The expression in the intestine was the highest, and it was found that the sea cucumber has the ability to synthesize GLA and STA with LA and ALA, but the ability to further synthesize EPA, ARA and DHA may be limited.

At the same time, the polyunsaturated fatty acid elongase 5 gene in sea cucumber was cloned and found to be expressed in the body wall, gonad, respiratory tree and intestine of sea cucumber. Cell experiments in yeast medium demonstrated that

ELOVL5 has the ability to extend 18:3n-6 and 20:5n-3 to 20:3n-6 and 22:5n-3, respectively. The ability of unsaturated fatty acids, meanwhile, also shows that the elongation ability of sea cucumber is weakened with the growth of carbon chain, and the synthesis ability of high unsaturated fatty acids may be affected by the composition of unsaturated fatty acids in feed. further research.

In this study, ALA has the same trend of EPA and DHA in the wall of sea cucumber, which further suggests that sea cucumber has the ability to synthesize highly unsaturated fatty acids, but its synthetic ability remains to be further demonstrated.

Effect of ARA Content in Feed on Antioxidant Capacity of Sea Cucumber in Sea Cucumber

The body will produce a large amount of active oxygen (ROS) during metabolism, such as superoxide anion (O₂⁻) and hydrogen peroxide (H₂O₂). ROS will have a series of negative effects on the animal body. For example, the body's oxidative stress response, the inactivation of metabolic related enzymes, and even the destruction of cell integrity. Superoxide dismutase (SOD), catalase (CAT) and carnitine palmitoyltransferase-1 (T-AOC) are the main antioxidant enzymes in the body, which can eliminate internal ROS. To avoid the oxidation of fatty acids, reduce the toxic effects of ROS, and thus protect organisms from oxidative damage, play a key role in the body's antioxidant (Chen et al., 2015).

Therefore, SOD, CAT and T-AOC activities can reflect the body's ability to resist oxidative stress and indirectly reflect the level of aquatic animal immunity. In this experiment, the activities of SOD, T-AOC and CAT in the intestinal tract of 0.36% and 0.51% ARA diet group were significantly higher than those in the control group (P<0.05). When the ARA content of the feed was further increased to 0.98%.

The vigor of the people showed a downward trend, which indicated that the addition of suitable ARA to the feed could increase the activity of antioxidant enzymes and increase the antioxidant capacity of sea cucumber. These results are similar to previous studies.

When the ARA content in the feed was 0.56%, the SOD activity in the serum of juvenile fish was significantly higher than that in the control group. At the same time, the results in the large-scale carp showed that when the ARA content in the feed was 0.37%-0.60%, the experimental fish activity of SOD in serum and liver was significantly improved, and the fish body had strong antioxidant capacity. In addition, similar conclusions were obtained in related studies of oyster (*Ostrea gigas thunberg*) and turbot.

Malondialdehyde (MDA) is the final decomposition product obtained by free radical-induced lipid peroxidation. Its content may indirectly reflect the degree of damage and antioxidant capacity of aquatic animals. The higher the content, the more serious the damage to the body. At the same time, it is pointed out that the occurrence of lipid peroxidation is closely related to the level of unsaturated fat.

In this study, when the ARA content in the feed was higher than 0.59%, the MDA content in the gut of the sea cucumber was significantly higher than that in the other treatment groups (P<0.05), which indicated that when the ARA content was too high, the lipid peroxidation of sea cucumber was higher. The reaction is strong and the body's antioxidant capacity is impaired. Therefore, it is further explained that when the content of HUFA in the feed is too high, lipid peroxidation is easily caused, and the antioxidant system of the body is destroyed.

Effect of ARA Content in Feed on the Activities of FAS, ACC and CPT-1 in the Intestinal Tract of Sea Cucumber

Acetyl-CoA carboxylase (ACC) is a key enzyme in the conversion of acetyl-CoA to malonyl-CoA during the initiation of fatty acid synthesis. Fatty acidsynthase (FAS) catalyzes the repeated extension of malonyl-CoA to form 16 and 18 carbon-saturated fatty acids, which in turn produces palmitic acid under the action of palmitoyl-ACP thioesterase.

It can be seen that FAS and ACC are key enzymes in the process of fatty acid synthesis, and the magnitude of their activity directly affects the progress

of fatty acid synthesis. In recent years, related research reports that polyunsaturated fatty acids in feed can regulate the activity and expression of related enzymes in fatty acid synthesis and oxidation to some extent (Wu et al., 2015).

With the increase of ARA level in feed, the FAS activity and expression level of sputum liver decreased significantly. Studies showed that the activity of FAS in liver, muscle and kidney of large yellow croaker was proportional to the ratio of linolenic acid/linoleic acid (ALA/LA).

The increase was also reduced; at the same time, the study also indicated that the higher polyunsaturated fatty acids in the diet inhibited FAS and ACC activity in the liver of mice. This may be because polyunsaturated fatty acids can inhibit the activity of 6-phosphate dehydrogenase and malic enzyme at a certain level, which restricts the production of reduced phosphotriester nucleotide (NADPH), thereby inhibiting FAS activity. Therefore, it can be explained that polyunsaturated fatty acids in feed can inhibit the synthesis of fatty acids in the body, and the inhibitory effect of n-3PUFA is higher than that of n-6 PUFA.

The results of this experiment showed that the activities of FAS and ACC in the intestinal tract of sea cucumber decreased significantly with the increase and decrease of ARA content in the feed, which further indicated that the fatty acid composition (especially HUFA) in the feed can play a certain role in the fatty acid anabolism of the body. Regulatory effect, when the content of HUFA is high, it inhibits the activity of enzymes related to fatty acid synthesis.

Carnitine palmitoyltransferase-1 (CPT-I) is a key point regulating mitochondrial β oxidation, and its activity can affect the oxidative decomposition rate of fatty acids. At the same time, the results of this experiment showed that when the content of ARA in the feed increased, the activity of CPT-1 in the intestinal tract of the sea cucumber increased first and then decreased, and the content of ARA which was too high or too low inhibited the activity of CPT-1. Similar results were also reported in the experiment of sputum.

The expression of CPT-1 gene in the liver increased first and then decreased with the increase of

ARA content in feed. The high content of ARA inhibited the activity of CPT-1. At the same time, it has also been shown in the study of large yellow croaker that conjugated linoleic acid can have a certain effect on CPT-1 activity in the liver. This may suggest that the activation of the PPAR- α gene may differ in the composition and content of fatty acids. Too high or too low PUFA content may make the activation weak or inhibit, and studies have shown that different fatty acid composition in the feed has a different effect on peroxisome proliferator-activated receptor alpha (PPAR-alpha) activity, and CPT-I is a target gene of PPAR-alpha, so its activity is affected by the fatty acid composition of the feed (Xie et al., 2016).

Conclusion

In summary, this study demonstrates that the proper amount of ARA in the feed has a certain promoting effect on the growth of sea cucumber. Based on the analysis of growth and antioxidant capacity index, when the ARA content in the feed is 0.36%-0.51%, the sea cucumber has better growth performance and antioxidant capacity. The regression analysis of weight gain rate and ARA content indicates that sea cucumber.

The optimum content of ARA in the feed is 0.51%. At the same time, the study also showed that the content of ARA in feed also had certain effects on the fatty acid composition and lipid metabolism in the intestinal tract of sea cucumber, and the related metabolic mechanism needs further study.

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