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RESEARCH REPORT

Effects of dietary botanical and synthetic astaxanthin on E/Z and R/S isomer composition, growth performance, and antioxidant capacity of white shrimp, *Litopenaeus vannamei*, in the nursery phase

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Abstract

Astaxanthin (AST) is a beneficial dietary supplement in shrimp farming. However, the different effects of synthetic AST (S-Ast) and natural AST (N-Ast) on the postlarvae is unclear. These effects were compared by supplementation with a gradient of AST (0, 50, 70, 90, 140 ppm) for 35 days. Growth parameters including weight and length gain, carotenoid content, AST content and isomer composition, antioxidant capacity (superoxide dismutase, catalase, glutathione peroxidase), survival rate and mRNA expression levels for antioxidant enzymes in *Litopenaeus vannamei* postlarvae were measured. The results indicated that postlarvae given N-Ast had significantly higher growth performance and AST content. Although there were different ratios of AST optical isomers in the diets, there was a similar optical isomer content in juvenile shrimp. The cis/trans AST ratio and antioxidant enzyme (SOD and GPx) activities in N-Ast90 were the highest among all groups. Cumulative mortality in N-Ast90, N-Ast140 and S-Ast70 were significantly lower than in the control and N-AST50. The mRNA expression levels for the antioxidant enzymes (cMnSOD and GPx) also increased for N-Ast90-fed shrimp under stress conditions. These results suggest that an epimerase may exist *in vivo* and that an appropriate level of botanical AST in diets was approximately 90 ppm for *L. vannamei* postlarvae.

Key Words: astaxanthin; *L. vannamei* postlarvae; *Vibrio*

Introduction

The Pacific white shrimp, *Litopenaeus vannamei*, constitutes a considerable part of the cultured shrimp around the world (Liu *et al*., 2014). Environmental stresses, i.e. intensive culture and environmental deterioration, induce the generation of excessive reactive oxygen species (ROS) in crustaceans, and lead to disease (Flegel, 1997; Cesaratto *et al*., 2004; Pelicano *et al*., 2004; Ji *et al*., 2011). AST, the dominant carotenoid pigment found in shrimp, cannot be synthesized *de novo* by the animals themselves (Yamada *et al*., 1990). It must be derived from their feed. AST can reduce oxidative damage generated under stress conditions through its powerful antioxidant properties (Supamattaya *et al*., 2005; Wang *et al*., 2006; Díaz *et al*., 2014). This AST property has generated considerable interest in the pigment and its potential functions. This interest has prompted us to analyze the impacts of AST on the physiological and biochemical characteristics of shrimp and on pathogen resistance in shrimp.

AST is a red pigment widely applied in crustacean and salmonid aquaculture as a feed additive (Chien *et al*., 2003; Choubert *et al*., 2006; Félixvalenzuela, 2006). Among other things, it can regulate immune responses and improve disease resistance (Zhang *et al*., 2013). Synthetic AST is most commonly used for feed supplementation, and there are only a few reports related to the use of botanical AST in shrimp aquaculture. It has been shown that dietary supplementation with botanical AST (derived from *Haematococcus pluvialis*) can significantly influence immune indicators in the

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hemolymph of *L. vannamei*, increase the expressions of SOD and GPx in the hepatopancreas, and increase resistance to white spot syndrome virus (WSSV) infection (Wang *et al*., 2015). It has also been reported that botanical AST has a positive impact on the color, weight and antioxidant ability in shrimp; however, the optimum concentration differs widely among species and developmental stages (Parisenti *et al*., 2015). Some studies have suggested that dietary supplementation with synthetic AST enhances growth in *Penaeus monodon* and shortens the intermolt stage for postlarvae of the prawn *Penaeus japonicas* (Torrissen, 1995; Petit, 1997). Putman (1992) believed that supplementation with synthetic AST to increase pigmentation would increase consumer acceptance, as well as accelerate sexual maturity, improve fertilization and egg survival, and embryo development. Little is known about any difference between botanical and synthetic AST when used as dietary supplementation for *L. vannamei* postlarvae.

A previous study found that supplementation with *H. pluvialis* cell powder had no significant effects on survival and ovarian development, but did enhance shrimp coloration, antioxidant levels in tissues, and improved the proximate composition of female *Eriocheir sinensis* (Long *et al*., 2017). Botanical and synthetic AST showed no significant differences in comparisons except for flesh AST content (Chien and Shiau, 2005). Here, using *H. pluvialis* powder and synthetic AST as the feed additives, we investigated the growth performance, AST and carotenoid contents and antioxidant parameters in growing *L. vannamei* juveniles. The aim of this study was to provide practical suggestions for diet formulation and quality improvement of reared postlarvae.

Materials and Methods

Experimental diets

Experimental groups were fed a basal diet supplemented with various amounts of *H*aematococcus *pluvialis* cell powder (Yunnan Alphy Biotech Co., Ltd, China), designated as N-Ast50 (with 50 ppm carotenoids), N-Ast90 (with 90 ppm carotenoids), N-Ast140 (with 140 ppm carotenoids). In addition, there was one negative control group (no AST added) and one positive control group with synthetic AST (Carophyll® Pink 10%, DSM, S-Ast70, with 70 ppm carotenoids). The proximate compositions, total carotenoids and AST contents of all diets are presented in Table 1. All ingredients were completely mixed together and water was added to shape pellets. Subsequently, the pellets were air-dried at room temperature and then kept at -18 °C until used. The AST content and composition of diets were analyzed using high-performance liquid chromatography (HPLC, Agilent 1200; Agilent Technologies, Santa Clara, CA, USA) according to the protocol of Yuan (Ronneberg *et al*., 1980; Okada *et al*., 1994). The chromatograms of diets obtained using silica and chiral columns are presented in Fig. 1E and Fig. 1F. The data indicated that the cis/trans AST ratios were 0.13 and 0.2 in S-Ast70 and N-Ast diets, respectively. The AST optical isomer ratio in N-Ast was 3S, 3'S : 3R,3'S : 3R,3'R = 30:3:1, and that in the S-Ast70 diet was 6:2:1.

Experimental design

Litopenaeus vannamei postlarvae with initial weights of 0.01 ± 0.001 g (mean ± SD) were pre-cultured at the Xindadi Aquaculture Co., Ltd, Weifang, China. Subsequently, postlarvae were transferred into indoor plastic drums (length × width = 1 m × 1.2 m) with 800 L water, and divided into five groups, each in triplicate (each replicate had 2000 individuals). The postlarvae were fed five times daily at 5:00, 9:00, 13:00, 17:00, and 21:00 with an initial ration of approximately 1.6 g/10,000 individuals, which was increased 20% every day, for 35 days. Diets were slightly adjusted, based on the feeding response, water temperature and residual feed. A water depth of 70 cm was maintained and 30% of the water was refreshed in the second week. The pH, salinity, water temperature, ammonia-nitrogen and dissolved oxygen were maintained at 7.8 - 8.2, 24 - 27‰, 28 - 31 °C, 0.06 - 0.08 mg/L, and 8.0 - 9.0 mg/L, respectively. After 35 days, juvenile shrimp were fasted for 24 h, and weighed. Then half of the juvenile shrimp were stored in liquid nitrogen for future analysis. The remaining animals were challenged with *Vibrio parahaemolyticus* as described below.

Table 1 Proximate composition, total carotenoid and astaxanthin contents of experimental diets (% dry weight)

Items	Control	S-Ast70	N-Ast50	N-Ast90	N-Ast140
Proximate composition					
Ash (%)	16.00	16.00	16.00	16.00	16.00
Moisture (%)	8.00	8.00	8.00	8.00	8.00
Crude protein (%)	37.79	37.59	37.62	37.62	37.62
Crude lipid (%)	8.50	8.32	8.32	8.32	8.32
Carotenoid (mg/kg)	18.35	74.38	47.59	95.19	142.78
Astaxanthin (mg/kg)	0.40	26.27	22.94	45.87	68.81

Note: N-Ast50, 90 and 140 indicate that the final carotenoid contents are 50 ppm, 90 ppm and 140 ppm, respectively. S-Ast70 means the final carotenoid content is 70 ppm.

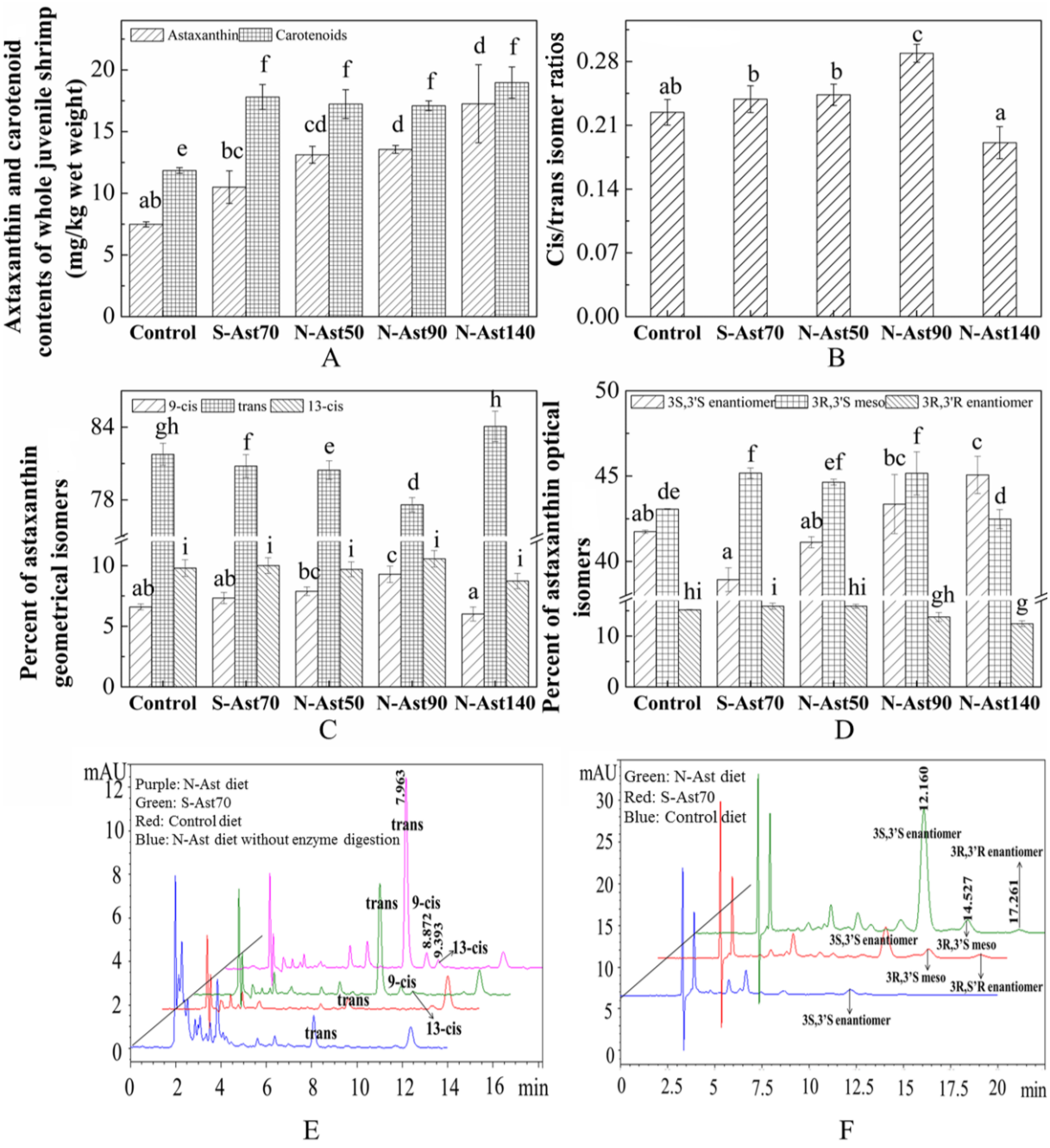

Fig. 1 The carotenoid and astaxanthin contents (A), cis/trans isomer ratios (B), astaxanthin geometrical isomer percentages (C), astaxanthin optical isomer percentages (D), HPLC chromatograms of three diets using a silica column (E) and HPLC chromatograms of diets using a chiral column (F), in *Litopenaeus vannamei* postlarvae after a 35-day feeding trial. The significant differences are indicated by different superscripts (a, b, c, d, e and f) ($P < 0.05$, n = 3).

Determination of carotenoids and astaxanthin content and composition

The surface water on thawed juvenile shrimp was removed using paper towels and then the animals were weighed and placed into 10 mL centrifuge tubes. Subsequently, the juvenile shrimp were cut into fragments with operating scissors and homogenized with an S10 homogenizer (Ningbo Xinzhi biotechnology Co., Ltd). Total carotenoids were extracted with acetone and measured with a UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan) at 478 nm, according to the method of Johnston (Johnston *et al.*, 2000). After enzymatic digestion with cholesterol esterase (EC 3.1.1.13, Wako, Japan), AST was measured by HPLC at 478 nm following the protocol of Yuan (Ronneberg *et al.*,

1980; Okada *et al*., 1994). AST geometrical isomers were separated using a silica column (4.6 mm × 150 mm, particle size = 5 μm, Phenomenex, Inc. USA), with a mobile phase of hexane and acetone (83:17, v/v). AST optical isomers were analyzed with a chiral column (460 mm × 250 mm, particle size = 5 μm, Daicel Chiral Technologies Co., Ltd, Shanghai, China), with a mobile phase of acetonitrile and methyl t-butyl ether (65:35, v/v). The injection volume for both was 60 μL at a flow rate of 1.0 mL/min.

Assessment of immune indices

Each thawed hepatopancreas was placed into 2 mL pre-cooling physiological saline solution and homogenized in an ice bath with an S10 homogenizer, and then centrifuged at 1,700 g for 10 min at 4 °C to obtain supernatant for further analysis. The activities of SOD, CAT and GPx in the hepatopancreas were measured with a spectrophotometer at room temperature at 550 nm, 405 nm and 412 nm respectively, according to the manufacturer's instructions. The enzyme detection kits were provided by Nanjing Jiancheng Bioengineering Institute (Jiangsu, China).

Challenge with V. parahaemolyticus and expression of cMnSOD and GPx mRNAs

On the 35th day, the hepatopancreases of three juvenile shrimp from each drum were sampled and placed in RNAstore reagent (TaKaRa 9750; TaKaRa Bio, Tokyo, Japan) and then stored in liquid nitrogen for later examination of the mRNA expression levels of cMnSOD and GPx. Then, 60 randomly selected, juvenile shrimp from each group were placed into 25 L plastic drums with 15 L water. *V. parahaemolyticus* was added to the water every 24 h to maintain the bacterial density at 3×10^8 cfu/mL. The dead shrimp were counted every 24 h. After this, the hepatopancreases of three live juvenile shrimp from each drum were sampled for further analysis of mRNA expression levels. Hepatopancreases from animals not challenged with *V. parahaemolyticus* were taken as control.

Total RNAs were extracted with RNAiso Plus (TaKaRa 9750; TaKaRa Bio, Tokyo, Japan) according to the manufacturer's instructions. The RNA concentration and purity were verified with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., MA, USA) (A260/A280) and its integrity was quantified by electrophoresis on a 1% agarose gel. The cDNA synthesis was conducted using a PrimeScript™ RT reagent kit with gDNA eraser (Perfect real time) (TaKaRa 9750; TaKaRa Bio, Tokyo, Japan) based on the manufacture's instructions. Primers of each gene were designed based on the published *L. vannamei* cDNA sequence using Primer 3 software as presented in Table 2 (Wang *et al*., 2017). All primers were produced by Ruiboxingke Biological Technology Co., Ltd. (Qingdao, China). Reaction conditions were also optimized. Real-time quantitative RT-PCR was executed using SYBR®*Premix Ex Taq*™ Ⅱ (TliRNaseH Plus) (TaKaRa RR820A; TaKaRa Bio, Tokyo, Japan) according to the manufacture's instructions. After amplification, a melting curve analysis verified that there was only one amplified product. Expression levels of genes were calculated with the comparative C_T method ($2^{-\Delta\Delta CT}$), and the values mean n-fold difference was compared with the control.

Data analysis

Statistical analyses were performed with IBM SPSS Statistics 19.0 (SPSS, Chicago, IL, USA). Data were expressed as mean ± standard derivation (SD). One-way ANOVA was used to examine whether significant differences existed among the groups. Homogeneity of data was determined with the Duncan's multiple range test. A probability (P) value < 0.05 was regarded as significant.

Results

Total carotenoid content and astaxanthin composition

The carotenoid content of all experimental groups (i.e. control, S-Ast70, N-Ast50, N-Ast90 and N-Ast140) showed no significant differences; however, all values were higher (P < 0.05) than that of the control (Fig. 1A). The average AST level of postlarvae fed N-Ast140 was higher than all other diets. The AST content of postlarvae fed N-Ast90 and N-Ast140 were significantly higher (P < 0.05) than that of postlarvae in other groups, particularly for N-Ast140, in which the AST/total carotenoid ratio was the highest.

Table 2 Primers for real-time quantitative RT-PCR

Gene name	GenBank number	Reference	Primer sequence (5'-3')	Annealing temperature (°C)	Product (bp)
Cytosolic manganese superoxide dismutase (cMnSOD)	DQ005531	Gomez-Anduro *et al*. (2006)	(F)ATCACTCACGGACTGGTTCC (R)GAGAGAAACGCCCTTGTGAC	59	219
Glutathione peroxidase (GPx)	AY973252	Fu *et al*. (2007)	(F)CGTGCAAAAAGGACCTTGGG (R)ATACGCGATGCCCCTAACAC	54.5	231
β-actin	AF300705y	Sun *et al*. (2007)	(F)GTGCCCATCTACGAGGGATA (R)TAGGACTTCTCCAGCGAGGA	56.5	233

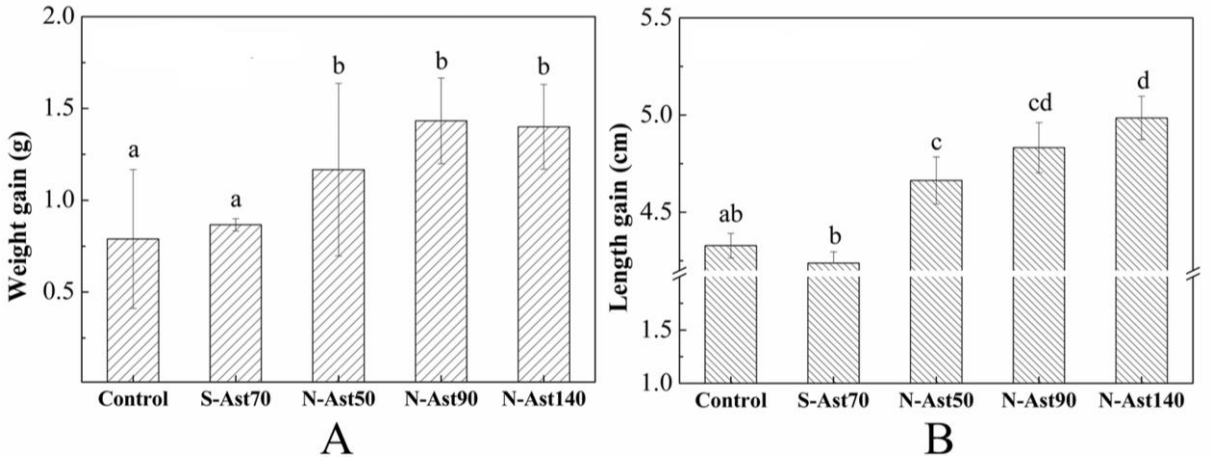

Fig. 2 The weight and length gains of *L. vannamei* postlarvae after a 35-day feeding trial. Data with different letters (a, b, c, and d) are significantly different ($P < 0.05$, n = 30).

Quantification of astaxanthin geometrical isomers

Among the main AST geometrical isomers (i.e. all-trans, 9-cis and 13-cis), the all-trans isomer was the dominant form in postlarvae (Fig. 1C). The proportion of the 9-cis isomer presented in postlarvae fed N-Ast90 was significantly higher ($P < 0.05$) than that of others. The proportion of the trans isomer found in postlarvae fed N-Ast140 was significantly higher than that for the other diets ($P < 0.05$). The 13-cis isomer of postlarvae showed no significant differences ($P < 0.05$) between control and experimental groups. The cis/trans ratio was highest in N-Ast90 (Fig. 1B).

Quantification of astaxanthin optical isomers

The optical isomers of AST in white shrimp postlarvae include the 3S,3'S isomer, 3R,3'S meso form and 3R,3'R isomer, among which the 3S,3'S isomer and 3R,3'S meso form are the major forms. The 3S,3'S isomer of postlarvae fed N-Ast140 was significantly higher ($P < 0.05$) than in other groups. The 3R,3'S meso content of postlarvae fed N-Ast90 and S-Ast70 were significantly higher ($P < 0.05$) than they were in others. The 3R,3'R isomer contents of postlarvae of all experimental groups were similar (Fig. 1D).

Growth performance with different supplemented diets

The weight and length gain of shrimp postlarvae fed botanical AST were both higher than those of the control and S-Ast70 after 35 days (Fig. 2). No significant differences for these two parameters was found between S-Ast70 and the control. The weight gain and length gain had no significant differences among N-Ast groups; however, the average weight and length gain both increased with increasing AST concentration but with only weight gain for animals fed N-Ast140.

Antioxidant enzyme parameters

Although the results were not uniform, all the highest values for antioxidant enzyme activities were found in the N-Ast groups. SOD, CAT and GPx activities were increased over the control ($P < 0.05$) the most in N-Ast90, N-Ast140 and N-Ast90, respectively (Table 3).

Table 3 Hepatopancreas antioxidant enzyme activities of *Litopenaeus vannamei* postlarvae after a 35-day feeding trial. Values are mean ± standard deviation (SD). Mean values in the same row for each type of experiment with different letters (a and b) are significantly different ($P < 0.05$, n = 3).

Dietary treatments Hepatopancreas parameters	Control	S-Ast70	N-Ast50	N-Ast90	N-Ast140
SOD (unit mg^{-1} $protein^{-1}$)	10.53 ± 0.92^{a}	12.89 ± 0.73^{ab}	14.07 ± 2.33^{ab}	17.32 ± 1.71^{b}	10.64 ± 1.22^{a}
CAT (unit mg^{-1} $protein^{-1}$)	4.48 ± 0.47^{a}	4.72 ± 0.89^{a}	4.60 ± 0.61^{a}	5.10 ± 1.31^{a}	7.93 ± 1.18^{b}
GPx (unit mg^{-1} $protein^{-1}$)	123.63 ± 5.95^{a}	136.44 ± 12.99^{a}	109.08 ± 4.79^{a}	197.76 ± 40.29^{b}	108.24 ± 14.15^{a}

Note: N-Ast50, 90 and 140 indicate that the final carotenoid contents are 50 ppm, 90 ppm and 140 ppm, respectively. S-Ast70 indicates the final carotenoid content is 70 ppm.

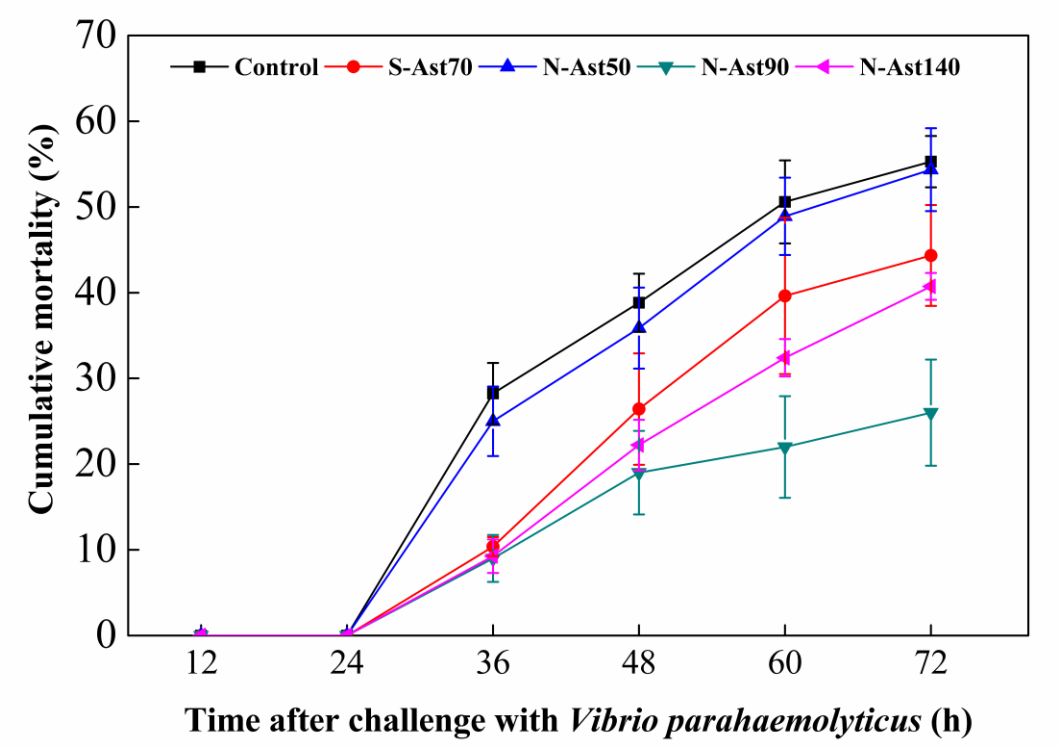

Fig. 3 The cumulative mortality (%) of *L. vannamei* postlarvae after being challenged with *V. parahaemolyticus*.

Postlarval challenge with V. parahaemolyticus and expression of cMnSOD and GPx mRNAs

After being challenged with 3×10^8 cfu/mL *V. parahaemolyticus* for 72 hours, the cumulative moralities of postlarvae in all experimental groups were determined (Fig. 3). The cumulative morality of postlarvae in N-Ast90 and N-Ast140 were significantly lower ($P < 0.05$) than that for postlarvae in other groups.

The cMnSOD mRNA expression levels of unchallenged postlarvae in S-Ast70 and all levels of N-Ast were significantly increased ($P < 0.05$) compared with the control. Its expression level was not significantly different ($P > 0.05$) among groups (Fig. 4A).

The GPx mRNA expression levels of unchallenged postlarvae were significantly higher ($P < 0.05$) than the control, with the highest in N-Ast90. Its expression level was not significantly different ($P > 0.05$) in any of four groups receiving AST (S-Ast70, N-Ast50, N-Ast90 and N-Ast140) (Fig. 4B).

Postlarvae in 15 L of water were challenged for 72 hours with 3×10^8 cfu/mL *V. parahaemolyticus*. The cMnSOD mRNA expression levels of challenged postlarvae in all experimental groups were significantly down-regulated ($P < 0.05$) compared to levels in the unchallenged animals. Its expression level in postlarvae fed N-Ast90 was significantly less downregulated ($P > 0.05$), but other groups had no significant differences ($P > 0.05$) from each other after being challenged with *V. parahaemolyticus* (Fig. 4A).

The GPx mRNA expression levels of challenged postlarvae in all experimental groups also were significantly down-regulated ($P < 0.05$). Its expression level in postlarvae fed S-Ast70 was significantly more downregulated ($P < 0.05$) than the others, which showed no significant differences from each other after being challenged with *V. parahaemolyticus* (Fig. 4B).

Discussion

AST is a valuable dietary ingredient for crustacean juveniles and plays a prominent role in intermediary metabolism (Niu *et al.*, 2012; Daly *et al.*, 2013). The exact mechanism making botanical AST better than synthetic AST from a growth perspective is not completely understood. According to studies, 6-7 hours after oral intake of trans AST, the AST concentration in plasma is significantly elevated, with the proportion of the all-trans configuration diminished while the cis configuration increased, especially 13-cis (Qsterlie *et al.*, 2000). The cis configuration, especially 9-cis, has demonstrated a higher antioxidant capacity *in vitro* as compared with the all-trans configuration (Liu and Osawa, 2007). This indicates that the cis/trans ratio can be an indication of antioxidant ability. The cis/trans ratios in S-Ast70 and the various N-Ast diets were 0.13 and 0.2, respectively. In terms of thermodynamic structure, the trans configuration is more stable, whereas the cis, with higher antioxidant capacity, is more active (Britton, 1995). The cis isomer level was

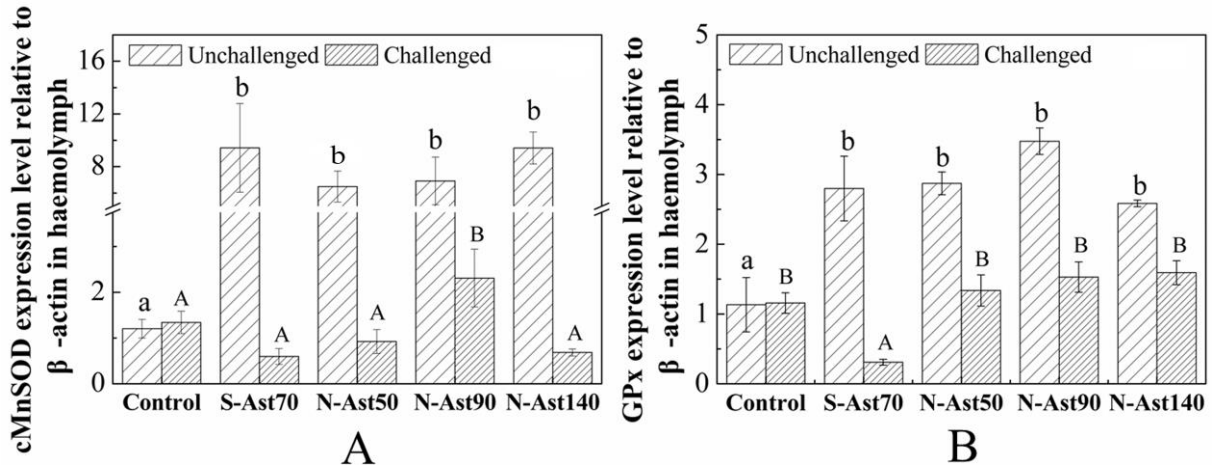

Fig. 4 SOD, CAT and GPx mRNA expression profiles of *L. vannamei* postlarvae fed various experimental diets. The animals were examined by RT-PCR before and after being challenged with *V. parahaemolyticus*. The actin gene was used for internal calibration. Vertical bars represented the mean ± SD. Significant differences are indicated with the different letters (a, b, A and B) ($P < 0.05$, n = 3).

higher in the N-Ast diets; however, no significant increase of the cis isomer was found in postlarvae fed N-Ast. We suppose that the cis isomer is consumed directly to neutralize existing oxidant stress in the postlarvae. The results showed that the cis/trans isomer ratio was significantly higher in animals fed N-Ast90, not N-Ast140. It is presumed that the 9-cis AST might, as an antioxidant, participate directly in immune reactions without configuration transformation *in vivo*. Accordingly, the 9-cis isomer content was lower in animals fed diet N-Ast140 than in animals fed the other diets.

In this study, the ratio of the optical isomers 3S,3'S : 3R,3'S : 3R,3'R in the N-Ast diets was about 30:3:1, and that in the S-Ast70 diet was 6:2:1. The ratio in the diets with N-Ast agrees with previous studies (Grung *et al.*, 1992; Turujman *et al.*, 1997). However, the ratio in the diet with S-Ast70 was not in accord with previous reports on synthetic AST, where the 3S,3'S : 3R,3'S : 3R,3'R ratio was ca. 1:2:1 (Lorenz and Cysewski, 2000). However, the distribution of these AST optical isomers in juvenile shrimp was 5:4:1 to 3:3:1, which was not so extremely different as that in the diets. The results above strongly indicate that juvenile shrimp might have isomerization ability in addition to selective absorption and deposition. Carotenoids usually can hydroxylate into AST irreversibly. In this connection, existing AST might be involved in dehydroxylation first, and then hydroxylates again *in vivo*. It is likely that an epimerase exists in the AST metabolic pathway in juvenile shrimp. However, in the absence of additional evidence, this possibility needs further examination.

Several previous studies have found that dietary supplementation with *H. pluvialis* powder significantly enhances the length and weight gain of *L. vannamei* (Chuchird *et al.*, 2015; Parisenti *et al.*, 2015). *P. monodon* had a higher weight gain through supplementation with botanical AST and capsanthin (Supamattaya *et al.*, 2005). This study demonstrates that dietary supplementation with N-Ast90 and N-Ast140 significantly enhances both length and weight gain of *L. vannamei* juveniles. A previous study found that dietary supplementation with 50 ppm synthetic AST had no significant influence on the growth of *P. monodon* juveniles (Boonyaratpalin *et al.*, 2001). It has also been reported that neither 50 ppm of botanical AST nor 100 ppm of synthetic AST had a significant effect on the weight gain of kuruma prawn (*Marsupenaeus japonicus*) postlarvae (Chien and Shiau, 2005). This situation is consistent with part of the present study in that no significant difference was found between the growth performance of control and shrimp fed S-Ast70. However, the present study found that N-Ast50 had a significant effect on shrimp growth performance. According to a previous report, AST was preferable to canthaxanthin because it produced nature-identical pigmentation and was more efficiently deposited (Storebakken *et al.*, 1987). It is highly likely that the different AST types, presented in diets, were selectively absorbed, which may also help to explain why the AST content of juvenile shrimp in N-Ast50 was higher than that of S-Ast70. Alternatively, it can be assumed that N-Ast in the supplementation can be better assimilated, converted and deposited in juvenile shrimp. It is unclear why weight gain of animals in the N-Ast140 treatment appeared to be slightly less (but not significantly so) than in the N-Ast50 treatment. Excessive absorption of dietary carotenoids might lead to side effects such as reported for female *E. sinensis* (Long *et al.*, 2017). Petri and Lundebye (2007) proved that the organ distribution content of high doses of AST in rats after oral application was not increasing all the time. AST beyond some threshold may generate side effects, including energy-consumption during metabolism and excretion. The slightly lower weight gain may be

related to this. This interesting phenomenon needs to be investigated further. Our results suggest that dietary supplementation with botanical AST was more effective than with S-AST in promoting postlarval growth and AST accumulation.

SOD, CAT and GPx are major endogenous antioxidant enzymes. Their expression levels reflect the level of reactive oxygen species, and protect tissue cells from oxidative damage (Liu *et al.*, 2007). Higher SOD values indicate that there are more superoxide radicals in cells that need to be removed. Higher CAT and GPx values reflect that there is more H_2O_2 in tissues that needs to be eliminated. Overall, the antioxidant activity generated by N-Ast was relatively higher than that by S-Ast70. The level of cis geometrical isomers, known to have greater antioxidant activity than the all-trans isomers, was higher in botanical AST than in synthetic AST. SOD and CAT activities were both significantly decreased with increasing concentrations of dietary AST in *Hyphessobrycon callistus* and *L. vannamei* (Wang *et al.*, 2006; Zhang *et al*., 2013). Some studies have indicated the AST has more powerful ROS quenching activity than CAT and SOD, and that it could protect individuals from oxidative damage. However, the study also indicated that SOD, CAT and GPx activities of *E. sinensis* all exhibited a tendency of "low-high-low" with gradient concentrations of the *H. pluvialis* powder (Long *et al.*, 2017). In the present study, SOD and GPx activities of juvenile shrimp both presented the "low-high-low" profile as well. CAT activity of juvenile shrimp fed N-Ast140 was significantly higher than in the other groups. These results suggest that appropriate levels of dietary botanical AST could enhance the expression of the enzymes. Moreover, AST itself can eliminate ROS, which would decrease the substrate level for the antioxidant enzymes (Chuchird *et al*., 2015). The lower levels of substrates might lead to the decreased expression of antioxidant enzymes (GPx). AST participates in various metabolic processes, but it should be maintained at a proper level. Higher AST concentration is not necessarily better in the cultivation of shrimp.

In the present study, after the *L. vannamei* were challenged with *V. parahaemolyticus*, the cumulative mortality of juvenile shrimp fed N-Ast90 and N-Ast140 were significantly lower ($P < 0.05$) than that for other treatments. According to a previous study, provision of synthetic AST in the diet increased the survival of *L. vannamei* and significantly improved total hemocyte count, phagocytosis activity, phenoloxidase activity, and SOD activity (Chuchird *et al*., 2015). This is in the agreement with our results. The immune protective effects of AST might be attributable to its antioxidant property to counteract the stress from pathogens.

Our results strongly indicate that dietary supplementation with AST improves the tolerance of juvenile shrimp to the *Vibrio* stress. Growth performance and resistance to stress are important considerations for industrial aquaculture, but diet cost must also be considered carefully. Thus, the N-Ast90 diet would be preferable overall.

The SOD enzymes constitute a first-line defense that transforms superoxide anions ($O^{2-\cdot}$) into hydrogen peroxide (H_2O_2) and oxygen molecules. It plays a prominent role in the oxidative damage defense system (Campacórdova *et al*., 2002; Parrilla-Taylor and Zenteno-Savín, 2013). In the present study, postlarvae fed N-Ast or S-Ast70 had higher expression levels of cMnSOD mRNA than the control under normal conditions. After being challenged with *V. parahaemolyticus*, cMnSOD mRNA expression levels were significantly reduced, usually below the control level. Among these experimental groups, postlarvae fed N-Ast90 had significantly higher ($P < 0.05$) mRNA expression levels than other groups. GPx removes the H_2O_2 produced by the first-line defense system, thus helping to neutralize the effects of oxidative stress. GPx, even at a low concentration, can transform H_2O_2 (Nordberg and Arnér, 2001). The postlarvae fed N-Ast90 had slightly higher GPx mRNA expression levels than the control animals and animals fed other rations under normal conditions. After being challenged with *V. parahaemolyticus*, the expression levels of GPx significantly decreased as compared to the unchallenged animals. Postlarvae fed N-AST had higher GPx mRNA expression levels than S-Ast70 fed animals, and the expression tendency was similar to that of cMnSOD mRNA. These results indicate that dietary botanical AST effectively relieves environmental oxidative damage through higher gene expression levels of antioxidant enzymes, which could counteract stress induced by reactive oxygen species in *L. vannamei* postlarvae.

Conclusions

In summary, different ratios of AST optical isomers in diets had no significant effects on juvenile shrimp. This result suggests that an astaxanthin epimerase may exist *in vivo*. Dietary botanical AST can improve growth performance, AST content and resistance to pathogenic bacteria. N-Ast50 appeared to have a more significant effect than S-Ast70 except cumulative mortality, although their AST concentrations are similar. Our present data indicate that the appropriate concentration of botanical AST in diets is around 90 ppm for *L. vannamei* postlarvae.

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