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Survival, growth, sexual maturity and tissue histamine accumulation of the mysis, *Neomysis awatschensis* and *N. japonica Nakazawa*, fed histamine supplemented diets

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ABSTRACT

Estuaries are often subjected to intensive aquaculture activities. Finfish and crustacean culture frequently involve the use of trash fish, which can contain high amounts of histamine. This has the potential to be a major ecological concern since this trash fish often becomes a feed source for naturally occurring small aquatic animals, such as mysis. The present study evaluated the effects of dietary histamine supplementation on survival, growth, sexual maturity and tissue accumulation of two estuarine mysis, *Neomysis awatschensis* and *N. japonica Nakazawa*.

Both *N. awatschensis* and *N. japonica Nakazawa* were fed specially formulated diets containing 0 (control), 1, 2 and 4 g/kg supplemented histamine (5 replicates per treatment; 110 neonates per replicate) from the time they became capable of effectively feeding on formulated diets (10 and 17 day-post-hatch for *N. awatschensis* and *N. japonica Nakazawa*, respectively). The feeding experiment continued until the mysis reached sexual maturity. Our current findings indicate that dietary histamine had negative effects on both mysis species although their sensitivity to histamine appeared to be species-specific. Results showed that survival was significantly lower for *N. awatschensis* fed diet containing 4 g/kg supplemented histamine (56.8%) when compared to that of the control (78.3%), while for *N. japonica Nakazawa*, survival was significantly lower in both 2 (52.1%) and 4 g/kg (51.5%) histamine treatments than in the control group (74.3%) ($p<0.05$). Histamine containing diets also significantly impacted body length of sexual matured *N. japonica Nakazawa*, however, no significant differences on either body weight or length were detected for *N. awatschensis* ($p>0.05$). It generally took longer for both males and females of the two mysis species to reach sexual maturity when they were fed diets containing higher levels of histamine and the differences were often significant ($p<0.05$). Compared to the control, the whole tissue histamine concentration increased significantly for *N. japonica Nakazawa* fed diets containing 2 and 4 g/kg supplemented histamine ($p<0.05$), however, no significant differences in tissue histamine levels were found among all treatments for *N. awatschensis*. These results suggest that the consumption of stale aquaculture feeds containing histamine could significantly impact naturally existing aquatic animals that play an important role in the marine food web and ecosystem.

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1. Introduction

Over the past decades, in an effort to satisfy increasing demands for seafood, aquaculture has grown rapidly worldwide. This rapid expansion of aquaculture industry has been particularly prevalent in Asian countries, such as China. In this region, aquaculture is mainly conducted in nearshore coastal and estuarine regions, and trash fish are often extensively utilized as feeds by the industry (e.g. for grouper and crab farming) (Muslima et al. 2009) largely due to their ready local availability and often lower costs compared to dry formulated feed. However, the extensive use of trash fish in aquaculture has led to concerns regarding potential harmful effects of biological amines, such as histamine, to the health of the ecosystem and naturally occurring aquatic animals since stale trash fish contains high level of biogenic amines. The biogenic amine concentration is a quality criterion of fish meals as it indicates spoilage (Pike and Hardy, 1997; Tapia-Salazer et al., 2001; Vinci and Antonelli, 2002). One of the major biogenic amines, histamine is produced by the bacterial decarboxylation of L-histidine, which has a low molecular weight and possesses a range of biological activities (Ten-Brink et al., 1990).

The effects of dietary histamine have been reported for mammals (Taylor, 1986; Ababouch, 1990), chickens (Harry and Tucker, 1976; Huisman et al., 1992) and commercially important aquacultured species, such as penaeid shrimps (Tapia-Salazar et al, 1998, 2001) and fishes (Watanabe et al., 1987; Fairgrieve et al., 1998; Opstvedt et al., 2000). For mammals, histamine is a threat to food safety as it causes food poisoning (Taylor, 1986; Ababouch, 1990). The presence of histamine at

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high levels in feeds has also been shown to cause increased mortality, decreased weight gain, lower feed consumption (Harry et al., 1975; Osuna, 1985) and gizzard lesions (Harry and Tucker, 1976) in chickens. However, the effects of dietary histamine on aquaculture animals are less clear. Lovell (1989) suggested that high concentrations of dietary histamine caused growth reduction in fish. A decline in feed consumption in the rainbow trout *Oncorhynchus mykiss* when fed diets supplemented with histamine at 2 g/kg (Fairgrieve et al., 1998) or putrescine at 13.3 g/kg (Cowey and Cho, 1992) has also been reported. However, although Watanabe et al. (1987) and Fairgrieve et al. (1994) observed that dietary histamine supplementation caused intestinal damage in *O. mykiss*, no significant effects on feed intake or weight gain of the fish was detected. In contrast to these earlier reports, more recently, Tapia-Salazar et al. (2001) found that dietary histamine supplementation at 0.6–4.8 g/kg had no negative effects on feed consumption, feed conversion ratio and survival of the blue shrimp *Litopenaeus stylirostris*. Moreover, these authors observed an improvement in weight gain when histamine was supplemented at 1.2–2.4 g/kg (Tapia-Salazar et al., 2001).

While the effects of dietary histamine on aquaculture species have been studied, to date, little attention has been paid to aquatic animals that naturally exist in aquatic systems, and which are likely to feed on leftover feeds from aquaculture farms. Stale aquaculture feeds, including trash fish and rancid formulated feeds, are often released into natural water bodies by either water exchange or feeding for cage aquaculture, this may in turn become food for other small aquatic animals. The potential effects of histamine on species that form important parts of aquatic ecosystems are of a particular concern. Mysis shrimp are often dominant benthic fauna in shallow coastal waters and estuaries and play an important role in marine food web and energy flow (Mauchline, 1980; Rolland and Fulton, 1982). For instance, they are known to be the exclusive natural diets for newly settled juvenile Japanese flounder, *Paralichthys olivaceus* (Mauchline 1980; Minami, 1997; Sudo, 2003). In addition, mysis are also generally sensitive to marine pollution, and are commonly used as indicator species for various pollutants (McKenney and Matthews, 1990; Verslycke et al., 2007).

Estuaries are often subjected to anthropogenic inputs including intensive aquaculture activities and as a result there has been increasing interest in using brackish water mysis as a model species for toxicological tests (Ghekiere et al., 2007; Verslycke et al., 2007). Consequently, the present study was conducted to clarify potential effects of dietary histamine on survival, growth, sexual maturity and tissue accumulation of two abundant estuarine mysis species in China, *N. awatschensis* and *N. japonica Nakazawa* (Liu and Wang, 2000).

2. Materials and methods

2.1. Mysis stock culture

The stock cultures of both mysis species were maintained according to the method described by Verslycke and Janssen (2002). In short, both *N. awatschensis* and *N. japonica Nakazawa* were cultured in a recirculating system. The salinity of the culture medium was 15 psu, which was obtained by diluting high saline natural salt brine with filtered (120 μm) de-chlorinated tap water. The water temperature of the cultures was measured daily and maintained at 25.0 ± 1.0 °C while a 12 h:12 h light–dark photoperiod was kept.

2.2. Experimental diets

A basal diet was formulated (Table 1) based on nutritional requirements for penaeid shrimp recommended by Tacon (1989) and Akiyama et al. (1991). The experimental diets were prepared by supplementing the basal diet with 1, 2 and 4 mg/kg histamine (H7125; histamine (C5H9N3) ≥ 97.0%; 111.5 g/mol; Sigma, St. Louis, MO). Each diet was analyzed to determine moisture (AOAC, 1990, method 920.36), crude protein (Tecator, 1987), crude lipid (Tecator, 1983) and ash (AOAC, 1990, method 942.05). The actual histamine content in each experimental diet was confirmed using HPLC (high performance liquid chromatography) according to Tapia-Salazar et al. (2000) (Table 2).

Table 1
The composition of the basal diet (control).

Ingredients	%
Fish meal	28.0
Rapeseed meal	10.0
Soya bean meal	22.0
Wheat flour	18.4
Squid meal	5.0
Shrimp meal	5.0
Molting hormone	0.1
Vitamin mix[a]	1.5
Mineral mix[b]	1.5
Lecithin	3.0
Fish oil	1.0
Yeast	3.0
Binder	1.5
Analyzed composition	
Moisture	10.2 ± 0.0
Crude protein	35.1 ± 0.0
Crude lipid	14.7 ± 0.6
Ash	9.8 ± 0.3

Source of the ingredients: Shanghai Yongnong Feed, China.
[a] Vitamin mix: 1 kg of diet contained vitamin A, 10,000 IU; vitamin D, 2500 IU; vitamin K, 64 mg; thiamin, 60 mg; riboflavin, 250 mg; pyridoxine, 60 mg; α-tocopherol acetate, 500 mg; L-ascorbic acid, 4.5 g; calcium pantothenate, 240 mg; niacin, 60 mg; folic acid, 12 mg; biotin, 50 mg; cyanocobalamine, 4 mg.
[b] Mineral mix: 1 kg of diet contained $Ca(H_2PO_4)_2$, 10 g; $MgSO_4 \cdot 7H_2O$, 2.4 g; KCl, 4.5 g; NaCl, 2.1 g; $FeSO_4 \cdot H_2O$, 155 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSO_4 \cdot H_2O$, 80 mg; $MnSO_4 \cdot H_2O$, 30 mg; KI, 11.7 mg; $CoCl_2 \cdot 6H_2O$, 4.8 mg; Na_2SeO_3, 2.4 mg.

2.3. Survival, growth and sexual maturity experiment

The formulated diets containing different levels of supplemented histamine were fed to *N. awatschensis* and *N. japonica Nakazawa* from 10 and 17 day-post-hatch (DPH) respectively and until experimental animals reached sexual maturity. Prior to this, the newly released neonates of both mysis species were fed newly hatched *Artemia* nauplii to satiation daily. The timing of commencement of feeding on the formulated diets for each mysis species was based on results of our pilot experiments, in which the effective ingestion of formulated feeds was shown to only occur after a period of development from newly hatched neonates of both mysis species, which corresponded to the appearance of male secondary sexual organ.

Synthetic seawater was used for the feeding trial and each diet treatment had five replicates, with each replicate consisting of 110 newly hatched neonates housed in a 10 L (37 × 24 × 11 cm) glass aquarium filled with 5 L water. During the experiment, the bottom of each aquarium was siphoned daily to remove any detritus and leftover feed, at the same time a fifth of the water was then exchanged with salinity pre-adjusted new water. After water exchanges, the formulated diets containing different levels of histamine were added to the corresponding aquaria to feed the mysis to satiation. The water temperature was maintained at 25.0 ± 1.0 °C and photoperiod kept at light:dark = 12 h:12 h throughout the experiment. The pH, dissolved

Table 2
Histamine concentration in the experimental diets (g/kg).

Histamine supplemental level (g/kg)	Actual analyzed histamine level
0	29.77 ± 0.59
1	1034.82 ± 22.93
2	2048.66 ± 24.07
4	4037.57 ± 5.38

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