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Effects of T-2 toxin on growth, immune function and hepatopancreas microstructure of shrimp (*Litopenaeus vannamei*)

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ABSTRACT

T-2 toxin (T-2) is a trichothecene-A-type mycotoxin produced by the fungus *Fusarium* spp., which can contaminate animal feed. It is toxic to many living cells especially the hepatocytes and bone marrow cells. The effects of dietary T-2 on growth, biochemical and immunity parameters, and histopathological changes in the hepatopancreas of white shrimp (*Litopenaeus vannamei*) were studied. Shrimp with an initial body weight of 3.5 ± 0.5 g were fed five diets (n = 50/group) containing 0, 0.5, 1.2, 2.4, or 4.8 mg kg^{-1} T-2. Dietary exposure to T-2 significantly decreased shrimp growth rate and specific growth rate ($P < 0.5$), but not the fat content, compared with the control group. Exposure to T-2 damaged the hepatopancreas microstructure in a dose-dependent manner and this may have contributed to a lower hemolymph albumin concentration and lower phenoloxidase activity. Total hemocyte counts and activity of hemolymph glutamic-pyruvic transaminase > glutamic-oxaloacetic transaminase and alkaline phosphatase enzymes increased initially in a dose-dependent manner but declined thereafter. This study showed that T-2 is a multi-organ toxin with effects on the hemolymph, immune system, and hepatopancreas of shrimp. Collectively, these effects may have contributed to the inhibition of growth and decreased survival rate leading to a lower yield and quality of shrimp meat with resultant economic loss.

Statement of relevance

It is proposed that hemolymph phenoloxidase enzyme activity could be used as a cheap non-specific biomarker for initial screening of shrimp exposed to even low T-2 toxin concentrations, instead of more expensive LC-MS/MS T-2 analysis — although a dose response was not evident at high exposure concentrations.

1. Introduction

T-2 is a trichothecene mycotoxin produced by the fungus *Fusarium* spp., an infrequent contaminant in animal feed and human food (Krska et al., 2007). T-2 is hepatotoxic and also affects the more active proliferating cells such as the bone marrow cells, leukomonocytes and nephrocytes (Zou et al., 2011). T-2 toxicity in humans can lead to apoptosis in blood lymphocytes, leukopenia, granulocytopenia, and depression of bone marrow hematopoiesis, which can result in an inability to overcome infections (Chaudhari et al., 2009; Parent-Massin, 2004; Vlata et al., 2005). T-2 and ochratoxin can synergistically decrease the production of IL-2 and the expression of plasma interferon-gamma (IFN-γ) (Xue et al., 2010). In quails, T-2 has been shown to reduce activity in enzymes such as alkaline phosphatase (AKP) in blood, which plays an important role in the innate immune system, and glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT), which have historically been used as indicators of tissue damage (Madheswaran et al., 2004; Nemcsok and Boross, 1982).

The effects of trichothecenes on aquatic animals are not well known. T-2 at 2 mg·kg^{-1} decreased the number of lymphocytes and caused hepatopancreas necrosis in black tiger shrimp (Bundit et al., 2006). T-2 at doses >2.5 mg·kg^{-1} reduced body weight, feed ingestion, feed conversion and hemoglobin concentration in rainbow trout (Poston et al., 1982). Feeding T-2 to catfish at 1 mg·kg^{-1} decreased intestinal immunity and increased mortality by up to 84% (Manning et al., 2005).

Abbreviations: AKP, alkaline phosphatase; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; PO, phenoloxidase; THC, total hemocyte count.

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T-2 contamination of shrimp feed is not well documented and only a few reports on the effects of T-2 toxin on shrimp exist, mostly from South Korea, Japan, and Thailand (Anukul et al., 2013). We have previously shown that T-2-producing *Fusarium* spp. exist in the shrimp farming environment, including the feed (Qi, 2013). This research reports on the effects of feed T-2 toxin on growth, immunity-physiological parameters and hepatopancreas histopathology in white shrimp (*Litopenaeus vannamei*).

2. Materials and methods

2.1. Experimental diets and feeding trials

Two isoproteic and isolipidic (based on proximate analysis) shrimp feeds, control and T-2-added, were prepared according to the method of Dai et al. (2013). Feed pellets were oven-dried at 50 °C, packed in high density polyethylene bags, and stored at −20 °C until use.

White shrimp ($n = 250$, initial body weights (W_i) = 3.5 ± 0.5 g) from a shrimp breeding base in Donghai Island, Zhanjiang, were randomly distributed into 25 test aquaria ($35 \times 25 \times 15$ cm^3), and acclimatized for 7 days to laboratory conditions: water temperature 25 ± 1 °C (heated with submersible 1000 W heaters), pH 7.5 ± 0.1, salinity 10 ± 1‰, and saturated with dissolved oxygen. Approximately a third of the water in each aquarium was replaced daily at ~9 a.m. The shrimp were fed three times a day with a commercial feed at the rate of 5% body weight per day. The shrimp body lengths were measured before and after the feeding trail. Groups of shrimp ($n = 50$/group) were fed diets containing 0 (control), 0.5, 1.2, 2.4, or 4.8 mg·kg^{-1} of T-2 for 20 days. On day 21, the body weights (W_f) of surviving shrimp were recorded. Shrimp from each group ($n = 27$ per group) were anesthetized with ice, sacrificed, and their hemolymph collected for analysis of immunological and physiological parameters. Three shrimp from each group were decapitated and their hepatopancreases removed for histopathology.

2.2. Shrimp body weights and morphology

At the end of the experiment the following growth indices were calculated as described by Qi et al. (2015):

1) Weight gain (%) = $((W_f - W_i) / W_i)$
2) Specific growth rate (SGR) = $[(\ln W_f - \ln W_i) / (t_f - t_i)] \times 100\%$
3) Relative fatness (%) = $W_f / BL^3 \times 100$
4) Survival rate (%) = $100 (N_i - N_f) / N_i$

where W_i and W_f are the initial and final average body weights at times t_i (day 1) and t_f (day 21) respectively. BL refers to the final body length. N_i and N_f are the initial (=50) and final numbers of shrimp in each group.

2.3. Humoral and cellular analyses

2.3.1. Hemolymph extraction

Hemolymph was extracted from abdominal sinusoids and mixed with an equal volume of sterile anticoagulant (sodium citrate 1.32%, citric acid 0.48%, glucose 1.47%). A portion of this was used to determine the total number of hematocytes, albumin concentration, and phenoloxidase (PO) activity. The remainder was stored at 4 °C overnight, centrifuged at 3000 rpm for 6 min, and the hemolymph serum removed for measurement of AKP, GPT and GOT.

2.3.2. Total hematocyte count (THC)

Ten microliters of hemolymph-EDTA was added to 90 μL of 0.4% trypan blue solution, and a 10 μL aliquot of this solution was placed in a Neubauer chamber for 1 min and then transferred to a hemocytometer to measure THC using a microscope (Olympus CKX41, Tokyo, Japan) at 200× magnification.

2.3.3. Phenoloxidase activity

Phenoloxidase activity was determined according to Wu et al. (2012). A cell lysate of hemolymph was obtained by sonication, followed by centrifugation and the supernatant assayed for PO activity. Fifty microliters of the supernatant and 200 μL of 0.01 mol·L^{-1} L-DOPA aqueous solution were mixed, incubated for 20 min at 25 °C, and the absorbance read at 490 nm using a microplate reader (Thermo MULTISKAN MX3, Shanghai, China). One unit of PO activity was defined as an increase in absorbance of 0.001 min^{-1}·mg protein^{-1}.

2.3.4. Albumin concentration

Ten microliters of hemolymph lysate supernatant was mixed with 2.5 mL bromocresol green solution, kept at room temperature for 10 min and the optimal density measured at 628 nm using a spectrophotometer (Jingke 722S, Shanghai, China) as described by the kit instructions of Nanjing Jiancheng Bio-Reagent Company (China) with bovine serum albumin as standard.

2.3.5. Activity of AKP, GOT, and GPT in hemolymph lysate

Alkaline phosphotase, GOT and GPT enzymes were assayed using detection kits (Nanjing, China) according to the manufacturer's instructions. One unit of AKP enzymatic activity corresponded to 1 mg of phenol liberated per 100 mL cell-free hemolymph at 37 °C for 15 min. AKP activity was expressed as U·100 mL^{-1} hemolymph. GOT and GPT activities were measured at 37 °C and expressed as U per milligram of protein in hemolymph. One unit of GPT or GOT enzymatic activity corresponded to the product pyruvate to oxidize NADH to NAD^+ and a decline in absorbance at 340 nm of 0.001 in 1 mL of hemolymph.

2.4. Histopathology

The hepatopancreases from three shrimp per dose were fixed in 10% formalin for 24 h, dehydrated in an alcohol series (50% to 95%), and samples embedded in paraffin. Sections 0.5 mm thick were taken using a microtome, stained with hematoxylin and eosin, and histopathological changes observed under a microscope (Olympus CKX41, Tokyo, Japan).

2.5. Data analyses

Analysis of variance (ANOVA) and Duncan's new multiple range test were used to determine statistically significant differences between treatments at the 95% ($P < 0.05$) confidence level.

3. Results

3.1. Effect of T-2 on shrimp growth parameters (Table 1)

Shrimp weight gain and specific growth rate decreased significantly ($P < 0.05$) at all T-2 doses and was lowest at the highest dose. T-2 also affected shrimp survival rate. However, the lowest survival rate was observed at the two lowest doses (0.5 and 1.2 mg·kg^{-1}·feed) and was significantly different ($P < 0.05$) to the control. There was no significant effect of T-2 on the relative fatness of the shrimp.

Table 1
Shrimp morphological characteristics following dietary exposure to T-2 toxin for 20 days.

Diet (mg·kg^{-1})	Weight gain* (%)	Specific growth* rate (% d^{-1})	Relative fatness* (%)	Survival rate* (%)
0.0	22.38 ± 0.63^b	1.01 ± 0.02^b	1.92 ± 0.06^a	92.22 ± 1.92^c
0.5	18.30 ± 1.12^a	0.84 ± 0.05^a	1.96 ± 0.13^a	78.89 ± 5.09^b
1.2	18.12 ± 0.82^a	0.83 ± 0.03^a	1.82 ± 0.14^a	72.22 ± 1.92^a
2.4	19.10 ± 1.37^a	0.87 ± 0.06^a	1.86 ± 0.09^a	91.11 ± 3.85^c
4.8	17.77 ± 1.81^a	0.82 ± 0.08^a	1.84 ± 0.04^a	88.89 ± 1.92^c

* Values followed by the same letter in a column are not significantly different ($P = 0.05$).