

## Effects of ochratoxin A and deoxynivalenol on growth performance and immuno-physiological parameters in black tiger shrimp (*Penaeus monodon*)

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### Abstract

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Ochratoxin A (OTA) and deoxynivalenol (DON), naturally occurring contaminants of animal feed, have been implicated in several mycotoxicoses in farm livestock but there is little information on their toxicity in aquatic invertebrates. Therefore, in the present study an 8-week feeding trial was conducted on black tiger shrimp (*Penaeus monodon*) to assess the effects of OTA and DON on growth performance, haemolymph parameters and histopathology of shrimp. Results showed that feed supplemented with DON caused no effect on growth or survival rate of the shrimp. However, shrimps fed DON feed diet with 1,000 ppb showed significantly higher growth performance. No significant difference in total haemocyte counts (THC) was found in shrimp fed mycotoxins-supplemented feed. Feeding high level of OTA (1,000 ppb) caused a decrease in phenoloxidase (PO) activity. Although no histopathological change was observed, decrease in serum

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alkaline phosphatase (ALP), serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels in shrimp fed OTA or DON indicated that mycotoxin may impair the functioning of hepatopancreatic cells. Since no adverse effect occurred with the highest levels of OTA and DON (1,000 and 2,000 ppb, respectively) on haemolymph parameters and no residue was detected at the completion of the 8-week feeding period, it can be concluded that shrimp feeds occasionally contaminated with OTA or DON have no negative impact on the shrimp culture industry.

**Key words :** toxin, ochratoxin A, deoxynivalenol, growth performance, immuno-physiological parameters, black tiger shrimp, *Penaeus monodon*

### บทคัดย่อ

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ผลของ ochratoxin A และ deoxynivalenol ต่อการเจริญเติบโต ปัจจัยทางภูมิคุ้มกันและ  
สรีรวิทยาในกุ้งกุลาดำ (*Penaeus monodon*)

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ochratoxin A (OTA) และ deoxynivalenol (DON) เป็นท็อกซินที่ผลิตจากเชื้อรา มีรายงานมีการปนเปื้อนในอาหารสัตว์และเป็นพิษต่อสัตว์เลี้ยงหลายชนิด แต่ข้อมูลความเป็นพิษของท็อกซินเหล่านี้ในสัตว์น้ำมีค่อนข้างจำกัด การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลต่อการเจริญเติบโต องค์ประกอบเลือดและภูมิคุ้มกัน และการเปลี่ยนแปลงทางเนื้อเยื่อของกุ้งกุลาดำที่ได้รับอาหารผสม OTA ในระดับ 100, 200 และ 1,000 ppb และอาหารผสม DON ที่ระดับ 500, 1,000 และ 2,000 ppb อย่างต่อเนื่องเป็นเวลา 8 สัปดาห์ ผลการศึกษาพบว่าอัตราการเจริญเติบโตและอัตราการรอดของกุ้งที่ได้รับอาหารผสม DON ทุกระดับไม่แตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญ อย่างไรก็ตามพบว่ากุ้งที่ได้รับอาหารผสม DON ในระดับ 1,000 ppb มีแนวโน้มการเจริญเติบโตสูงขึ้นเมื่อเปรียบเทียบกับกลุ่มควบคุม นอกจากนี้กุ้งที่ได้รับอาหารผสมท็อกซินทั้งสองชนิดไม่มีการเปลี่ยนแปลงของปริมาณเม็ดเลือดรวม แต่พบว่ากุ้งที่ได้รับอาหารผสม OTA ในระดับ 1,000 ppb มีระดับความว่องไวของเอนไซม์ฟีนอลออกซิเดสลดลง จากการตรวจสอบไม่พบการเปลี่ยนแปลงทางพยาธิสภาพของเนื้อเยื่อกุ้ง แต่พบว่ากุ้งที่ได้รับอาหารผสมท็อกซินมีระดับของเอนไซม์ ALP, SGOT และ SGPT ลดลง ซึ่งอาจเป็นผลจากความเป็นพิษของท็อกซินต่อการทำงานของเซลล์ตับและตับอ่อนของกุ้ง จากผลการศึกษาพบว่าการได้รับท็อกซิน OTA และ DON ในระดับความเข้มข้นสูง (1,000 และ 2,000 ppb ตามลำดับ) ส่งผลกระทบต่อเปลี่ยนแปลงองค์ประกอบเลือดของกุ้งค่อนข้างน้อย และไม่พบการตกค้างของท็อกซินในเนื้อเยื่อกุ้งหลังจากได้รับท็อกซินอย่างต่อเนื่องเป็นเวลา 8 สัปดาห์ จึงสรุปได้ว่าอาหารกุ้งที่อาจมีการปนเปื้อนของ OTA และ DON (ในระดับสูงถึง 1,000 หรือ 2,000 ppb ตามลำดับ) ไม่ได้เป็นปัจจัยเสี่ยงต่อสุขภาพกุ้งหรือผลกระทบต่ออุตสาหกรรมเพาะเลี้ยงกุ้ง

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Mycotoxins are toxic metabolites produced by filamentous fungi growing on agricultural crops and products. Production of mycotoxins by fungi can also occur during processing and storage of

harvested feed materials when environmental conditions are suitable. It has been established that mycotoxins pose a health risk to livestock and, as a consequence, may cause economic loss either by

unfavourable effects on the animals themselves, or by an increased potential for detrimental health effects in human beings consuming contaminated products (Lioi *et al.*, 2004). Mycotoxins represent a diverse group of secondary fungal metabolites, which vary widely in their chemistry and toxicology. A large number of mycotoxins have been identified. The most frequently occurring mycotoxins include aflatoxins, ochratoxins and trichothecenes (Sudakin, 2003).

Ochratoxin A (OTA), the secondary metabolites of some *Aspergillus* and *Penicillium* strains, is usually the most abundant of the ochratoxins in contaminated feedstuffs. It is also considered to be the most toxic of the ochratoxin series (Marquardt and Frohlich, 1992; Manning *et al.*, 2003). OTA is a mycotoxin food and feed-contaminant known to induce nephro- and hepato-toxicity as well as a tubular-interstitial nephropathy in humans and other animals (Froquet *et al.*, 2003; Petrik *et al.*, 2003). OTA has been linked to the fatal human kidney disease referred to as Balkan endemic nephropathy (Marquardt and Frohlich, 1992). Ochratoxicosis of poultry (Huff *et al.*, 1974; Burns and Dwivedi, 1986) and swine (Krogh, 1991) results in diminished performance, nephropathy, hepatotoxicity and reduced immunocompetence. Degeneration and necrosis of kidney and liver were observed in rainbow trout intraperitoneally injected with OTA (Doster *et al.*, 1972) and channel catfish (Manning *et al.*, 2003).

Deoxynivalenol (DON), an example of trichothecenes, is a mycotoxin produced by fungi of the *Fusarium* genus, i.e. *Fusarium culmorum* and *Fusarium graminearum*, which are abundant in various cereal crops and processed grains (Eriksen and Alexander, 1998). DON inhibits the synthesis of DNA and RNA and protein synthesis at the ribosomal level. The toxin has a haemolytic effect on erythrocytes. An acute dose of DON can induce emesis in pigs, whereas at lower concentration in the diet it reduces growth and feed intake and impairs the immune system of pigs (Rotter *et al.*, 1996; Eriksen and Alexander, 1998; D'Mello *et al.*, 1999; Eriksen and Pettersson, 2004).

Studies on toxicity of mycotoxins in aquatic

invertebrates have been conducted mainly for aflatoxins. It has been reported that dietary aflatoxin B<sub>1</sub> adversely affected growth performance, feed conversion, apparent digestibility coefficients and caused histopathological changes of the Pacific white shrimp (*Penaeus vannamei*) (Osrowski-Meissner *et al.*, 1995), *P. stylirostris* (Lightner, 1993) and *P. monodon* (Boonyaratpalin *et al.*, 2001; Bintvihok *et al.*, 2004). However, the effects of other dietary mycotoxins, in particular OTA and DON on black tiger shrimp (*P. monodon*) have not been investigated. Therefore, the present study was undertaken with *P. monodon* to describe the effects of OTA and DON on growth performance, immuno-physiological parameters, histopathological changes, as well as the residues of toxins in shrimp tissues.

## Materials and Methods

### 1. Experimental diets

Seven isonitrogenous and isocaloric diets were formulated to contain equal amounts of all ingredients, except the level of OTA or DON (supplied by Biomin Innovative Animal Nutrition GmbH, Austria) as follows: diet 1 contained no mycotoxin (control diet), diets 2, 3 and 4 contained 100, 200 and 1,000 ppb OTA, respectively. Diets 5, 6 and 7 contained 500, 1,000 and 2,000 ppb DON, respectively (Table 1). All the diets were processed by a meat grinder and stabilized with about 30% moisture and then made into pellets. This process was followed by drying in an air flow oven at 60°C until the moisture content was less than 10%. The dry pellets were kept in plastic bags in a refrigerator until use.

### 2. Experimental animals

Apparently healthy *P. monodon* juveniles, weighing ca. 2 g, were obtained from a commercial farm and acclimatized in the laboratory for 2 weeks before experimentation. Tests were carried out with six replicate groups each consisting of twenty shrimps housed in 20 l tanks. In all treatment, the shrimps were fed four times daily with an experimental diet at set levels depending on satiation

**Table 1. Composition of experimental diets used (7 isonitrogenous and isocaloric diets were formulated to contain equal amounts of all ingredients, except the level of OTA or DON).**

Ingredient (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Fish meal	28	28	28	28	28	28	28
Shrimp head meal	10	10	10	10	10	10	10
Squid meal	5	5	5	5	5	5	5
Wheat gluten	6	6	6	6	6	6	6
Soy bean meal	10	10	10	10	10	10	10
Wheat flour	20	20	20	20	20	20	20
Rice flour	14.42	14.42	14.42	14.42	14.42	14.42	14.42
Fish oil	2	2	2	2	2	2	2
Lecithin	2	2	2	2	2	2	2
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.06	0.06	0.06	0.06	0.06	0.06	0.06
BHT	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Zeolite	1.5	1.5	1.5	1.5	1.5	1.5	1.5
OTA*	-	2	4	20	-	-	-
DON**	-	-	-	-	5	10	20

\* Standard OTA is 5 ppm in starch

\*\* Standard DON is 10 ppm in starch

based on to water temperature and molting cycle. The shrimps were reared for 8 weeks in a flow-through seawater system with flow rate of 2 l per min. Weight gain, survival and feed conversion ratio were recorded every two weeks. After an 8-week feeding trial, haemolymph was sampled for analysis of immuno-physiological parameters. Shrimp tissues were also collected for histopathological studies.

### 3. Haemolymph parameters

#### 3.1 Total haemocyte counts (THC)

Haemolymph was withdrawn from the base of the walking legs using 1-ml sterile syringe with a 25 gauge needle. Haemolymph was diluted with 0.15% trypan blue solution. The mixture was then placed on haemocytometer to measure THC using standard procedure.

#### 3.2 Phenoloxidase (PO) activity

PO activity was assayed as described by Smith and Söderhäll (1983) using L-dihydroxyphenylalanine (L-DOPA) as a substrate. To obtain

lysates, haemocytes were disrupted by sonication. The lysate supernatants were assayed for PO activity using a spectrophotometer to measure the OD<sub>490nm</sub>. The concentration of haemocyte lysate protein was determined by Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as a standard. One unit of PO activity was defined as an increase in absorbance of 0.001 min<sup>-1</sup> mg protein<sup>-1</sup>).

#### 3.3 Analysis of serum alkaline phosphatase (ALP), glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvic transaminase (SGPT) and calcium levels

After withdrawal of haemolymph, the analysis of ALP, SGOT and SGPT in blood serum was conducted using an automated analyzer (Boehringer Mannheim Automated Analysis, Hitachi 717). Calcium levels were analyzed according to the method described by Takeuchi (1988).

### 4. Analysis of mycotoxin residue

After the 8-week feeding trials, mycotoxin

residue in shrimp cephalothorax and musculature was analyzed using the high performance liquid chromatography (HPLC) method by Biomin Innovative Animal Nutrition GmbH (Austria).

### 5. Histopathological studies

After feeding trials, live shrimps were fixed in Davidson's fixative (Bell and Lightner, 1988). Samples were then processed for routine histological preparation (Humason, 1979). Tissue sections were stained with hematoxylin and eosin and histopathological changes were observed under microscope.

### 6. Statistical analysis

An analysis of variance (ANOVA) and Duncan's new multiple range test were used to examine for significant differences among treatments at 95% confidence level (for statistically significant differences, it was required that  $P < 0.05$ ).

## Results

### 1. Effect of OTA and DON on growth performance

After the 8-week feeding trial, shrimp fed with diets containing either OTA or DON did not show any difference in growth rate and weight gain compared to the control, except for the 1,000

ppb DON fed group. Shrimps fed DON feed diet with 1,000 ppb showed significantly higher growth performance, compared to the control. However, feed supplemented with either OTA or DON caused no effect on survival of the shrimp (Tables 2, 3).

### 2. Effect of OTA and DON on immuno-physiological parameters

After the 8-week feeding trial, shrimps fed with various concentrations of OTA or DON exhibited no difference in the numbers of haemocytes in blood circulation, compared to the control. The results of PO activity analysis showed that feeding with high level of OTA (1,000 ppb) caused significant decreasing of PO activity. However, PO activity was not significant different in the groups fed with various concentrations of DON (Table 4).

A significant decrease in ALP level in serum of shrimps fed OTA or DON was observed. SGOT and SGPT levels tended to be higher in shrimps fed with high concentration of OTA; on the other hand; these values tended to be lower in shrimps fed with higher concentration of DON. No significant difference occurred in calcium level in shrimps fed mycotoxins and controls (Table 5).

### 3. Histopathological studies and mycotoxin residue

No pathological change was noted in gills,

**Table 2. Growth rate of black tiger shrimp (*P. monodon*) during feeding trial with diets containing various levels of mycotoxins. Results are shown as mean (g) with standard deviation.**

Diet	Feeding period (week)				
	0	2	4	6	8
1 (control)	2.41±0.01 <sup>a</sup>	4.10±0.24 <sup>ab</sup>	6.70±0.47 <sup>b</sup>	8.92±0.72 <sup>ab</sup>	11.33±0.97 <sup>ab</sup>
2 (100 ppb OTA)	2.42±0.01 <sup>a</sup>	4.23±0.29 <sup>bc</sup>	6.67±0.77 <sup>b</sup>	8.85±0.98 <sup>ab</sup>	11.52±1.32 <sup>ab</sup>
3 (200 ppb OTA)	2.42±0.01 <sup>a</sup>	4.10±0.28 <sup>abc</sup>	6.62±0.34 <sup>b</sup>	9.03±0.57 <sup>b</sup>	11.42±0.70 <sup>ab</sup>
4 (1,000 ppb OTA)	2.41±0.08 <sup>a</sup>	3.92±0.18 <sup>a</sup>	5.98±0.35 <sup>a</sup>	8.14±0.51 <sup>a</sup>	10.57±0.83 <sup>a</sup>
5 (500 ppb DON)	2.42±0.01 <sup>a</sup>	4.35±0.14 <sup>bc</sup>	6.95±0.32 <sup>b</sup>	9.25±0.45 <sup>b</sup>	12.03±0.72 <sup>bc</sup>
6 (1,000 ppb DON)	2.41±0.01 <sup>a</sup>	4.44±0.16 <sup>c</sup>	7.23±0.20 <sup>b</sup>	9.70±0.39 <sup>b</sup>	12.75±0.79 <sup>c</sup>
7 (2,000 ppb DON)	2.41±0.01 <sup>a</sup>	4.35±0.16 <sup>bc</sup>	7.09±0.27 <sup>b</sup>	9.48±0.35 <sup>b</sup>	12.07±0.43 <sup>bc</sup>

Values followed by the same letter in a column do not differ significantly at  $p = 0.05$

**Table 3. Weight gain, specific growth rate, feeding rate and percentage survival of black tiger shrimp (*P. monodon*) fed with diets containing various levels of mycotoxins for 8 weeks. Results are shown as mean with standard deviation.**

Diet	Percent weight gain	Specific growth rate (% per day)	Feeding rate (% per day)	Percentage survival
1 (control)	363.47±43.18 <sup>bc</sup>	1.72±0.20 <sup>a</sup>	4.05±0.35 <sup>a</sup>	96.67±5.58 <sup>a</sup>
2 (100 ppb OTA)	371.77±58.98 <sup>bc</sup>	1.62±0.21 <sup>ab</sup>	3.78±0.33 <sup>ab</sup>	97.78±5.44 <sup>a</sup>
3 (200 ppb OTA)	370.13±34.45 <sup>bc</sup>	1.62±0.10 <sup>ab</sup>	3.75±0.13 <sup>ab</sup>	100.00±0.00 <sup>a</sup>
4 (1,000 ppb OTA)	334.89±35.97 <sup>c</sup>	1.78±0.15 <sup>a</sup>	4.02±0.19 <sup>a</sup>	97.78±3.44 <sup>a</sup>
5 (500 ppb DON)	395.10±35.39 <sup>ab</sup>	1.51±0.15 <sup>b</sup>	3.59±0.28 <sup>b</sup>	98.89±2.72 <sup>a</sup>
6 (1,000 ppb DON)	427.76±37.45 <sup>a</sup>	1.44±0.10 <sup>b</sup>	3.48±0.13 <sup>b</sup>	100.00±0.00 <sup>a</sup>
7 (2,000 ppb DON)	398.75±17.55 <sup>ab</sup>	1.47±0.04 <sup>b</sup>	3.50±0.04 <sup>b</sup>	100.00±0.00 <sup>a</sup>

Values followed by the same letter in a column do not differ significantly at p = 0.05

**Table 4. Immunological parameters of black tiger shrimp (*P. monodon*) fed with diets containing various levels of mycotoxins for 8 weeks. Results are shown as mean with standard deviation.**

Diet	THC (x10 <sup>7</sup> cells ml <sup>-1</sup> )	PO activity (unit)
1 (control)	7.20±2.34 <sup>a</sup>	377.72±157.10 <sup>a</sup>
2 (100 ppb OTA)	6.65±1.71 <sup>a</sup>	324.65±102.83 <sup>a</sup>
3 (200 ppb OTA)	8.03±2.16 <sup>a</sup>	345.04±108.45 <sup>a</sup>
4 (1,000 ppb OTA)	7.73±2.67 <sup>a</sup>	218.74± 87.88 <sup>b</sup>
5 (500 ppb DON)	7.90±2.08 <sup>a</sup>	305.06±111.30 <sup>a</sup>
6 (1,000 ppb DON)	6.56±2.43 <sup>a</sup>	306.90±124.26 <sup>a</sup>
7 (2,000 ppb DON)	6.26±1.60 <sup>a</sup>	342.74±114.62 <sup>a</sup>

Values followed by the same letter in a column do not differ significantly at p = 0.05

**Table 5. Haemolymph parameters of black tiger shrimp (*P. monodon*) fed with diets containing various levels of mycotoxins for 8 weeks. Results are shown as mean with standard deviation.**

Diet	ALP (U/L)	SGOT (U/L)	SGPT (U/L)	Ca (mg %)
1 (control)	26.89±14.08 <sup>a</sup>	708.40±113.17 <sup>a</sup>	399.10±54.53 <sup>ab</sup>	58.90±2.08 <sup>a</sup>
2 (100 ppb OTA)	18.64±13.28 <sup>b</sup>	511.73±55.49 <sup>cd</sup>	314.18±43.68 <sup>c</sup>	63.09±4.32 <sup>a</sup>
3 (200 ppb OTA)	12.40±6.13 <sup>bc</sup>	567.30±91.11 <sup>bc</sup>	353.30±66.12 <sup>bc</sup>	62.00±4.57 <sup>a</sup>
4 (1,000 ppb OTA)	13.60±5.89 <sup>b</sup>	648.80±130.75 <sup>ab</sup>	406.80±79.58 <sup>ab</sup>	63.00±4.37 <sup>a</sup>
5 (500 ppb DON)	14.00±8.43 <sup>b</sup>	650.60±145.81 <sup>ab</sup>	420.10±93.63 <sup>a</sup>	61.50±6.70 <sup>a</sup>
6 (1,000 ppb DON)	4.27±2.15 <sup>c</sup>	457.91±99.38 <sup>de</sup>	300.27±62.12 <sup>cd</sup>	59.73±4.22 <sup>a</sup>
7 (2,000 ppb DON)	11.20±5.25 <sup>bc</sup>	401.20±46.40 <sup>e</sup>	244.60±33.12 <sup>d</sup>	58.60±3.69 <sup>a</sup>

Values followed by the same letter in a column do not differ significantly at p = 0.05

**Table 6. Mycotoxin residues in whole shrimp after 8-week feeding trial.**

Diet	OTA (mg/kg)	DON (mg/kg)
1 (control)	< 4.4*	< 50*
2 (100 ppb OTA)	< 4.4*	< 50*
3 (200 ppb OTA)	< 4.4*	< 50*
4 (1,000 ppb OTA)	< 4.4*	< 50*
5 (500 ppb DON)	< 4.4*	< 50*
6 (1,000 ppb DON)	< 4.4*	< 50*
7 (2,000 ppb DON)	< 4.4*	< 50*

\* Minimum detection level of an automated analyzer.

hepatopancreas, lymphoid organ, haematopoietic tissues, nervous tissues or antennal gland in shrimp fed OTA or DON. These results were in correlation with non-detectable level of these mycotoxin residues in whole shrimp carcass (Table 6).

### Discussion

The worldwide contamination of foods and feeds with mycotoxins is a significant problem of great agro-economic importance. The economic impact of mycotoxins includes toxic effects on humans and other animals with resultant increased health care, veterinary care costs and reduced livestock production (Hussein and Brasel, 2001).

It has been reported that some dietary mycotoxins, e.g. aflatoxin B<sub>1</sub> affect growth performance, survival and immune systems of black tiger shrimp. In addition, aflatoxins can cause histological changes in the hepatopancreas of black tiger shrimp (*P. monodon*) (Boonyaratpalin *et al.*, 2001) and Pacific white shrimp (*P. vannamei*) (Ostrowski-Meissner *et al.*, 1995).

In the present study, slight reduction in body weight gain was observed after 8 weeks for shrimp fed diets containing 1,000 ppb OTA. In contrast, shrimps fed DON feed diet with 1,000 ppb showed significantly higher growth performance, compared to the control. Hamilton *et al.* (1985) described increased feed intake and weight gain of White Leghorn and broiler chickens, fed for 28 days, with diets containing DON up to 4.1

mg/kg, as tolerance of the animals to a high toxin level. After the 8-week feeding trial, both THC and PO activity of experimental shrimp were not affected by OTA or DON, although the elimination of toxins and other foreign substances in crustaceans was reported as the role of haemocytes as well as proPO activating system (Söderhäll and Cerenius, 1992).

It has been reported that aflatoxin B<sub>1</sub> causes atrophy of hepatopancreatic tubules, necrosis and infiltration of fibroblastic tissue between the hepatopancreatic tubules. Also R-cells became smaller in size and haemocyte infiltration in hepatopancreatic tissues of black tiger shrimp (Lavilla-Pitogo *et al.*, 1994; Boonyaratpalin *et al.*, 2001; Bintvihok *et al.*, 2004). In this experiment, shrimp fed OTA and DON diet developed no histopathological change. However, there is some indirect evidence of impairment of mycotoxins to the hepatopancreas; the activity of ALP which is a hepatopancreatic enzyme that functions in detoxification tended to be decreased. Additionally, it was also found that SGOT and SGPT which are hepatic enzymes that function in breaking down of amino acids (Stryer, 1988) tended to decrease in shrimps fed OTA and DON. These observations indicate that hepatopancreatic tissue of shrimp is sensitive to dietary OTA and DON, though no histopathological lesion of the tissue was found.

At the completion of the feeding trial, OTA and DON residue was not detected in shrimp cephalothorax or musculature suggesting that feed

contaminated with OTA or DON poses a very low risk to shrimp production. A value of 10 mg DON/kg feed has been set for feeds offered to cattle and chickens, for pigs the advisory level is set at 5 mg/kg (Trucksess *et al.*, 1995). Though contamination of mycotoxins can occur, a number of options are available for limiting adverse effects in livestock, i.e. proper storage of raw materials for feed production. Additionally, DON levels have been reported to be reduced by as much as 75% during milling and other forms of physical treatment (Charmley and Prelusky, 1994). These indicate low likelihood of contamination with high-level mycotoxins in shrimp feed and it can be concluded that OTA and DON have no negative impact to shrimp culture industry.

In summary, the present study provides the first evidence of shrimp feed contaminated with OTA and DON causes very low risk to shrimp health. It is suggested that levels of OTA and DON in shrimp feed should not exceed 1,000 and 2,000 ppb respectively.

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