

# CHAPTER I

## INTRODUCTION

### 1.1 Background and rationale of the study

Aquaculture has been developed from extensive to intensive system of which over 60% of operating cost is feeding cost (FAO, 2014). In the present, the trend of using plant as feed ingredient, especially plant protein source, has continuously increased to reduce the feeding cost resulting from rising price of fishmeal. The feed formulation of herbivorous fish (e.g. tilapia, grass carp, common carp and crucian carp) can be included high percentage of plant protein source (approximately 34-51% of total feed ingredient in aquafeed) such as soybean meal, corn gluten meal, wheat gluten meal, soybean concentrate etc. (Rana et al., 2009; Tacon et al., 2009). Nevertheless, the higher inclusion of plant ingredients in fish feed is increased, the greater risk of contamination of mycotoxins (toxic metabolites produced by fungi) in fish feed becomes. The mycotoxin-producing fungi are commonly found in agricultural commodities used as animal feedstuffs (CAST, 2003; D'Mello and Macdonald, 1997; Leung et al., 2006; Pietsch et al., 2013; Sudakin, 2003; Whitlow et al., 2010). Contaminated plant ingredients can cause impair health and growth performance of fish leading to significant economic losses. Though the precise estimation of worldwide economic costs involved mycotoxin contamination is not clearly mentioned, potentially economic costs in the United State were approximately \$932 and \$6 million/year for crop and livestock losses, respectively (CAST, 2003; Wu, 2007).

Agricultural commodities are commonly contaminated with many mycotoxins produced mainly by fungus among several genera, e.g. *Aspergillus*, *Penicillium* and *Fusarium* if improper stored. Although these fungi normally produce hundred mycotoxins, the significant mycotoxins which are potentially cause adverse impacts on fish health and growth performance include aflatoxin B1 (AFB<sub>1</sub>), ochratoxin A (OTA), trichothecenes (e.g. deoxynivalenol: DON and T-2 toxin), zearalenone (ZON) and fumonisin B1 (FB<sub>1</sub>) (Hussein and Brasel, 2001; Richard, 2007).

The occurrence of *Fusarium* mycotoxins, particularly deoxynivalenol and zearalenone, is common in feedstuff worldwide (Morgavi and Riley, 2007). A survey of worldwide prevalence of mycotoxins in 3,349 samples (e.g. cereal grain and finished feed) in 2012 demonstrated quite a high percentage of mycotoxin prevalence like aflatoxin (Afla, 25%), ZON (46%), DON (64%), fumonisin (FUM, 56%) and OTA (31%) (BIOMIN's Mycotoxins Report, 2012). The average concentrations of each *Fusarium* mycotoxins detected in samples were ranged from 0.01 to 0.4 ppm ZON; 0.1 to 2.8 ppm DON and 0.3 to 0.8 ppm FUM, respectively (BIOMIN's Mycotoxins Report, 2012). The mycotoxin survey program from 2009 to 2011 indicates DON is the most common detectable mycotoxin found in agricultural commodities (BIOMIN's Mycotoxins Report, 2011; Rodrigues and Nährer, 2012). Interestingly, the samples of commercial fish feed which were designed for cyprinids collected from the central Europe. Average concentrations of DON and ZON detected in samples were 0.23 ppm DON and 0.07 ppm ZON, respectively; and the maximal concentration of a sample was 0.83 ppm DON (Pietsch et al., 2013).

Deoxynivalenol (DON) is a trichothecene mycotoxin, which are mainly produced by *Fusarium graminearum* and *F.culmorum*, frequently occurs in wheat, corn, soybean meal, barley, rice bran and other feed ingredients (Binder et al., 2007; Rodrigues and Nährer, 2011). DON is a potent inhibitor of protein synthesis at the cellular level reducing protein synthesis concerned with poor growth performance (Dänicke et al., 2006; Feinberg and McLaughlin, 1989; Pestka et al., 2004). The unavoidable consumption of feeds containing DON by farmed animals decreases their health and growth performance. Noticeable symptoms from consumption of high DON concentrations are vomiting, diarrhea and feed refusal. Impacts of exposure to low concentration of DON include the decreases in growth rate and feed intake, histopathological alteration in organs and either immunosuppression or immunostimulation (Hooft et al., 2011; Pestka, 2007; Pestka and Smolinski, 2005; Tiemann et al., 2006; Ueno, 1984; Woodward et al., 1983)

Several studies have stated that DON affects farmed animals and aquatic species (Bergsjø et al., 1993; Dillenburger et al., 2001; Eriksen and Pettersson, 2004; Forsyth et al., 1977; Hooft et al., 2011; Pestka, 2007; Pinton et al., 2008; Prelusky et al., 1994; Trigo-Stockli et al., 2000; Young et al., 1983). Nevertheless, the effects

of DON on animals vary depending on numerous factors, including differences in nutritional and health status prior to exposure, differences in environmental conditions and differences in the source of DON (Rotter, 1996). Susceptibility to DON among terrestrial animal species ranged from the most sensitive to less sensitive to DON as pigs > mice > rats > poultry  $\approx$  ruminants was reported by (Rotter, 1996). Pigs are considered as the most sensitive animal species to DON as low as 1 to 2 ppm. The decrease of growth rate and feed intake are affected when oral exposure to DON at these concentrations. Furthermore, pigs exhibited feed refusal and vomiting when receiving high concentrations of DON up to 12 and 20 ppm, respectively (Forsyth et al., 1977; Young et al., 1983). On the other hand, the growth performance of poultry and ruminants were not significantly affected by consumption of dietary DON at greater concentration than 6 ppm (Charmley et al., 1993; Ingalls, 1996; Trenholm et al., 1985).

In fish, only a few studies have reported adverse impacts of dietary DON on commercial fish, including rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) (Hooft et al., 2011; Manning, 2005; Manning et al., 2013; Woodward et al., 1983). Rainbow trout (initial body weight = 50 g/fish) fed corn artificially contaminated with graded concentrations of 1 to 12.9 ppm DON for eight weeks caused the significant reductions in weight gain, feed conversion efficiency (FCE) and feed intake (Woodward et al., 1983). Moreover, rainbow trout showed a complete feed refusal when fed the high concentration of dietary DON at 20 ppm (Woodward et al., 1983). The other study on the effects of dietary DON on rainbow trout was conducted by Hooft et al. (2011) confirming the adverse impacts of DON originating from corn naturally contaminated with *Fusarium* mycotoxins on the health performance by using diets containing graded concentrations of DON at 0.3, 0.8, 1.4, 2.0 and 2.6 ppm for feeding rainbow trout (initial body weight = 24 g/fish) for eight weeks. The results of 8-week feeding trial showed significant linear or quadratic decreases in growth rate, weight gain, feed intake, feed conversion efficiency, retained nitrogen, recovered energy, energy retention efficiency, nitrogen retention efficiency and protein content of carcass, but apparent digestibility coefficients of crude protein and gross energy were not observed. Moreover, fish fed with dietary DON at 2.6 ppm exhibited some histopathological alterations of liver, such as fatty

infiltration and, pyknosis and karyolysis in hepatocytes (Hooft et al., 2011). Whereas, Manning (2005) and Manning et al. (2013) reported that channel catfish were much more resistant to dietary DON than rainbow trout. The reduction in growth or feed consumption of catfish (initial body weight = 5.0 g/fish) were not caused by feeding diets containing with DON up to 10.0 ppm for six weeks; however, growth rate and feed conversion efficiency became poorer when catfish fed with dietary concentrations of 15.0 and 17.5 ppm DON (Manning, 2005). Besides, Manning et al. (2013) reported that feeding of diets containing DON (0, 2.5, 5.0, 7.5 and 10 ppm) from naturally contaminated wheat for seven weeks did not affect weigh gain, feed intake or survival rate of fingerlings channel catfish (initial weigh = 5.9 g/fish). Obviously, among two fish species have different response to DON exposure.

Farmed animals and fish (e.g. pigs and rainbow trout) appear to have different sensitivities to dietary DON among. Additionally, detectable concentrations of DON in animal feeds that affect some animal species are higher than maximal concentrations (0.5 ppm) of DON in guideline value, set by European Commission (Commission Recommendation, 2006). Because of different sensitivities among farmed animals and fish, it is therefore necessary to investigate impacts of DON on growth performance and health of herbivorous and omnivorous fish species for a clear understanding. Particularly, tilapias which are an herbivorous and/or omnivorous species have a higher risk to expose to diets contaminated with *Fusarium* mycotoxins, especially DON, than carnivorous fishes due to the consequence of high inclusion of plant ingredients in dietary formulation. Tilapias are able to derive protein from both animal and plant resources; consequently, large portions of plant ingredients can be included in fish feed for tilapias. Tilapia became a candidate for aquaculture because of their fast growth, tolerance to a wide range of environmental conditions, resistance to stress and disease, ability to reproduce in short generation time and acceptance of artificial feeds (De Silva, 2004; El-Sayed, 2006). Tilapias and other cichlids are as the second most important farmed fish species in the world, surpassed only by carps. World aquaculture productions of tilapia and other cichlids has increased from 1.4 to 4.5 million tone between 2002 and 2012 with a sale value of over \$7 billion (FAO, 2012). Owing to aforementioned reasons, the red tilapia (*O. niloticus* × *O. mossambicus*) was used as an experimental animal in the present study.

The objectives of the present study were: to examine effects of experimental diets containing DON from naturally contaminated wheat on growth performance and health of red tilapia (*O. niloticus* × *O. mossambicus*).

## **1.2 The scope and limitation of the study**

The experiment was conducted to investigate the adverse impacts of dietary wheat naturally contaminated with DON on growth performance, selected health indices of juvenile red tilapia (*O. niloticus* × *O. mossambicus*). Fish were fed with experimental diets contained graded concentrations of DON for eight weeks under an aquaculture laboratory condition.

## **1.3 Experimental hypothesis**

Feeding of graded concentrations of dietary DON from wheat naturally contaminated with *Fusarium* mycotoxins to juvenile red tilapia (*O. niloticus* × *O. mossambicus*) caused adverse effects on the growth rate and health.

## **1.4 Objectives of the study**

The specific objectives of this study were as follows:

1.4.1 To examine the effects of experimental diets on growth performance of red tilapia (*O. niloticus* × *O. mossambicus*), namely body weight gain, growth rate (measured as thermal-unit growth coefficient, TGC), feed intake, feed conversion efficiency and mortality.

1.4.2 To examine the health of red tilapia affected by feeding of dietary DON from naturally contaminated wheat on hepatosomatic index (HSI), haematocrit (Hct), selected hepatic enzymes, namely plasma aspartate aminotransferase (AST) and plasma alanine aminotransferase (ALT).

1.4.3 To investigate whether the histopathological changes in organs, namely liver, spleen, kidney and intestines were associated with feeding of dietary DON from naturally contaminated wheat.