# CHAPTER V
# DISCUSSION AND CONCLUSION

## 5.1 Chemical composition

All experimental diets were formulated to be isointrogenous and isoenergetic according to (National Research Council, 2011). However, dietary protein level in experimental diets of the present study was below the protein requirement suggested by NRC (2011). Growth rate (TGC = 0.108) and feed conversion efficiency (FCE = 0.71 gain/feed) of fish fed control diet (Diet 1) was in normal ranges (Bowen, 1987; National Research Council, 2011). Although the level of crude protein used in this study was below recommended levels according to NRC (2011), most of the growth indices were in the optimum range suggested that nutrients provided in this experiment were sufficient.

## 5.2 Determination of mycotoxins in five experimental diets using HPLC and LC-MS/MS

In order to formulate graded DON concentrations of five experimental diets for feeding red tilapia, wheat naturally contaminated with *Fusarium* mycotoxins (41 ppm DON) as the source of DON were replaced with graded portions of clean wheat (uncontaminated wheat) in Diet 2, 3, 4, and 5 as 1.2, 2.5, 5.0 and 10.0 g per 100 g of diet, respectively.

According to feeding formulation used in this study, five experimental diets were found to be mainly contaminated with *Fusarium* mycotoxins, especially DON, analyzed by both HPLC and LC-MS/MS techniques. Similarly, the recent surveys of worldwide contamination of feedstuffs and finished feed with mycotoxins conducted by Rodrigues and Nährer (2012a) and Pietsch et al. (2013) which *Fusarium* mycotoxins, especially DON, ZON and FUM, were found to contaminate in most samples. Rodrigues and Nährer (2012b) reported that among the most well-known mycotoxins, namely Afla, DON, ZON, FUM and OTA, which were the most commonly occurring mycotoxins analyzed by ELISA and HPLC, were *Fusarium*

mycotoxins: DON with 65% of positive samples (1,695 samples) tested by ELISA, followed by FUM, Afla, ZON and OTA with 48%, 24%, 21% and 17% of positive samples, respectively. Whereas, results of mycotoxins analyzed by HPLC showed that FUM (56%) and DON (50%) were greater percentage of positive samples (4,363 samples) than other mycotoxins, including ZON (44%), Afla (31%), and OTA (27%). Besides, determination of *Fusarium* toxins in commercial fish feed (Pietsch et al., 2013) revealed that a total of 11 fish feed samples collected from central Europe contained DON as more than 80% of positive samples tested by HPLC; meanwhile, ZON was detected in all samples by HPLC. In terms of types of samples, *Fusarium* mycotoxins, such as DON, ZON and FUM, were notably contaminated in all cereal grain as animal feedstuffs. Most of the cereal grains as animal feedstuffs, in particular, contaminated with DON were: soybean meal, wheat, DDGS, straw, barley and other feed ingredients at average concentrations of 0.06, 1.4, 2.9, 0.9, 1.7 and 0.2 ppm DON. Interestingly, the highest concentrations of DON in this survey analyzed by HPLC were obtained from wheat and barley with 49 and 14 ppm, respectively (Rodrigues & Nährer, 2012b). Similarly, the concentration of DON in wheat used as the ingredient of experimental diets (Diet 2 to 5) in the present study was found to contain with 41 ppm DON analyzed by HPLC. Furthermore, graded concentration of dietary DON (ranging from 0.07 to 1.15 ppm) originated from the replacement of clean wheat with contaminated wheat (41 ppm DON) appeared to be in the practical range of analyzed DON concentrations (average concentration was 0.3 ppm DON; maximal concentration was 0.8 ppm DON) in commercial fish feed reported by Pietsch et al. (2013). Nevertheless, the concentrations of dietary DON and ZON used in the present study do not exceed the guideline value for complete finished feed (5 ppm DON and 0.5 ppm ZON) regulated by Commission Recommendation (2006).

The co-occurrence of fungal metabolites in feed ingredients and finished feeds were reported in many studies (Binder et al., 2007; Pietsch et al., 2013; Placinta et al., 1999; Rodrigues and Nährer, 2012b). Some studies recently reported the occurrence of non-regulated metabolites (e.g. siccanol, aurofusarin, 15-hydroxyculmorin and culmorin) in feed ingredients and feeds; unfortunately, the further information of those metabolites are not available (Abia et al., 2013; Schwartz et al., 2011; Streit et al., 2013; Sulyok et al., 2010).

Likewise, mycotoxins analysis by LC-MS/MS in the present study did not detected only regulated metabolites but also non-regulated metabolites contaminating in most diets. *Fusarium* metabolites were the most frequently occurring metabolites in the total number of 50 detected metabolites in experimental diets. Although there were 50 detected metabolites in experimental diets, only six fungal metabolites found to be graded concentrations in Diet 1 to 5 based on of graded proportion of the clean wheat replaced with contaminated wheat in the dietary formulation. Surprisingly, some metabolites unexpectedly contaminated in experimental diets of the present study with high concentrations of aurofusarin, 15-hydroxyculmorin and culmorin. Detected concentrations of those metabolites in Diet 1 to Diet 5 ranged from 0.01 to 2.46 ppm aurofusarin, 0.06 to 1.83 ppm 15-hydroxyculmorin and 0.20 to 1.39 ppm culmorin. Likewise, Abia et al. (2013) and Streit et al. (2013) reported that non-regulated metabolites (e.g. *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* metabolites) were found at very low concentrations in foodstuffs, such as nuts, maize and soybean.

Comparison of analytical methods: numerous existing analytical methods have been used for screening mycotoxins in different types of sample; that was reviewed in Turner et al. (2009) and Krska and Molinelli (2009). It is impossible to use only one standard for mycotoxin analysis owing to the variety of chemical structure of metabolites (Turner et al., 2009) and each of analytic methods has limitation of detection. Those methods are: liquid‑liquid extraction (LLE), supercritical fluid extraction (SFE), solid phase extraction (SPE), thin layer chromatography (TLC), HPLC, gas chromatography (GC), and capillary electrophoresis (CE) and ELISA. The HPLC method tends to be popular as a routinely practical analysis for mycotoxin determination including surveys of worldwide contamination of mycotoxins in food and feed (Binder et al., 2007; Rodrigues and Nährer, 2012b; Turner et al., 2009). However, due to limitation of those analytical methods; for instance, (1) analytical methods have to be separated to screen each mycotoxin of interest since each method is specific on each metabolite and matrices such as corn, wheat and barley, (2) it would take time in case of determination of large number of samples, (3) non-regulated metabolites and masked mycotoxins (e.g. DON-Glucoside) cannot be detected by routine analysis due to the lack of standard solution, and (4) those

methods are not sensitive enough to detect a trace of metabolites. Hence, the LC-MS/MS, which is the recently analytical technique, has been developed for screening mycotoxins in various matrices. It is able to detect wider range of different metabolites, such as mycotoxins, non-regulated metabolites and undetectably masked mycotoxins, in various matrices than HPLC (Turner et al., 2009; Berthiller et al., 2006; Syndenham et al., 1996; Sulyok et al., 2010). The causes of differently detectable concentrations of DON using HPLC and LC-MS/MS in the present study could account for differences in method performance characteristics of analyses, including extraction efficiency, limit of detection, available standard solution, precision and trueness of the method (Sulyok et al., 2006; Sulyok et al., 2007, 2010). However, using either HPLC or LC-MS/MS for DON analysis in this study, the results appeared the similar trends of graded concentrations of DON. Besides, some of recent studies have chosen LC-MS/MS as a tool of mycotoxin analysis in cereal grains and feeds (Maul et al., 2012; Streit et al., 2013).

A comparison of concentrations of mycotoxins contaminated in all diet analyzed by HPLC and LC-MS/MS in the present study: two sets of the same five dietary samples were analyzed to screen for types and concentrations of mycotoxins occurring in experimental diets using HPLC and LC-MS/MS. The results of detected mycotoxins analyzed by differently analytic methods showed different concentrations and types of mycotoxins in the same experimental diets. In particular, analyzed DON (ranged from 0.12 to 3.74 ppm) by HPLC were higher than those (ranged from 0.07 to 1.15 ppm DON) by LC-MS/MS. Regression curve between graded concentrations of DON and response of red tilapia shows that the relationship between weight gain and analyzed DON by LC-MS/MS ($R = -0.992$) was greater than those by HPLC ($R = -0.954$). Consequently, detected concentrations of DON by LC-MS/MS were more reasonable to be account for the practical concentrations of DON in the present study.

### 5.3 Growth performance and mortality of red tilapia

Diets contaminated with DON negatively affected growth rate, feed intake and feed conversion efficiency in accord with similar earlier observations on rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) (Woodward et al., 1983; Hooft et al., 2011; Manning, 2005).

In channel catfish, weight gain and feed intake were not affected by graded concentrations of dietary DON neither naturally contaminated sources of wheat and corn, nor purified form of DON ranging from 0 to 10.0 ppm DON (Manning, 2005; Manning et al., 2013); however, fish fed the high concentrations of dietary DON (15.0 and 17.5 ppm DON) naturally contaminated sources of wheat showed the trend of reduction in growth rate and poorer feed conversion efficiency (Manning, 2005). The results demonstrated no toxicity of DON for channel catfish at dietary concentration of 10 ppm DON. Thus, it was concluded that channel catfish is more tolerant to DON exposure than red tilapia in the present study. In cases of rainbow trout observed by Woodward et al. (1983) and Hooft et al. (2011), the responses of rainbow trout fed low dietary concentrations of DON as low as 1 ppm displayed reduction in growth rate, feed intake and feed conversion efficiency. Woodward et al. (1983) and Hooft et al. (2011) reported that reduction in feed intake ($R^2 = 0.98$), weight gain ($R^2 = 0.99$) and feed efficiency ($R^2 = 0.91$), has a significant relationship with increasing dietary concentrations of DON ranging from 1 to 12.9 ppm. It is relative to the finding of Hooft et al. (2011), weight gain and growth rate (TGC) decrease linearly ($P<0.0001$) with increasing dietary concentrations of DON ranging from 0.3 to 2.6 ppm; in addition, feeding of graded concentrations of dietary of DON resulted in a significant linear ($P<0.0001$) and quadratic ($P<0.0125$) reduction in feed intake and feed conversion efficiency of rainbow trout. However, the observed differences in sensitivity of rainbow trout to DON among two studies could be accounted by using different sources of DON in which Woodward et al. (1989) used artificially infected corn and Hooft et al. (2011) used naturally contaminated corn; additionally, the size of fish used in the study of Hooft el at. (2011) was two times smaller (initial body weigh = 23 g/fish) than those (initial body weigh = 50 g/fish) used in the study of Woodward et al. (1983). Hooft et al. (2011) compared the sensitivity, in terms of weight gain, of rainbow trout in which among two studies (Hooft et al., 2011;

Woodward et al., 1983), and pig by adjusting DON intake in terms of metabolic body weight and days, suggesting that rainbow trout in the study of Hooft et al. (2011) was more susceptible to DON than those of Woodward et al. (1983). Anyhow, the finding of Woodward et al. (1983) was about the same as immature pigs in the finding of Smith et al. (1997).

The consequence of using a naturally contaminated ingredient with fungi as mycotoxin source causes greater negative effects than using a purified form of DON because the intake of combined mycotoxins can lead to synergistic or additive effects (Eriksen & Pettersson, 2004; Placinta et al., 1999; Speijers and Speijers, 2004). In the present study, naturally contaminated wheat (41 ppm DON) as feed ingredient to be mixed in experimental diets, which were found to mainly contaminated with graded concentrations of *Fusarium* mycotoxins (e.g. DON and ZON), *Fusarium* metabolites (aurofusarin, rubrofusarin, culmorin and 15-hydroxculmorin), and *Alternaria* metabolites (e.g. alternariol, AOH; alternariol monomethyl ether, AME). In the studies of Hooft et al. (2011) and Woodward et al. (1983), which were reported that the experimental diets were contaminated with the trace amounts of 0.6 and 4 ppm ZON, respectively, in addition to DON. In the present study, Diet 5 (the highest concentration of dietary DON) was contaminated with 0.98 ppm ZON. Based on previously findings of Woodward et al. (1983) and Hooft et al. (2011), it suggested that the amount of ZON, which did not exceed 1 ppm, did not cause any deleterious effect on red tilapia performance in the present study. Certainly, published reports on effect of dietary ZON on pigs appearing the most sensitive animal to ZON are stated that hyperestrogenism in female swine is caused by feeding of dietary concentrations of over 1 ppm ZON (CAST, 2003; Mostrom, 2012; Zinedine et al., 2007).

In terms of *Alternaria* metabolites, these fungal metabolites are not as notable as regulated mycotoxins (e.g. DON, OTA, Afla, FUM and ZON), available information of their toxicity on animals is limited (European Food Safety Authority, 2013; Ostry, 2008). The metabolites have been received attention due to the low naturally occurring contamination concentrations in feed and feed ingredients (Streit et al., 2013). There are over 70 metabolites of *Alternaria* metabolite group, but only some of them are reported to be harmful to animals. Those metabolites include AOH, AME, tenuazonic (TeA) and altertoxins (ATX). Both of the TeA and ATX are stated

be the most acutely toxic in *Alternaria* metabolites; on the other hand, AOH and AME are not very acutely toxic to animals (Ostry, 2008). Griffin and Chu (1983) demonstrated that an injection of to 1000 µg AOH/egg, and 500 µg AME/egg, respectively, to 7-day-old chicken embryos were not caused reduced weight gain, mortality or teratogenic effects; whereas TeA exhibited $LD_{50}$ (50% lethal dose) of 548 µg TeA/egg. It is in accord with the finding of Sauer et al. (1978), feeding of diets contaminated with high concentrations of 24 ppm AME and 39 ppm AOH, respectively for 21 days did not significantly reduce weight gain and cause alteration of organ tissues on gross and microscopic examinations (Sauer et al., 1978). To my knowledge, no reports on negative effects of dietary AOH and AME on health and growth performance of fish are available, the trace concentrations of AOH (0.12 ppm in Diet 5) and AME (0.15 ppm in Diet 5) in the present study, which were over 160 times lower than those of AOH and AME in the study of Griffin et al. (1983) and Sauer et al. (1978). It suggested that the highest concentrations of AOH (0.12 ppm) and AME (0.15 ppm) in the present study did not cause any deleterious effects on red tilapia performance.

Some *Fusarium* metabolites, culmorin, 15-hydroxyculmorin, aurofusarin and rubrofusarin, regarded as less well-known metabolites were detected in experimental diets of the present study. The concentrations of individual metabolites in naturally occurring fungus grains are normally at trace concentrations (Streit et al., 2013). Culmorin and 15-hydroxyculmorin are often found to simultaneously occur with trichothecene mycotoxins, in particularly DON. Ghebremeskel and Lengseth (2000) reported that there was a strong correlation between the amount of DON occurrence in grains (e.g. wheat, oat and barley) and the amount of culmorin and 15-hydroxyculmorin occurrence. The ratio between the total amount of DON and culmorin compounds ranged from 0.14 to 1.07 ppm in the samples. This is similar to the present study, the detected ranges of DON and culmorin compounds in Diet 5 (the highest concentrations of fungal metabolites) were 1.15 ppm DON, 1.39 ppm culmorin, 1.83 ppm 15-hydroxyculmorin, respectively. Thus, the inclusion of plant ingredients naturally contaminated with *Fusarium* mycotoxins in fish feed does not only have unawares risk to DON contamination but also contamination of culmorin compounds in fish feed. There is very little published information on effect of

culmorin and 15-hydroxyculmorin in animals; the only reported study on the toxicity of culmorin in animals was published by Rotter et al. (1992). Pigs (initial body weight = 22.8 kg/pig) were fed on either three diets contained 6 ppm DON, 2 ppm culmurin or dietary combination of 6 ppm DON and 2 mg culmorin for 21 days. The results showed that only pigs fed diet contained 6 ppm DON significantly had the reduced weight gain and feed intake. The adverse effects of red tilapia performance observed in present study were not associated with feeding of dietary culmorin or interaction between DON and culmorin in diets (Rotter et al., 1992).

In case of 15-hydroxyculm, only few reports were published by Ghebremeskel and Langseth (2001) and Langseth (1998). The 15-hydroxyculmis simultaneously presents with other *Fusarium* mycotoxins (e.g. DON) and metabolite (e.g. culmorin) in wheat, oat and barley samples. (Ghebremeskel and Langseth, 2001) showed a strong correlation between the amount of DON and 15-hydroxyculm occurrence in grain samples; concentration presents of culmorin and 15-hydroxyculm were similar to DON concentrations in naturally contamination grains. However, information of 15-hydroxyculm toxicity is still lacking. Only in vitro study (MTT-cell culture) conducted by Langseth (1998), crude extraction of *F.culmorum* isolated from rice showed significantly toxicity in swine kidney cells. The types of major metabolites in rice sample extraction contaminated with *F.culmorum* strain analyzed for qualification included DON, 3-acetyl-DON, culmorin and 15-hydroxyculm.

In the present study, aurofusarin detection was ranged from 0.01 to 2.46 ppm aurofusarin in Diet 1 to Diet 5, respectively. Aurofusarin is the *Fusarium* metabolite produced by *F.graminearum*. Little information on aurofusarin toxicity is available, the only published studies reported by Dvorska and Surai (2001). Feeding of diet contaminated 26.4 ppm aurofusarin from *F.graminearum* cultured material for eight weeks significantly changed fatty acid profile resulting in decreasing in nutritional quality of Japanese quails (*Coturnix japonica*) egg yolk (Dvorska et al., 2001). The high concentrations of aurofusarin (24.6 ppm) used in the study of Dvorska and Surai (2001) were over ten times greater than the highest concentrations of aurofusarin (2.46 ppm) detected in present study.

Even if the strong evidence of DON toxicity in rainbow trout and pigs as low as 1 ppm DON (Forsyth et al., 1977; Hooft et al., 2011; Rotter, 1996; Smith et al.,

1997; Woodward et al., 1983; Young et al., 1983), it is still unclear to conclude that the poor performance of red tilapia observed in the present study were caused by DON contaminated in experimental diets. The relationship between dietary concentrations of mycotoxins/metabolites, such as DON, rubrofusarin and 15-hydroxyculmorin and performance parameters, such as body weight gain, TGC, feed conversion efficiency and feed intake of red tilapia is shown in Figure 24. The results showed that the depression of performance parameters of red tilapia was related to increasing concentrations of DON, rubrofusarin and 15-hydroxyculmorin in experimental diets. To the best of my knowledge, the results of the present study indicate that the poor performance of red tilapia may be caused by DON alone or the possible interaction between of DON and *Fusarium* metabolites, namely 15-hydroxyculmorin and rubrofusarin. This remains to be investigated.

There are evidences demonstrated that intake of combined mycotoxins would lead to be a possible higher risk for having detrimental impact on animals than intake of a single mycotoxin (D'Mello and Macdonald, 1997; Harvey et al., 1996; Harvey et al., 1995; Kubena, Edrington, Harvey, Buckley et al., 1997; Kubena, Edrington, Harvey, Phillips et al., 1997).

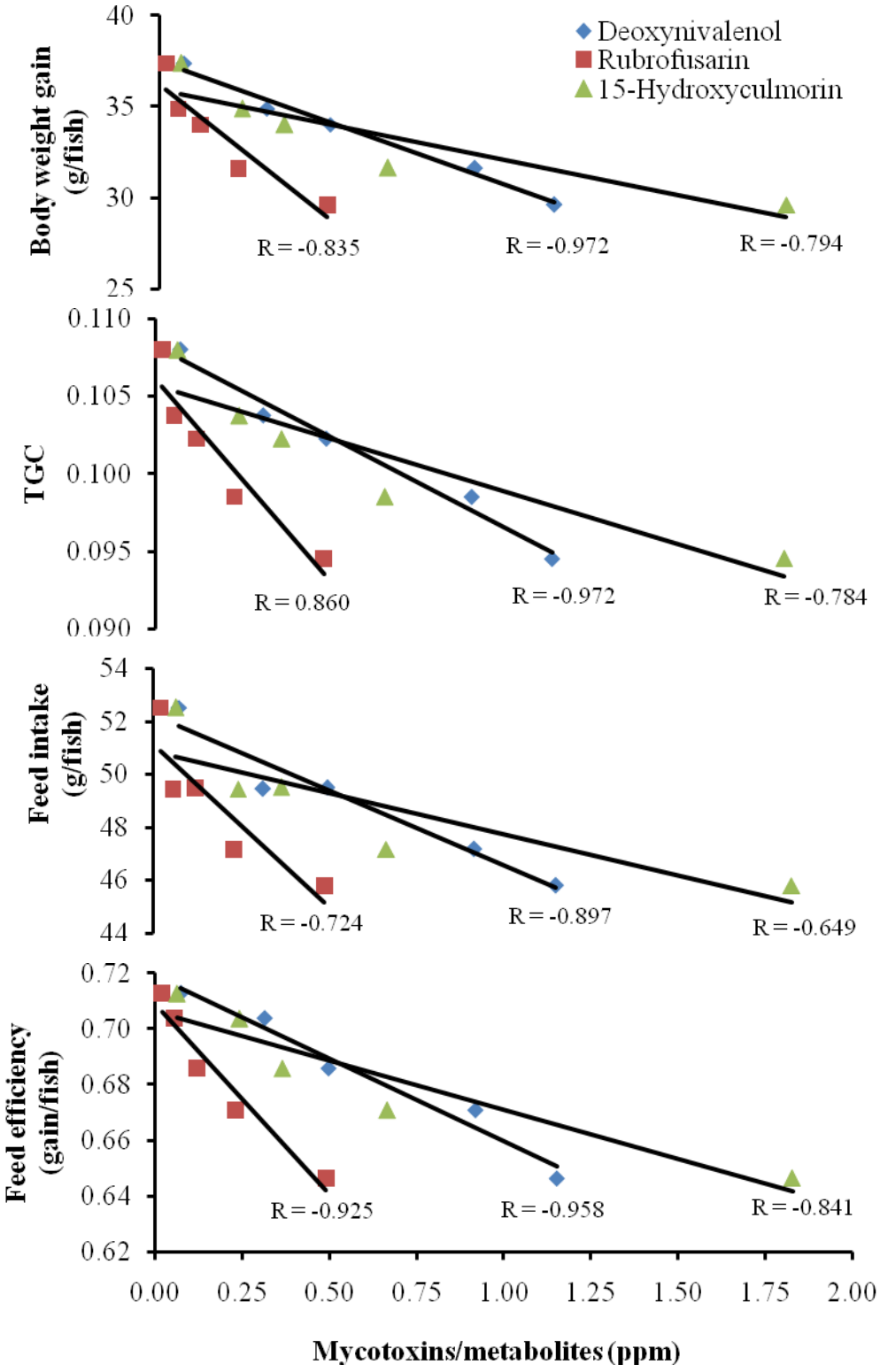


**Figure 24** Relationship between the amount of mycotoxin/metabolite present in five dietary samples and parameters (weight gain, feed intake, TGC and feed efficiency) of red tilapia performance

The differences in response varied among species may be explained by differences in absorption, distribution, metabolism and elimination of DON (Rotter, 1996). Poultry and cattle are tolerant to DON because of a low degree of absorption and their abilities of intestinal microorganisms to convert DON to DOM-1 which is finally rapidly eliminated through similar portions of urine and faces (He et al., 1992; King et al., 1984; Swanson et al., 1987). Conversely, intestinal microorganisms of pigs have a poor ability to detoxify DON and pigs have a rapidly systemic absorption of DON into blood (Dänicke et al., 2004; He et al., 1992)

Information on DON metabolism in fish is limited. Microorganisms from guts of fish play important roles in the transformation of DON to DOM-1 as non-toxic metabolite via deepoxidation and deacetylation. Microbes from guts of Brown bullhead catfish (*Ameiurus nebulosus*) were more capable to transform DON to DOM-1 than brown trout (*Salmo trutta*), pink salmon (*Oncorhynchus gorbuscha*) and other fishes (Guan et al. 2009). Besides, hepatic metabolism of DON studied in vitro by Maul et al. (2012) and Pietsch et al. (2011) revealed that fish hepatic microsomes are capable to transform DON to DON-3-glucuronide (less toxic metabolite). Among fish species rainbow trout appeared to be the most sensitive fish species to DON may be associated with the poor detoxification activity of DON to DOM-1 by microorganisms in guts and poor ability of hepatic microsomes to transform DON to DON-3-glucuronide (Guan et al., 2009; Maul et al., 2012; Pietsch et al., 2011). On the other hand, based on previous studies, channel catfish is regarded as the most tolerant fish species related to the ability of microorganisms in guts to convert DON to non-toxic metabolite (Manning, 2005; Manning et al., 2013; Guan et al., 2009). Food intake and weight gain were more sensitive to dietary DON in red tilapia than channel catfish, possibly reflecting differences in its metabolism.

The significant reduction in feed intake of red tilapia observed in the present study is in agreement with fish and pigs. The reduction of feed intake caused by *Fusarium* mycotoxins e.g., DON, T-2 toxin, fusaric acid and fumunisin B1, could be due to (1) the irritation of DON and co-occuring mycotoxins in gastrointestinal tract (Arnold et al., 1986; Rotter et al., 1994), and (2) the inhibition of gastric emptying (Fioramonti et al., 1993) and (3) disturbance of brain neurotransmitter, namely as serotonin (5-hydroxytryptamine, 5-HT) (Fitzpatrick et al., 1988; Swamy et al., 2002).

The elevation of brain serotonin, which is derived from brain tryptophan, is linked to behavioral changes such as loss of appetite, vomiting, emesis (Fioramonti et al., 1993; Smith, 1992). The inhibition of hepatic protein synthesis caused by *Fusarium* mycotoxins was related to aminoacidemia causing increasing blood and brain tryptophan resulting in increasing rate of serotonin synthesis in brain (Leathwood, 1987; Smith, 1992).

Poor performance of fish associated with feeding diets naturally contaminated with *Fusaium* mycotoxins observed in the present study is similar to previous studies on effects of *Fusarium* mycotoxins reported by Woodward et al. (1983) and Young et al. (1983), which appeared to be related to reduction of feed intake. Nevertheless, Hooft et al. (2011) showed rainbow trout pair-fed the control diet (0.3 ppm DON) had significantly higher growth rate, feed conversion efficiency, and whole body crude protein level than their counterparts fed the highest concentration of 2.6 ppm DON. This finding suggested effect of dietary DON on performance of rainbow trout was not simply related to a reduction of feed intake but also negative effects on nutrient metabolism (Hooft et al., 2011). Agreeably, Lun et al. (1985) reported the depression of pig performance may be resulted of disturbance of minerals absorption and (or) metabolism by feeding of dietary DON.

DON is considered not to be acute toxic to farmed animals; besides, it is well-known as the least toxic compound in trichothecene mycotoxins. DON is approximately 10 times less toxic than T-2 toxin (Rotter, 1996; Ueno, 1984). Manning et al. (2013) revealed that mortality of channel catfish fed diets containing high concentrations of 10 ppm DON did not appear during the 21-day trial. Conversely, feeding of diets containing dietary concentration of 5.0, 7.5 and 10.0 ppm DON improved survival rate of channel catfish when challenged with bacteria pathogen *Edwardsiella ictaluri* in the 21-day post-challenge trial. The author explained that the improved survival rate of catfish from infection with *E. ictaluri* in this study was due to presence of DON in diets (Manning et al., 2013). Notably, the severest symptoms of acute toxicity resulted from oral exposure to high concentrations of DON reported in pigs and rainbow trout include diarrhea, feed refusal and vomiting at proximately 12 and 20 ppm DON (Forsyth et al., 1977; Woodward et al., 1983; Young et al., 1983).

Though the mortality of red tilapia is statistically different among dietary treatments, it is not clear evidently that this observed mortality was caused by toxicity of DON contaminated in diets. Because the highest concentrations of DON (1.15 ppm DON, Diet 5) in the present study was at least 10 times lower than concentrations of acute toxicity of 12 ppm DON and 20 ppm DON that have been reported in previously published studies by Young et al. (1983) and Woodward et al. (1983), respectively. None of the previous researches reports negative impact of DON on mortality of fish, namely channel catfish and rainbow trout (Matejova et al., 2014; Hooft et al., 2011; Manning, 2005; Woodward et al., 1983). Furthermore, since red tilapia as the experimental animal were reared in indoor circulating water system, the laboratory conditions (e.g. water quality and management of disease infection) were properly and efficiently controlled in suitable ranges of requirements for tilapia culture. Nonetheless, considering in terms of survival rate, it was high survival rate ranging between 83% and 96% of total fish number in dietary treatments, the mortality observed in the present study could be thus accounted for a normal mortality of the experimental animal reared under laboratory conditions. Likewise, the normal survival rate of fish culture reported in numerous studies of the tilapia species under laboratory conditions (indoor circulating water system) were in range from 79.7% to 99.7% of total fish number in each experimental treatment (El-Sayed, 2002; Ridha, 2006; Suresh & Lin, 1992). Obviously, the mortality of red tilapia from all treatment groups in the present study at the last week of the experiment was lower than any other previous weeks. Possibly, the longer red tilapia exposed to dietary DON, the better the fish were adaptable. The explanation of normal mortality may possibly be differences in the health status and adaptable response to the laboratory conditions of experimental animals (Rotter, 1996).

### 5.4 Hematological and biochemical effects and HSI of red tilapia

Hematological and blood biochemistry variables are considered as potential indicators for health assessment in fishes (Houston, 1997; Roche and Bogé, 1996). Available information is limited concerning the hematological and biochemical effects of DON exposure in fish. Rizzo et al. (1992) reported that *Fusarium* mycotoxins (e.g. DON and T-2 toxin) induced hemolytic activity of red blood cell in

rat. Nevertheless, detrimental effects of DON on Hct, AST or ALT activities in blood sample collected at the end of experiment were not observed in the present study.

The result of the present study agreed with the finding of Manning (2005) and Matejova et al. (2014) that Hct of channel catfish fed diets containing high concentration of 17.5 ppm DON from naturally contaminated wheat was not affected Manning (2005). Similarly, Hct of one-year-old rainbow trout was not affected by feeding of diet containing 2 ppm DON for 23 days (Matejova et al., 2014). Harmoniously, the study of Pinton et al. (2008), Hct, AST and ALT of pigs (average body weight = 21 kg/ pig) fed low concentration of dietary DON (2.48 ppm) for eight weeks were not altered. On the other hand, Hct of pigs were influenced by feeding of high concentration of dietary DON (10 ppm, Lun et al., 1985). Likewise, Prelusky et al. (1994) reported effect of low concentration of dietary DON on hematological alteration, pigs fed diets containing 3.0 ppm DON from naturally contaminated corn had significantly lower Hct value than those fed on either control diet or diets containing 3.0 ppm purified DON.

DON toxicity on selected hepatic enzymes, e.g. AST and ALT, was investigated in one-year-old rainbow trout fed diet containing 2 ppm DON for 23 days, the results show that both of the AST and ALT of rainbow trout were not significantly different from rainbow trout fed a control diet. Conversely, the elevation of AST in pigs was affected by feeding dietary concentrations of purified DON. This alteration might be related to intake of dietary DON and/ or increase of age Drochner et al. (2006); nonetheless, negative effects of DON on ATS and ALT of pigs, hens and roosters were not observed in the studies of Yegani et al. (2006) and Pinton et al. (2008). The significant increase in AST and ALT activities indicates alteration of tissue protein metabolism probably resulting from necrosis of liver, kidney and heart (El-Sayed et al., 2009).

The effect of DON-induced toxicity on relative organ weight (e.g. liver, kidney and spleen) has been reported in pigs that the significant reduction in weights of liver and kidney were caused by feeding of diets containing over 3.0 ppm DON from naturally contaminated corn (Rotter et al., 1994; Swamy et al., 2002), but the finding of Trenholm et al. (1994) was revealed that the significant increases in weights of liver and stomach of pigs were influenced by feeding of diets containing

over 8.7 ppm DON from naturally contaminated wheat. In the present study, liver weight in red tilapia expressed as HSI was not affected by dietary DON. Similarly, the in vivo studies of (Yegani et al., 2006) and (Swamy et al., 2004), feeding of high concentrations of dietary DON from naturally contaminated cereals did not cause negative effect on organ weight (e.g. liver, kidney, spleen) of chickens. The increase in liver weight relative to body weight found in pigs influenced by feeding of dietary DON may be resulted from acceleration of live tissue growth to detoxify and/ or excrete DON from the body (Friend et al., 1986; Trenholm et al., 1994).

### 5.5 Histopathological changes in organs of red tilapia

The liver is considered as a good indicator of nutritional and toxic pathology because of its function in metabolizing compounds from the digestive tract (Hinton et al., 1988; Rašković et al., 2011).

In pigs, Tiemann et al. (2006) reported that histopathological effects in the livers of female pigs associated with exposure to 6.1 and 9.6 ppm DON. For histological examination showed the increase in thickness of interlobular connective tissue of the livers with increased collagen fibrils; furthermore, electron microscopy was used for ultra-structural examination of hepatic cell which was found certain changes associated with oral exposure to DON. These effects included the increment in autophagosomes, residual bodies and fatty droplets in the hepatic cytoplasm, loss of bound ribosomes from the endoplasmic reticulum membrane of the hepatocytes, and reduction in hepatic glycogen. In addition, a dose-dependent decrease in hepatic glycogen and increase in the deposition of hemosiderin in the livers were related to DON exposure (Tiemann et al., 2006). It confirms with the finding of Hooft et al. (2011), deleterious effects of health performance of rainbow trout were associated with feeding of diets containing low concentrations of DON for eight weeks. Some of the livers of fish fed dietary DON showed the histopathological alteration; for instance, subcapsular edema was found in livers of fish fed dietary level of 1.4 ppm DON. Moreover, the livers of fish fed dietary level of 2.6 ppm DON appeared focal areas of severe fatty infiltration and phonotypical alteration of the hepatocytes (Hooft et al., 2011). In the present study, most of fish fed experimental diets had healthy livers but only few livers of fish appeared both of the changes in gross examination in

liver and histopathological alteration. The finding of gross examination, subcapsule edema (formed like-round shape) was found in seven livers samples from fish fed Diet 2, 3 and 4 (0.31, 0.50 and 0.92 ppm DON). The finding of histopathological alteration, focal necrosis was observed in hepatic tissues of two fish fed Diet 3, 4 and 5 (0.50, 0.92 and 1.15 ppm DON). Furthermore, abundance of melanomacrophage aggregate was found in two liver samples of fish fed Diet 3 (0.50 ppm DON). Even though no information concerning the relationship between exposure to DON and effect of melanomacrophage exists in fish, melanomacrophage (a pigment-containing cell) is known as a biomarker for chemical indication (Agius & Roberts, 2003). Its function is related to destruction or detoxification endogenous and exogenous compounds (Haaparanta et al., 1996). Agius and Roberts (2003) indicates that development of melanomacrophage is associated with chronic inflammatory lesion. Lastly, cytoplasmic vacuolation was noticed in livers of fish fed Diet 2 and 3 (0.31 and 0.50 ppm DON). This alteration might be due to lipid accumulation or lack of glycogen in hepatocytes, which usually contain lipids and glycogen (Camargo and Martinez, 2007; Matos et al., 2007). Moreover, feeding of diet contaminated with 2 ppm DON to one-year-old rainbow trout for 23 days caused the severe hyaline droplet degeneration in tubular epithelium cells of the caudal kidney. This alteration in caudal kidney is caused by DON because the kidney functions as a major route of toxicant excretion; nonetheless, other organs, such as gill, liver, skin and spleen, did not show any alteration (Matejova et al., 2014). As results of the present study, the histopathological observation did not show a dose-response relationship between exposure to DON and histopathological alteration. Although histopathological change is not a specific tool for a specific toxicity, the alterations are basically associated with the response of hepatocytes to toxicant (Hinton et al., 1988). However, there is no clear evidence correlation of the histopathological observation and the exposure to DON.

### 5.6 Chemical composition in flesh of red tilapia

DON acts as an inhibitor of protein synthesis by binding to the 60S ribosome subunit (Ueno, 1984). Hooft et al. (2011) demonstrated that increasingly graded levels of DON (0.3 to 2.6 ppm) caused linearly decreasing contents of carcass protein of

rainbow trout, as well as, retained nitrogen (RN), recovered energy (RE), nitrogen retention efficiency (NRE) and energy retention efficiency (ERE) decreased with increasing levels of DON. The decreased protein contents in carcass of rainbow trout may be associated with reduction in the rates of protein synthesis resulted from oral exposure to DON (Hooft et al., 2011). In addition, the results of (Dänicke et al., 2006) indicated the ability of DON to inhibit protein synthesis expressed as fractional synthesis rate. Some of tissues, namely kidney, spleen and ileum of pigs orally exposed to 5.7 ppm DON from naturally contaminated wheat had significant decrease in protein synthesis. In the present study, proximate analysis of pooled samples of fish flesh, regarded as approximately 55-60% of whole body, was not able to reasonably refer to alteration caused by DON exposure in whole body carcass. Furthermore, carcass composition should have been determined before and after experiment to investigate EN, RE, NRE and ERE.