

TABLE OF CONTENTS

	Page
ABSTRACT (IN THAI)	i
ABSTRACT (IN ENGLISH)	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	ix
LIST OF ABBEVIATIONS	xii
CHAPTER I INTRODUCTION	1
1.1 Background and rationale of the study	1
1.2 The scope and limitation of the study	5
1.3 Experimental hypothesis	5
1.4 Objectives of the study	5
CHAPTER II LITERATURE REVIEWS	6
2.1 Tilapias	6
2.2 Global tilapia production and the use of fishmeal and plants as protein sources in tilapia culture	9
2.3 Fungi and mycotoxins	14
2.3 The worldwide contamination of mycotoxins in animal feeds and feed ingredients	15
2.5 Factors affecting fungal growth and mycotoxin production	21
2.6 <i>Fusarium</i> mycotoxin	24
CHAPTER III RESEARCH METHODOLOGY	48
3.1 Experimental animal	48
3.2 Experimental conditions	48
3.3 Experimental diets	49
3.4 Mycotoxin analysis	50
3.5 Sample collection	55

TABLE OF CONTENTS (Cont.)

	Page
3.6 Laboratory analysis	55
3.7 Calculations and statistical analysis	60
CHAPTER IV RESULTS	61
4.1 Chemical composition in experimental diets	61
4.2 Mycotoxin analysis of five experimental diets using HPLC and LC-MS/MS	61
4.3 Growth performance and mortality of red tilapia	64
4.4 Hematological and biochemical parameters and hepatosomatic index	64
4.5 Chemical composition in muscle of red tilapia	64
4.5 Histopathological changes in organs of red tilapia (n=12 per treatment)	65
CHAPTER V DISCUSSION AND CONCLUSION	77
5.1 Chemical composition	77
5.2 Determination of mycotoxins in five experimental diets using HPLC and LC-MS/MS	77
5.3 Growth performance and mortality of red tilapia	81
5.4 Hematological and biochemical effects and HSI of red tilapia	89
5.5 Histopathological changes in organs of red tilapia	91
5.6 Chemical composition in flesh of red tilapia	92
REFERENCES	94
APPENDICES	114
APPENDIX A Results of mycotoxin analysis	115
APPENDIX B Proximate analysis	118
VITAE	124

LIST OF TABLES

		Page
Table 1	Anti-nutritional compounds in plant ingredients used in dietary fish formulation and negative effects of anti-nutritional compound on fish	13
Table 2	Detected levels of worldwide mycotoxin contamination (ppm) in samples from different regions in 201	18
Table 3	Detected levels of mycotoxin contamination (ppm) in animal feed and feed ingredients in 2010	19
Table 4	Temperature and water activity (a_w) for optimal growth and mycotoxin production	23
Table 5	Chemical structures of type A, type B, type C, and type D trichothecenes	25
Table 6	Ingredient composition of five isonitrogenous and isoenergetic diets and concentrations of detected <i>Fusarium</i> mycotoxins and other fungal metabolites in diets analyzed by LC-MS/MS	54
Table 7	Twelve steps of the procedure of tissue processing in Shandon citadel 2000 tissue processor	58
Table 8	Mycotoxins in five experimental diets analyzed by HPLC (ppm)	62
Table 9	Effects of the dietary DON on weight gain, growth rate, feed intake, feed conversion efficiency and percentage of mortality of red tilapia (initial body weight = 4.3 g/fish; mean \pm standard error of mean) in the 8-week feeding trial	66
Table 10	Effects of the dietary DON on blood chemistry, hematocrit and hepatosomatic index of red tilapia (initial body weight = 4.3 g/fish) in the 8-week feeding trial	70

LIST OF TABLES (Cont.)

	Page
Table 11 Chemical composition in muscle (expressed as percentage of dry matter) of red tilapia (initial average weight = 4.3 g/fish) fed diets containing graded concentrations of DON for eight weeks	73

LIST OF FIGURES

		Page
Figure 1	(A) Feature of Nile tilapia (<i>Oreochromis niloticus</i>), (B) Mozambique tilapia (<i>O. mossambicus</i>)	8
Figure 2	Global aquaculture productions of farmed tilapias and other cichlids during 2002 to 2012	10
Figure 3	The percentage of global aquaculture production of tilapias and other cichlids (total 4,507,002 tonnes) from each region in the world in 2012	11
Figure 4	Trends of global prices of feed ingredients used in fish feed	12
Figure 5	The global mycotoxin occurrence between January and December in 2009 and 2011, the data is presented as percentage of positive samples tested for mycotoxins in feeds and feed ingredients (aflatoxin (Afla), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZON) and fumonisin B1 (FB1))	16
Figure 6	Chemical structures of type A, type B, type C, and type D trichothecenes	25
Figure 7	Chemical structure of Deoxynivalenol	26
Figure 8	Chemical structure of T-2 toxin	36
Figure 9	Chemical structure of Fumonisin B ₁	39
Figure 10	Chemical structure of zearalenone	42
Figure 11	Chemical structure of deoxynivalenol-3-glucoside	46
Figure 12	The catalytic activity of AST	56
Figure 13	The catalytic activity of ALT	57
Figure 14	Chemicals and procedure used for staining the tissue specimens until Mounting	59

LIST OF FIGURES (Cont.)

		Page
Figure 15	Comparison of the relationship between response of red tilapia in terms of weight gain of red tilapia and graded concentration of dietary DON analyzed by LC-MS/MS and HPLC	63
Figure 16	(A) Growth curves and (B) body weight gain of red tilapia (initial average weight = 4.3 g/fish) fed experimental diets containing 0.07 (control), 0.31, 0.50, 0.92 and 1.15 ppm DON for eight weeks (Standard error bars; n = 4 for each treatment)	67
Figure 17	(A) Thermal-unit co efficiency (TGC) and (B) Feed intake of red tilapia (initial average weight = 4.3 g/fish) fed experimental diets containing 0.07 (control), 0.31, 0.50, 0.92 and 1.15 ppm DON (standard error bar; n = 4 for each treatment)	68
Figure 18	A) Feed efficiency of red tilapia (initial average weight = 4.3 g/fish) fed experimental diets containing 0.07 (control), 0.31, 0.50, 0.92 and 1.15 ppm DON (standard error bar; n = 4 for each treatment), and (B) percentage of mortality of red tilapia in each week	69
Figure 19	(A) Plasma aspartate aminotransferase (AST) and (B) Plasma alanine aminotransferase (ALT) of red tilapia (initial average weight = 4.3 g/fish) fed with experimental diets containing 0.07 (control), 0.31, 0.50, 0.92 and 1.15 ppm DON for eight weeks (Standard error bars; n = 12 for each treatment)	71

LIST OF FIGURES (Cont.)

		Page
Figure 20	(A) Hematocrit (Hct) and (B) Hepatosomatic Index (HSI) of red tilapia (initial average weight = 4.3 g/fish) fed with experimental diets containing 0.07 (control), 0.31, 0.50, 0.92 and 1.15 ppm DON for eight weeks. (Standard error bars; n = 12 for each treatment)	72
Figure 21	Histological changes of liver of tilapia fed experimental diets containing 0.07 ppm DON (control diet) for eight weeks (H&E stain, scale bar = 179.48 μ m). (A) and (B) illustrate normal hepatic architecture consists of portal vein, hepatopancreas and sinusoids	74
Figure 22	Histological changes of liver of tilapia fed experimental diets containing 0.50 ppm DON (Diet 3) for eight weeks (H&E stain, scale bar = 179.48 μ m). (A) displays blood congest in central portal vein and sinusoids (arrows), and distribution of necrosis (arrowheads) (B) exhibits melanomacrophage aggregate at bile (arrow)	75
Figure 23	Histological changes of liver of tilapia fed experimental diets for eight weeks (H&E stain, scale bar = 179.48 μ m). (A) Fish fed with 0.31 ppm DON (Diet 2) displays the increase in connective tissue at the portal vein and subscapsular edema (arrows). (B) Fish fed diet containing with 0.31 ppm DON (Diet 2) exhibited cytoplasmic vacuolation	76
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	86

LIST OF ABBREVIATIONS

3-aDON	3-acetyl-deoxynivalenol
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
15-aDON	15-acetyl-deoxynivalenol
α -ZON	alpha zearalenol
β -ZON	beta zearalenol
%	percentage
μ g	microgram
AFB ₁	aflatoxin B1
ALP	serum alkaline phosphatase
ALT	plasma alanine aminostransferase
AST	plasma aspartate aminostransferase
a _w	water activity
°C	celsius
DDGS	dried distillers grains with soluble
DNA	deoxyribonucleic acid
DOM-1	de-epoxy deoxynivalenol
DON	deoxynivalenol
DON-3-Glu	deoxynivalenol-3- β -D-glucoside
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
FUM	fumonisin
FB ₁	fumonisin B ₁
g	gram
H	hydrogen
HSI	hepatosomatic index
Hct	hematocrit
H&E staining	haematoxylin and eosin staining
HPLC	High Performance Liquid Chromatography

LIST OF ABBREVIATIONS (Cont.)

Kg	kilogram
LC-MS/MS	Liquid Chromatography - Tandem Mass Spectrometry
mg	milligram
ng	nanogram
NIV	nivalenol
OAc	acetoxyl group
OCOCH ₂ CH(CH ₃) ₂	ester-link isovaleryl
OH	hydroxyl group
OTA	ochratoxin A
<i>O. mossambicus</i>	<i>Oreochromis mossambicus</i>
<i>O. niloticus</i>	<i>Oreochromis niloticus</i>
ppb	part per billion
ppm	part per million
RNA	ribonucleic acid
SOFIA	The State of World Fisheries and Aquaculture
ZON	zearalenone