CHAPTER II
LITERATURE REVIEWS

2.1 Tilapias

Tilapias are the freshwater fish belonging to the family Cichlidae. They are original from Africa and were introduced into many regions of the world. Historically, tilapias were expanding worldwide during the period from 1970 to 1990. According to FAO aquaculture production statistics, the number of countries practicing tilapia culture significantly increased to jump from 16 countries in 1970 to over 100 countries in 1990 (De Silva, 2004). Hence, tilapias become a candidate for aquaculture, particularly in developing countries, because of their fast growth, tolerance to a wide range of environmental conditions (e.g. temperature, salinity, low dissolved oxygen, etc.), resistance to stress and disease, ability to reproduce in short generation time and feed on low trophic levels and acceptance of artificial feeds immediately after yoked-sac absorption (De Silva, 2004; El-Sayed, 2006).

Based on FAO database, 12 tilapia species, namely six *Oreochromis* spp., *Sarotherodon* spp., and *Tilapia* spp., and five tilapia hybrids have been introduced into many regions in the world; for example, Europe, Asia-Pacific, South America, North America and Africa. However, among all tilapia species, Nile tilapia is the most important species in tilapia culture. Nonetheless, the tilapia hybrid strains, e.g. Nile tilapia x blue tilapia (*O. niloticus* x *O. aureus*), Nile tilapia x Wami tilapia (*O. niloticus* x *O. urolepis hornorum*), Nile tilapia x long-finned tilapia (*O.niloticus* x *O. macrochir*), Mossambique tilapia x Wami tilapia (*O. mossambicus* x *O. urolepis hornorum*) and Mossambique tilapia x Nile tilapia (*O. mossambicus* x *O. niloticus*), were developed to increase tilapia production and expand tilapia farming in brackish water reservoirs (Bartley et al., 2000; De Silva, 2004; De Verdal et al., 2014; Suresh and Lin, 1992).

In the history of tilapia hybrid, the beginning of tilapia hybrid culture was related to brackish water farming systems. The hybrid tilapia farming was association with intensive shrimp farming to contribute in part to the reproduction of green water

in ponds supplying good quality water for shrimp ponds, but hybrid tilapias was only by-product of shrimp production. In order to develop a saline tolerance tilapia strains that are able to growth fast, Red tilapia (*O. niloticus* × *O. mossambicus*) was bred between Nile tilapia (*O. niloticus*) and Mozambique tilapia (*O. mossambicus*) to be a fast growing and highly saline tolerance species. It is known that Nile tilapia has fast growth; however, it is not tolerance to live in saline water. On the other hand, Mozambique tilapia is a low growth species, but it is well tolerant to live in saline water (De Verdal et al., 2014; Suresh and Lin, 1992).

Nile tilapia has compressed body; caudal peduncle depth equal to length (Figure 1). Its scales are cycloid. A knob-like protuberance absents on dorsal surface of snout. Upper jaw length shows no sexual dimorphism. First gill arch comprises of 27 to 33 gillrackers. Lateral lines are interrupted. Spinous and soft ray parts of dorsal fin are continuous. Nile tilapia has dorsal fin with 16-17 spines; 11 to 15 soft rays; and anal fin with 3 spines and 10-11 rays. Caudal fin is truncated. Colour of pectoral, dorsal and caudal fins is reddish in spawning season; caudal fin with many black bars (Rakocy, 2005). As for Mozambique tilapia, it has deep bodied cichlid fish native to the eastward-flowing rivers of central and southern Africa1. It varies in its appearance due to its ability to interbreed with related species of cichlids. It can also alter characteristics such as body size and age at sexual maturity in response to differing environmental conditions. In general, both sexes have a long dorsal (upper) fin that starts from above the gills and continues along the majority of the upper body. The dorsal and anal fins are elongated towards the end of fish and easily reach to the caudal fin when depressed against the body. The caudal fin often has a red margin in adult fish (Encyclopedia of Life, 2014). Nile tilapias and Mozambique tilapias are classified according to the principle of fish taxonomy following as (Figure 1):

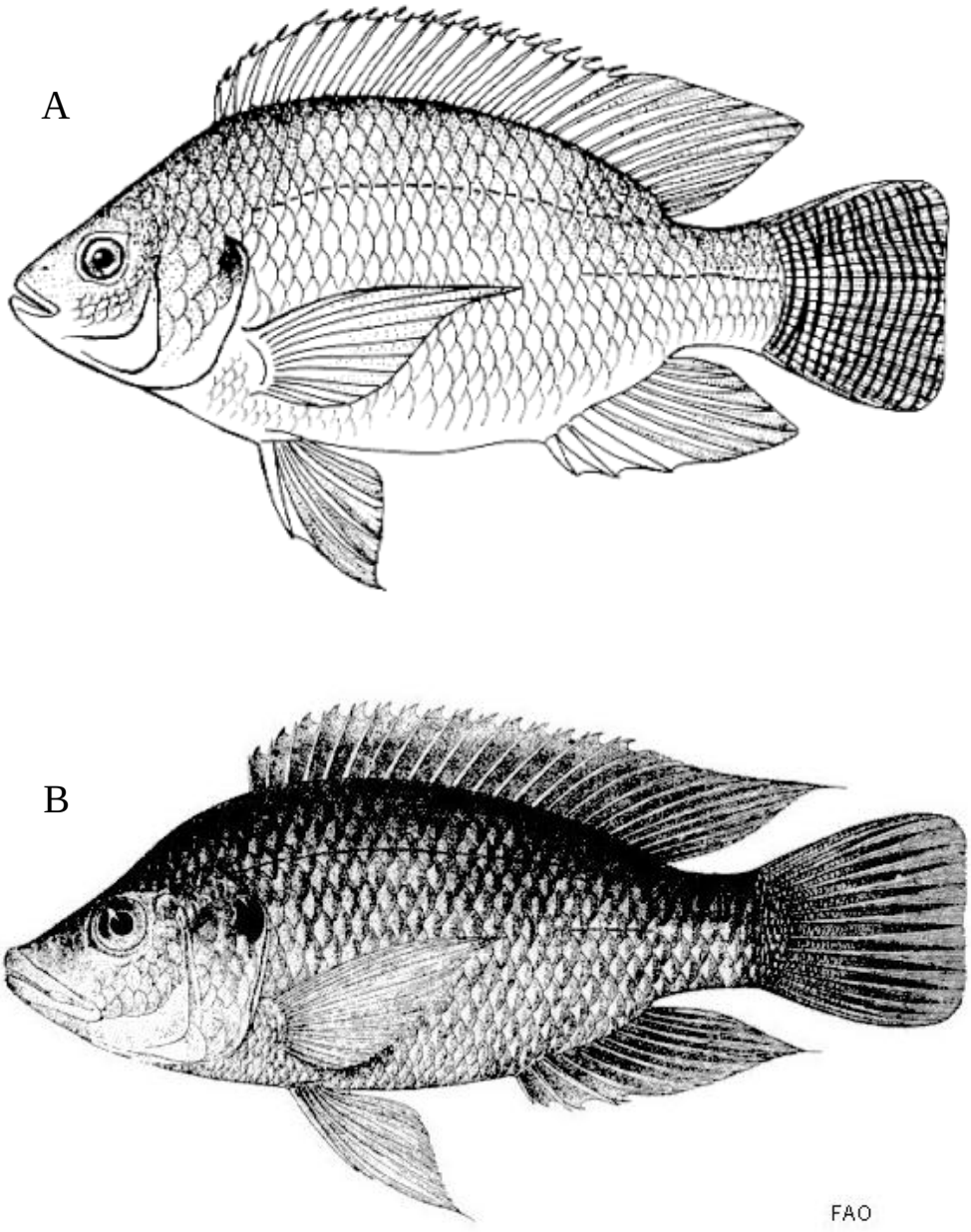

Figure 1 (A) Feature of Nile tilapia (*Oreochromis niloticus*), (B) Mozambique tilapia (*O. mossambicus)* (Encyclopedia of Life, 2014; Rakocy, 2005)

Phylum Chordata
Subphylum Vertebrata (Craniata)
Superclass Gnathostomata
Class Actinopterygii
Subclass Neopterygii
Superorder Acanthopterygii
Order Perciformes
Suborder Labroidei
Family Cichilidae
Genus Oreochromis
Species *niloticus* (Linnaeus)
Species *mossambicus*

2.2 Global tilapia production and the use of fishmeal and plants as protein sources in tilapia culture

Based on the data of world aquaculture production by fish species stated by FAO (2014), tilapias and other cichlids are the second most important farmed fish species in the world, surpassed by carps. The world aquaculture productions of tilapias and other cichlids have been increased from 1.4 million tonnes in 2002 to 4.5 million tonnes in 2012 with a sale value of more than $7 billion (Figure 2: FAO, 2012).

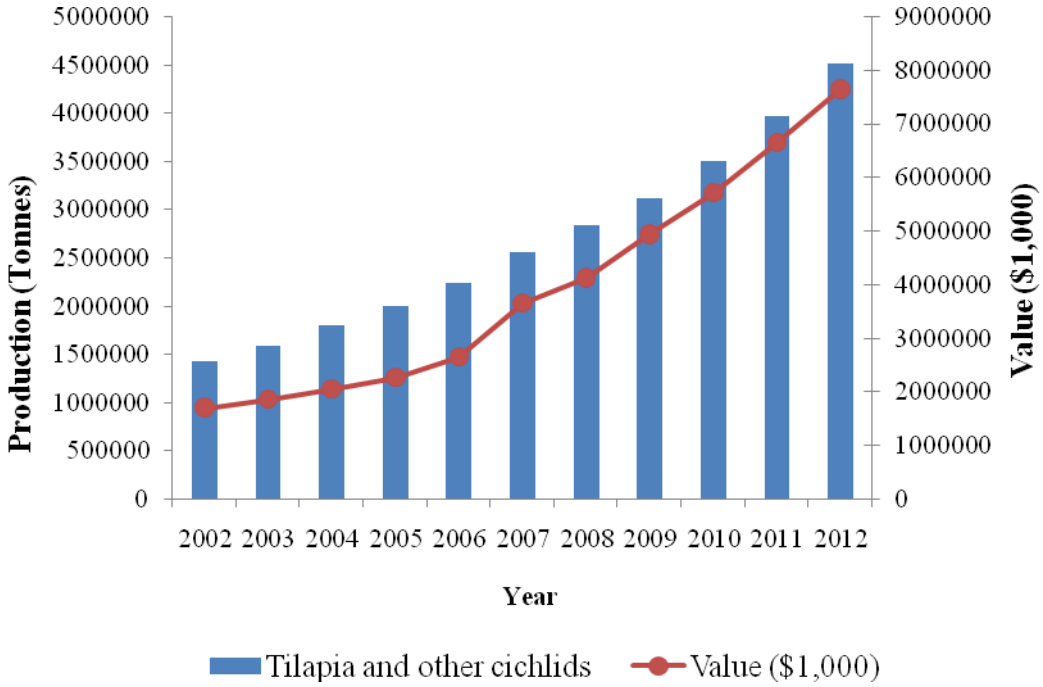

Figure 2 Global aquaculture productions of farmed tilapias and other cichlids during 2002 to 2012 (FAO, 2012)

Globally, the producers of farmed tilapias and other cichlids include Africa, America, Asia, Europe and Ocean. Nonetheless, the largest portion (3,122,134 tonnes) of world farmed tilapias and other cichlid productions were from the Asian continent as 69.27% of world total production (4,507,002 tonnes) of farmed tilapias and other cichlids in 2012 (Figure 3, FAO, 2012). Furthermore, Figure 3 showed the top five producers of farmed tilapias and other cichlids in Asia in 2012, including China, Indonesia, Philippines, Thailand and Bangladesh. Obviously, China is the largest producer of farmed tilapias and other cichlids with more than 1.5 million tonnes of farmed tilapias and other cichlids, it is considered as nearly 50% of total aquaculture productions (3,122,134 tonnes) of farmed tilapias and other cichlids in Asia. Chinese production is likely to raise approximately 1.3 million tonnes in 2010 to approximately 1.5 million tonnes in 2012. In contrast to China, the trend of Thai production of tilapias and other cichlids has slightly decreased from 179,355 tonnes in 2010 to 153,357 tonnes in 2012 (FAO, 2012)

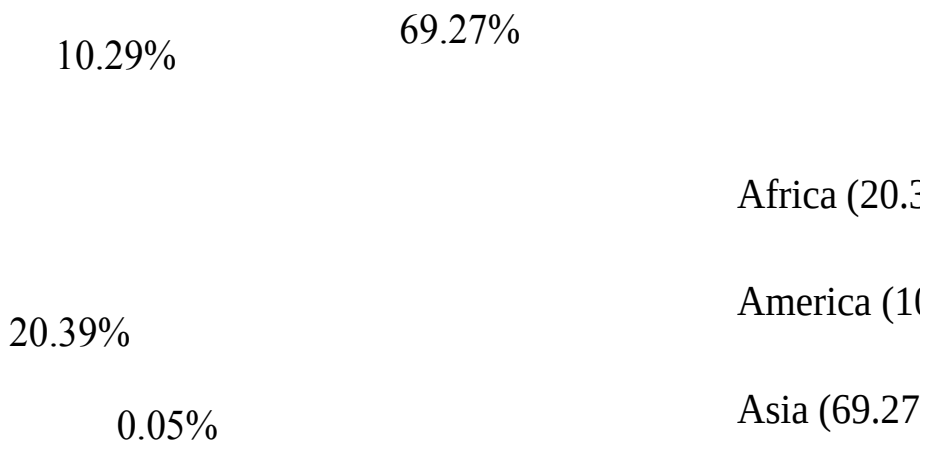

Figure 3 The percentage of global aquaculture production of tilapias and other cichlids (total 4,507,002 tonnes) from each region in the world in 2012 (FAO, 2012)

In global overview on the use of fishmeal and fish oil, Hasan and Halwart (2009) stated that aquaculture is the largest user of fishmeal, in case of pigs and poultry, it accounts for around a quarter of total usage of global fishmeal production. In aquaculture, fish feed, accounted for over 50% of operating cost in intensive aquaculture, consists of fishmeal as the most important source of protein and is much more expensive than other cereal grains as the usual sources of carbohydrates (El-Sayed, 2008; Rana et al., 2009). However, fishmeal and fish oil are two major dietary ingredients which contribute to increase fish feed price. In general, fish feed formulations of carnivorous finfish (e.g. salmon, seabass, seabream, eels and snakeheads) commonly require 20 to 40% of fishmeal in diets. While, herbivorous/ omnivorous finfish groups (e.g. tilapias, milkfish, grass carp, common carp and other cyprinids) require approximately 5% of fishmeal in diets. Crustaceans (e.g. marine shrimps, prawns, crabs and crayfish) require around 15 to 25% fishmeal in diets (Hasan and Halwart, 2009).

Though the tilapia farming has continually been expanded since last decade, rising of ingredient prices has become a major constrain. According to Rana et al. (2009), the world prices of fishmeal ranged between $500 and $700 per tonne during 2000 to 2005 (Figure 4). In 2008, the price of fishmeal jumped to $1,210 per tonne.

Moreover, not only the prices of fishmeal increased but also other feed ingredients commonly used for fish feed industry rose to 20-92% during 2007 and 2008. Consequence of rising fishmeal prices leads to the inclusion of alternative protein sources, particularly protein source of plants, in fish feed for tilapias. Many plant ingredients as protein source (fishmeal replacement) in fish feed include soybean meal, soy protein concentrate, cottonseed cake, corn gluten meal, wheat gluten meal (El-Sayed, 1999; Tacon et al., 2009).

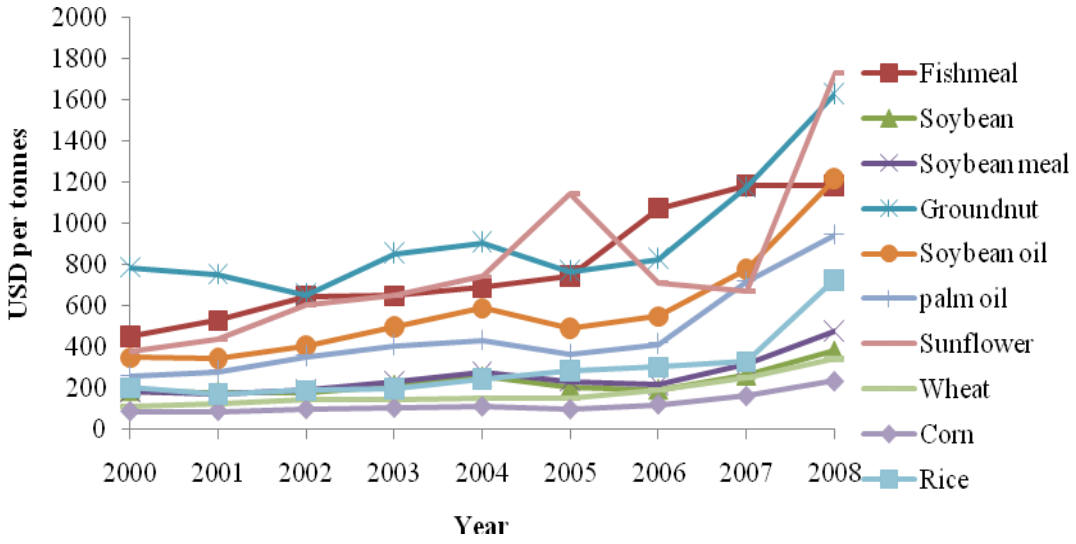

Figure 4 Trends of global prices of feed ingredients used in fish feed (Adapted from Rana et al., 2009)

Even though some plant ingredients are able to be highly substituted fishmeal as protein source in dietary formulation of herbivorous and omnivorous fishes, especially fish feed for farmed tilapias, there are some limitations of using alternative protein sources: nutritional limitation in terms of the balance of amino acid profile; for example, corn gluten meal is an important alternate protein source that is well digestible to fish widespread used in dietary formulation; however, corn gluten meal is substituted fishmeal in diets leading to deficiency of the amino acid, namely Lysine. Besides, cottonseed meal is a cheap plant protein containing high content of protein, but it contains low level of some amino acid, such as cystine, lysine and

methionine. Unfortunately, the substitution of cottonseed meal in diets composes of high level of gossypol, which is regarded as anti-nutritional compounds, in addition to the limitation of amino acid profile (El-Sayed, 1999; Hardy, 2010; Higgs et al., 1982; Inudo et al., 2004; Rana et al., 2009). The anti-nutritional compounds have been reported including phytic acid glucosinolate, saponin, tannins, soluble non-starch polysaccharides and gossypol (a phenolic anti-nutrient compound). The sources of anti-nutritional compounds and negative effects concerning with feeding diets containing with anti-nutritional compounds is summarized in Table 1. Lastly, the challenge related to the inclusion of plant ingredients, in particular plant by-products, in dietary formulation is also associated with unawareness contamination of mycotoxins in plant ingredients since fish feed formulation for farmed tilapias can be included high contents of plant ingredients as protein source resulting in higher risk of oral exposure to mycotoxins contaminating in fish feed (Hussein and Brasel, 2001; Rana et al., 2009; Richard, 2007).

Table 1 Anti-nutritional compounds in plant ingredients used in dietary fish formulation and negative effects of anti-nutritional compound on fish

Anti-nutritional compound	Source of plant ingredient	Negative effects on fishes	Reference
Gossypol	cottonseed meal	Affects reproductive, reduces growth and hematocrit	Hendricks and Bailey (1989)
Phyto acid glucosinolate	Soy protein concentrates, Soybean meal	Decrease in growth performance	Higgs et al. (1982)
Saponin	Soybean	Low feed intake, palatability	Bureau et al. (1998)
Mon-starch polysaccharides	Soybean meal	No toxicity, reduction of digestibility, interfere absorption of protein and lipids	Rana et al. (2009)
phytoestrogen	Soybean	affects male reproduction	Inudo et al.(2004)

2.3 Fungi and mycotoxins

Contamination of mycotoxins in foodstuffs and feedstuffs began to be interesting when aflatoxins were found to cause disease in turkey known as "Turkey 'X' disease" and also caused a hepatic cancer in farmed rainbow trout in 1935 (Morgavi and Riley, 2007b). Agricultural commodities as foodstuffs and feedstuffs are commonly contaminated with several mycotoxins, which are toxic secondary metabolites, mainly produced by the fungal genus, namely *Aspergillus*, *Penicillium* and *Fusarium*. Although these fungi normally produce hundred mycotoxins contaminating in cereal grains, there are some mycotoxins that are potential toxic in agriculture to cause adverse impacts on the health of human and animals resulting in significant losses of economics (CAST, 2003; Morgavi and Riley, 2007b). These are: aflatoxin B1, ochratoxin A (OTA), trichothecenes (e.g. deoxynivalenol: DON and T-2 toxin), zearalenone (ZON) and fumonisin B1 (FB_1) (Hussein and Brasel, 2001; Richard, 2007).

The aflatoxins, which are mainly produced by *Aspergillus flavus*, *A. parasiticus*, *A. nomius*, and *A. psuedotamarii*, are classified into four groups: aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin (AFG_1), and aflatoxin (AFG_2). Nonetheless, AFB_1 is taken an interest the most due to it is the most toxic to animals. For instance, the pathological effects of AFB_1 in animals are associated with hepatoxicity (liver damage), bile duct hyperplasia, hemorrhage in intestines and kidneys and carcinogenesis (CAST, 2003; Hussein and Brasel, 2001).

In 1930s, trichothecene mycotoxins, in particular DON, were suspected to cause feed refusal syndrome in animals. More than 180 trichothecene mycotoxins that have been known are produced by many fungi genera (e.g. *Fusarium*, *Cephalosporium*, *Myrothecium*, *Stachybotrys*, *Trichoderma*), but *Fusarium* fungi are considered to be the most economically significant producers of trichothecenes (Morgavi and Riley, 2007a; Mostrom and Raisbeck, 2012). Trichothecenes are generally divided into four types, including type A, B, C and D, only type A trichothecene (e.g. T-2 toxin) and type B trichothecene (e.g. DON) are regarded as an agricultural problem. The clinically pathological effects of trichothecenes are concerned in digestive disorder, hemorrhage, edema, dermatitis, and blood disorder (CAST, 2003; Eriksen and Pettersson, 2004; Mostrom and Raisbeck, 2012). Later, *Fusarium* mycotoxins caused

equine leucoencephalomalacia syndrome in horses by consumption of moldy corn in 1891 and later known as fumonisins. Fumonisins are cancer-promoting metabolites that are produced by *F. proliferatum* and *F. verticillioides*. There are two known fumonisins including FB_1 and fumonisin B_2 (FB_2). The FB_1 is more toxic to animals than FB_2 and it causes porcine pulmonary edema syndrome in pigs (Morgavi and Riley, 2007b; Voss et al., 2011). Lastly, ZON is one of the *Fusarium* mycotoxins, mainly produced by *F. graminearum*. Its adverse impact is distinctly associated with the reproductive problem in farmed animals, specifically in pigs, because it is estrogenic-like structure. Pigs exposed to ZON show pathological signs, such as vulvovaginitis, enlargement of uterus and mammary glands, and atrophy of testicles and ovaries (CAST, 2003).

2.4 The worldwide contamination of mycotoxins in animal feeds and feed ingredients

Cereal grains, including corn, barley, soybean, corn gluten meal, rice/bran, wheat, silage and straw, used as feed ingredients that are unavoidably contaminated with different concentrations and types of mycotoxins depending on the environmental conditions in each region. Since mycotoxins have been negatively associated with the illness of human and animals and also detectable concentrations of mycotoxin contamination are not constant, thus the annual mycotoxin survey program is conducted by Biomin to investigate the occurrence of mycotoxin contamination in different regions and types of feed crops (Rodrigues and Nährer, 2012a; Rodrigues and Nährer, 2012b). However, hundreds of mycotoxins and fungal metabolites have been reported that they contaminate in animal feeds and feed ingredients. The most important mycotoxin in terms of agriculture and animal production such as AFB_1, ZON, DON, FB_1 and OTA were detected in feed and feed ingredients randomly collected from around the world by the mycotoxin survey program of Biomin (Figure 5). The recent results of the survey program in 2011 showed the contamination of mycotoxins such as AFB_1, ZON, DON, FUM and OTA expressed as the percentage of positive samples were 27%, 40%, 59%, 51% and 27% of tested samples, respectively (Rodrigues and Nährer, 2011a; Rodrigues and Nährer, 2011b). A comparison of detected mycotoxins in feed and feed ingredients between 2009 to

2011 demonstrated that the contamination of some mycotoxins in feed and feed ingredients increased in 2011; for instance, the percentage of DON and OTA contamination increased from 58% to 59% and 26% to 27%, respectively (Rodrigues and Nährer, 2011a; Rodrigues and Nährer, 2012b).

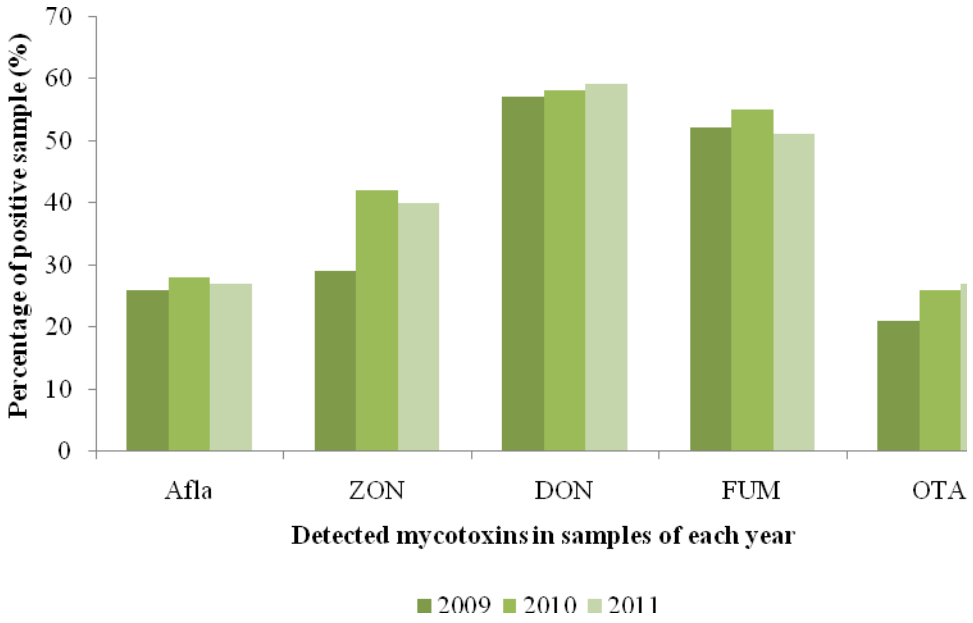

Figure 5 The global mycotoxin occurrence between January and December in 2009 and 2011, the data is presented as percentage of positive samples tested for mycotoxins in feeds and feed ingredients (aflatoxin (Afla), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZON) and fumonisin B1 (FB1) (Adapted from Rodrigues and Nährer, 2011a; Rodrigues and Nährer, 2012b).

The results of survey program of mycotoxin occurrence in animal feeds and feed ingredient from different world regions are shown in Table 2 and 3, respectively. Samples (e.g. corn, soybean, wheat/bran, corn gluten meal, rice/bran, DDGS (dried distillers grains with soluble), finished feed, silage, straw and barley) were collected from Asia, Oceania, Europe, North America, South America, Middle East and Africa to be analyzed for average and maximum concentrations of each mycotoxin.

Fusarium mycotoxins, particularly DON and FUM, were largely found in commodities. The highest percentages of DON contamination were found in Asia, Oceania, Europe and North America with 66%, 49%, 60% and 50% of tested samples, respectively, and FUM presented the highest percentages of 76%, 67% and 58% of tested samples in South America, Middle East and Africa. The average concentrations of DON in worldwide commodities were respectively ranged from 0.03 to 1.4 ppm, however, the most occurrence of DON presented as 59.3% of tested samples collected in Oceania (BIOMIN's Mycotoxins Report, 2011).

In terms of commodity types, DON and FUM presented the high concentrations and high average concentrations of their contamination in corn, wheat, DDGS and finished feed. The high concentrations of DON contamination was reported in wheat (49 ppm DON) and corn (26.1 ppm DON), respectively. The average contamination concentrations of DON in DDGS, corn gluten meal, wheat and corn were 3.1, 1.5, 1.1 and 1.0 respectively (Rodrigues and Nährer, 2011a).

Table 2 Detected levels of worldwide mycotoxin contamination (ppm) in samples from different regions in 2011

	AFB_1[a]	ZON[b]	DON[c]	FUM[d]	OTA[e]
Asia					
Number of tests	1337	1340	1364	1261	1219
Percent Positive (%)	36	53	66	52	28
Average (ppm)	0.03	0.1	0.6	0.9	0.002
Maximum (ppm)	2.2	11.3	41.4	77.5	40.0
Oceania					
Number of tests	191	213	207	179	191
Percent Positive (%)	6	26	49	12	11
Average (ppm)	0.002	0.3	1.4	1.2	0
Maximum (ppm)	1.8	23.3	49.3	4.8	0.01
Europe					
Number of tests	199	1089	1481	229	261
Percent Positive (%)	36	35	62	50	39
Average (ppm)	0.001	0.05	0.7	0.5	0.002
Maximum (ppm)	0.04	1.5	25.8	7.0	0.05
North America					
Number of tests	452	202	220	300	95
Percent Positive (%)	21	14	50	27	21
Average (ppm)	0.01	0.02	0.5	0.3	0.001
Maximum (ppm)	0.9	0.7	19.9	12.9	18.0
South America					
Number of tests	540	176	196	545	159
Percent Positive (%)	13	28	21	76	16
Average (ppm)	0.001	0.04	0.08	1.5	0.01
Maximum (ppm)	0.2	1.8	5.1	34.5	0.3
Middle East					
Number of tests	38	28	28	21	28
Percent Positive (%)	37	0	11	67	50
Average (ppm)	0.001	0	0.03	0.3	0.007
Maximum (ppm)	0.02	0	0.4	3.7	0.07

Table 2 Detected levels of worldwide mycotoxin contamination (ppm) in samples from different regions in 2011 (Cont.)

	AFB_1[a]	ZON[b]	DON[c]	FUM[d]	OTA[e]
Africa					
Number of tests	12	12	12	12	12
Percent Positive (%)	58	8	17	58	42
Average (ppm)	0.06	0.007	0.3	0.5	0.002
Maximum (ppm)	0.2	0.08	2.9	1.0	0.02

[a]aflatoxin (AFB_1); [b]ochratoxin A (OTA); [c]deoxynivalenol (DON); [d]zearalenone (ZON); [e]fumonisin B1 (FB_1)

(Adapted from BIOMIN's Mycotoxins Report, 2011)

Table 3 Detected levels of mycotoxin contamination (ppm) in animal feed and feed ingredients in 2010

	AFB_1[a]	ZON[b]	DON[c]	FUM[d]	OTA[e]
Corn					
Number of tests	590	681	704	729	337
Percent Positive (%)	28	46	72	75	9
Average (ppm)	0.02	0.1	1.0	1.9	0.001
Maximum (ppm)	4.7	3.3	26.1	53.7	0.09
Soybean					
Number of tests	93	103	109	87	87
Percent Positive (%)	20	14	32	2	21
Average (ppm)	0	0.005	0.08	0.007	0.001
Maximum (ppm)	0.006	0.08	1.02	0.3	0.02
Wheat/bran					
Number of tests	92	172	262	88	83
Percent Positive (%)	4	28	56	11	24
Average (ppm)	0	0.03	1.1	0.03	0.005
Maximum (ppm)	0.007	0.5	49.0	0.6	0.3
Corn Gluten Meal					
Number of tests	19	19	19	18	19
Percent Positive (%)	47	95	89	100	74
Average (ppm)	0.03	2.2	1.5	3.8	0.006
Maximum (ppm)	0.5	16.7	7.0	12.6	0.006

Table 3 Detected levels of mycotoxin contamination (ppm) in animal feed and feed ingredients in 2010 (Cont.)

	AFB_1[a]	**ZON[b]**	**DON[c]**	**FUM[d]**	**OTA[e]**
Rice/bran					
Number of tests	19	20	20	20	19
Percent Positive (%)	47	55	5	5	26
Average (ppm)	0.003	0.056	0.003	0.04	0.001
Maximum (ppm)	0.02	0.3	0.07	0.8	0.01
DDGS					
Number of tests	59	86	86	78	57
Percent Positive (%)	8	87	91	77	35
Average (ppm)	0.002	0.3	3.1	0.8	0.001
Maximum (ppm)	0.04	1.7	19.1	8.9	0.03
Finished Feed					
Number of tests	631	778	791	656	532
Percent Positive (%)	46	57	58	69	39
Average (ppm)	0.01	0.1	0.4	0.8	0.002
Maximum (ppm)	0.4	3.6	19.1	10.4	0.2
Silage					
Number of tests	253	320	359	239	233
Percent Positive (%)	2	36	48	19	14
Average (ppm)	0	0.08	0.7	0.2	0.001
Maximum (ppm)	0.009	2.1	14.3	3.1	0.04
Straw					
Number of tests	24	24	24	24	24
Percent Positive (%)	0	13	21	0	17
Average (ppm)	0	0.05	0.09	0	0
Maximum (ppm)	0	0.5	0.9	0	0.004
Barley					
Number of tests	7	186	329	329	6
Percent Positive (%)	0	9	60	0	25
Average (ppm)	0	0.009	0.7	0	0.003
Maximum (ppm)	0	0.5	14.1	0	0.02

[a]aflatoxin (AFB_1); [b]ochratoxin A (OTA); [c]deoxynivalenol (DON), [d]zearalenone (ZON); [e]fumonisin B1 (FB_1)

(Rodrigues and Nährer, 2011a)

2.5 Factors affecting fungal growth and mycotoxin production

Contamination of mycotoxins in agricultural commodities that mycotoxins are not only produced in commodities during storage; in fact, they have been formed by various mycotoxigenic fungi in moldy-infected crops at fields during plant development and prior to harvest (Miller, 1995). Contamination of mycotoxins in crops arises from the infection of fungi as plant pathogens that cause disease in crops, such as, head blight, ear rot of maize, seedling blight and foot rot of cereal. For example, *Fusarium* head blight is also known as "scab" or "foot rot", which is caused by *F. graminearum* and *F. culmorum*. These *Fusarium* fungi attack flowering stage under wet and cloudy condition (Snijders, 1990). Furthermore, the invasion of insects leads to increase sensitivity of plants to fungi (Doohan et al., 2003). Moreover, inappropriate agronomic practices including tillage system and cropping sequence also result in an accumulation of fungal spores and inoculum in soil, particularly cereal crop residues are considered as the significant sources of inoculum for fungi (Dill-Macky and Jones, 2000). The storage of commodities under improper conditions (high temperature and high humidity) is associated with fungal growth and mycotoxin production (CAST, 2003).

The climatic conditions, including temperature, humidity (referred to water activity (a_w)), light intensity and types of substrate differently affect the growth of fungi and mycotoxin production, especially temperature and water activity, are the most important factors that influence on differences in fungal species and fungal toxic types. Requirements of optimal temperature and water activity for growth of *Fusarium sp., Aspergillus sp.* and *Penicillium sp*. and mycotoxin production are shown in Table 4. *Aspergillus sp*. are able to grow in a wide range of temperature at 10-45 ˚C which is higher and wider than the ranges for growth of *Fusarium sp*. (at 15-30 ˚C) and *Penicillium sp*. (at 16-25˚C). In the other hand, requirement of water activity for growth of *Aspergillus sp*. ranging from 0.72 to 0.95 a_w which is quite dry and lower than a_w requirement of *Fusarium sp*. (0.86-0.99 a_w) and *Penicillium sp*. (0.96-0.97 a_w) (Cuero et al., 1987). Though *F. proliferatum* is able to grow at the low of 0.86 a_w on corn, the growth rate of *F. proliferatum* was slow at this a_w level and the amount of *F. proliferatum* yield also was little (Jiménez et al., 1996).

Mycotoxin productions vary with types of substrate and levels of a_w which are not the same as the optimal of fungal growth. A wide range of optimal a_w for each type of mycotoxin was variously reported. In cases of *Fusarium* mycotoxins production, Ramirez et al. (2006) and Hope et al. (2005) found the highest amount of DON production belonging to *F. graminearum* and *F. culmorum* were ranged from 0.98-0.99 a_w and 0.97-0.99 a_w, respectively. While requirements of a_w for ZON production by *F. graminearum* and *F. culmorum* were detected at 0.95 a_w and 0.97 a_w, respectively, which were lower than those of DON production (Jiménez et al., 1996). As for FUM production, the optimal level of a_w for FUM produced by *F. molin, F. proli* and *F. verti* was at 0.97 a_w in maize and corn, reported by Marin et al. (1995) and Samapundo et al. (2005). The *Aspergillus* and *Penicillium* mycotoxins, namely OTA, *A. ochraceus* produced the amount of OTA at 0.98 a_w which is higher than *P. verrucosum* (at 0.93 a_w). The optimal level of a_w for DON production is required higher those of a_w than any other type of mycotoxins (Arroyo et al., 2005; Ramos et al., 1998).

Table 4 Temperature and water activity (a_w) for optimal growth and mycotoxin production

Fungal species	Temperature (°C)		Water activity (a_w)		MCT	substrates	Ref.
	Range	Optima	Growth	Production			
A. flavus	10-45	33	0.78-0.80	0.83-0.87	Afla	Agar	a
A. parasiticus	10-42	32	0.78-0.80	0.87	Afla	Agar	a
A. ochraceus	25-30	30	0.96-0.98	0.98	OTA	Barley	b
	-	30	0.99	0.99	OTA	Barley	c
F. graminearum	25-30	25	0.95-0.99	0.995	DON	Wheat	d
	15-25	25	0.99	0.98-0.99	DON	Wheat	e
	16-28	28	0.97	0.95	ZON	Corn	f
	15-25	25	0.95-0.97	0.97	ZON	Corn	g
F. culmorum	15-25	-	0.98	0.97-0.99	DON	Wheat	e
	16-25	-	-	0.97	ZON	Corn	f
F. moliniforme	25-30	30	0.92-0.96	0.97	FB_1,	Maize	h
F. proliferatum	25-30	30	0.92-0.96	0.97	FB_2 FB_1,	Maize	h
	15-30	30	0.86-0.97	0.97	FB_2 FUM	Corn	i
F. verticillioides	15-30	30	0.92-0.97	0.97	FUM	Corn	i
P. verrucosum	16-25	25	0.96-0.97	0.93	OTA	Bread, Mai, Rice, Agar	a, j

- no report; OTA: ochratoxin; FUM: fumonisin; FB_1: Fumonisin B_1; DON: deoxynivalenol; ZON: zearalenol; a: Cuero et al. (1987); b: Ramos et al. (1998);
c: Pardo et al. (2004); d: Ramirez et al. (2006); e: Hope et al. (2005); f: Jiménez et al. (1996); g: Montani et al. (1988); h: Marin et al. (1995) m; i: Samapundo et al. (2005); j: Arroyo et al. (2005).

2.6 *Fusarium* mycotoxin

2.6.1 Trichothecene mycotoxins

Trichothecene is the group of mycotoxins mainly produced by several genera of fungi, especially fungal species of the genus *Fusarium*. The toxins are generally produced in agricultural crops and commodities, such as wheat, barley, oats, rye and maize that are used as human foods and animal feeds (Binder et al., 2007). Trichothecenes are sesquiterpenoids with a 12, 13-epoxide ring which is essential for the potential toxicity of trichothecenes (Desjardins et al., 1993); moreover, their structures (Figure 6) comprise of a double bond at C-9, 10 and a number of hydroxyl and acetoxyl groups, (Mostrom and Raisbeck, 2012). Generally, trichothecenes are classified into four categories (Table 5) depending on their functional groups and mycotoxin producing fungi (Ueno, 1980; Ueno et al., 1973). Type B trichothecenes contain a carbonyl group at the C-8 position. Though the type B trichothecenes, including deoxynivalenol (DON), 3-acetyl- deoxynivalenol (3-aDON), 15-acetyl-deoxynivalenol (15-aDON), nivalenol (NIV) and fusarenon X, are least toxic in the trichothecene groups, they are the most frequently detected toxic substrates. While, type A trichothecenes do not consist of a carbonyl group at the C-8 position and they are considered as the most toxic substrates in trichothecene group, including T-2 toxin, HT-2 toxin and diacetoxyscrirpentriol (Mostrom & Raisbeck, 2012; Ueno et al., 1973). Type C trichothecenes are characterized by a second epoxide function at C-7,8 and are not produced by *Fusarium* fungi. Lastly, type D trichothecenes trichothecenes contain a macrocyclic ring between C-4 and C-15 position with two ester linkages (Mostrom and Raisbeck, 2012; Sudakin, 2003). Although trichothecenes are divided into four categories, only type A and B trichothecenes are produced by *Fusarium* fungi, which are mostly found in crops, are associated with health problem in human and animals. Hence, type C and D trichothecenes are not mentioned any further (Eriksen and Pettersson, 2004; Rocha et al., 2005; Ueno, 1984).

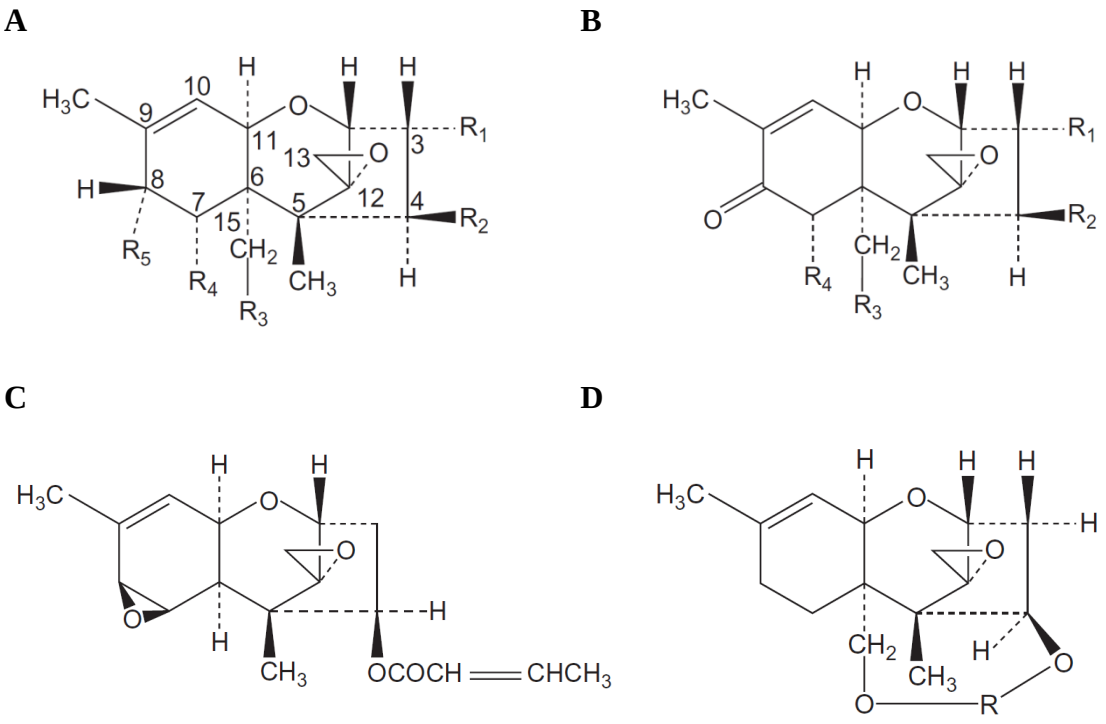

Figure 6 Chemical structures of type A, type B, type C, and type D trichothecenes (Mostrom and Raisbeck, 2012)

Table 5 Chemical structures of type A, type B, type C, and type D trichothecenes

Trichothecene	R1	R2	R3	R4	R5
Type A Trichothecene					
HT-2 toxin	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
T-2 toxin	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
Diacetoxyscrirpentriol	OH	OAc	OAc	H	H
Type B Trichothecene					
Deoxynivalenol	OH	H	OH	OH	O
3-acetyl- deoxynivalenol	OAc	H	OH	OH	O
15-acetyl- deoxynivalenol	OH	H	OAc	OH	O
Nivalenol	OH	OH	OH	OH	O
Fusarenon X	OH	OAc	OH	OH	O

OH: hydroxyl group; OAc: acetoxyl group; H: hydrogen; $OCOCH_2CH(CH_3)_2$: ester-link isovaleryl (Eriksen, 2003)

Toxicity of trichothecenes

The unavoidable consumption of foods and feeds contaminated with trichothecenes causes significant injuries to human and both of the terrestrial animals and fish (Friend et al., 1982; Hooft et al., 2011; Prelusky et al., 1994; Sudakin, 2003; Woodward et al., 1983). Trichothecenes are normally neither degraded nor hydrolyzed in the stomach after ingestion because they are stable at neutral and acidic pH (Eriksen, 2003; Ueno, 1987). The solubility of type A trichothecene is dissolved in non-polar solvents like ethyl acetate and diethyl ether. In contrast to type A trichothecene, type B trichothecenes are soluble in polar solvents like acetonitrile, alcohols and water (Ueno, 1987).

In terms of absorption and excretion, trichothecenes are rapidly absorbed and distributed to the blood stream; for instance, pigs absorb trichothecenes, such as T-2 toxin, DON and NIV into blood less than half an hour after oral exposure to the toxins and the trichothecenes are suddenly excreted after oral intake or intravenous exposure of the toxins (Eriksen and Pettersson, 2004). Because of rapid absorption, trichothecenes are rapidly within excreted with few days after oral exposure to the toxins rather than accumulate in body tissues. Urine is the main route of trichothecene excretion in case animals are injected with the toxins; additionally, feces are also the other route of toxin elimination in case animals are orally exposed to the toxins (Pollmann et al., 1985; Sudakin, 2003).

Metabolism of trichothecenes: there are some published reports reviewed on the kinetics of trichothecenes in animals (Eriksen and Pettersson, 2004; Mostrom and Raisbeck, 2012; Sudakin, 2003). The active sites on trichothecene skeleton of which the substrates become toxic include 12, 13-epoxytricothecene nucleus, the 9-ene moiety and the metabolic ester formed by parent hydroxyls. The metabolism of trichothecenes is involved in four metabolic reactions as detoxification of trichothecenes. For example, hydrolysis and oxidation (in Phase I) and glucuronide conjugation (in phase II) occur in body tissues, and reduction of the 12, 13-epoxide (de-epoxidation) is carried out by microbes in the gastrointestinal tract (Mostrom and Raisbeck, 2012; Sudakin, 2003).

In terms of hydrolysis, the C-9,10 double bond both of type A and B trichothecenes are hydrolyzed resulting in less toxicity (Eriksen et al., 2003; Eriksen,

2003). As for type A trichothecene, acetoxy group at C-4 of T-2 toxin and DAS, is de-acetylated by non-specific microsomal carboxyesterase (Ohta et al., 1978) and type B trichothecene, DON is directly deoxygenated at the oxide ring to a double bond resulting in a non-toxic excretable metabolite. Besides, glucuronidation is reported that it slowly takes place in pigs (Bauer et al., 1969). The de-epoxidation is regarded as an important reaction in the detoxification of trichothecenes in animals. The gut microbes in intestines of animals, such as ruminants and poultry, are able to detoxify with splitting off the epoxide righ at C12-13 of the toxin structures like T-2 toxin, DAS and DON by de-epoxidation (King et al., 1984; Swanson et al., 1988; Worrell et al., 1989).

The effects of trichothecenes in molecular level: trichothecenes, especially DON and T-2 toxin, are known as inhibitors of the protein synthesis by binding to the peptidyl transferase, which is an integral part of the 60S ribosomal subunit in animal cells that 9,10 double bond and the C-12,13 epoxide to play the role on inhibition of the protein synthesis (Feinberg and McLaughlin, 1989; Mostrom and Raisbeck, 2012). However, type A and B of trichothecene mycotoxins inhibit protein synthesis at different action site of the process such as initiation, elongation and termination. The initiation step of protein synthesis is exhibited by T-2 toxin and the elongation and termination steps of protein synthesis are inhibited by DON (Mostrom and Raisbeck, 2012). Besides, trichothecenes also inhibits both of the DNA and RNA synthesis resulting in a secondary effect of the inhibition of the protein synthesis (Eriksen and Pettersson, 2004). *In vivo* study on effect of dietary DON in pigs, protein synthesis was statistically reduced in kidneys, spleen and ileum of pigs fed dietary concentration of 5.7 ppm DON for four weeks, but decrease in protein synthesis of liver, skeletal and cardiac muscle, mesteric lymph nodes, duodenum, jejunum, pancreas and lung were not significantly observed in this study (Dänicke et al., 2006). Additionally, Poapolathep et al. (2002) reported that trichothecenes induce apoptosis (a form of programmed cell death) in thymus and spleen tissues. In a case of *in vitro* study in rat cells, T-2 toxin is demonstrated to be the inhibitor of protein synthesis in mitochondria and electron transport action (Mostrom and Raisbeck, 2012).

The effects of trichothecenes on brain neurotransmitter: several studies have demonstrated that trichothecenes (e.g. DON and T-2 toxin) alter brain regional

neurochemistry such as serotonin (5-hydroxytryptamine, 5-HT) in pigs and rats after exposure to trichothecenes leading to decrease in feed intake (Fioramonti et al., 1993; Fitzpatrick et al., 1988; Forsyth et al., 1977; Swamy et al., 2002). Serotonin is an important neurotransmitter in brain and also presents in the enterochromaffin cells of the intestinal mucosa (Fioramonti et al., 1993). Serotonin is synthesized from an essential amino acid tryptophan by the enzyme tryptophan 5-monooxygenase. The role of serotonin in the brain is involved in the triggering of the onset of sleep, mood, muscle coordination and feed intake (Leathwood, 1987). As evidence of a possible link between trichothecene-induced vomiting and serotonin activity in animals, the hepatic protein synthesis is inhibited by exposure to trichothecenes resulting in increased concentration of free amino acid in blood (hyperaminoacidemia). This circumstance subsequently leads to increased brain uptake of tryptophan from the gastrointestinal tract and crosses the blood brain barrier and finally causes the synthesis of serotonin in the brain (Dillenburger et al., 2001; Leathwood, 1987; Rotter, 1996; Smith, 1992). The similar effects of DON and T-2 toxin on serotonin activity in pigs and rats were studied by Swamy et al. (2002) and Fitzpatrick et al. (1988). The ratio of 5-Hydroxyindoleacetic acid (5-HIAA, 5-HT metabolite) to 5-HT ratio was elevated in hypothalamus and pons (a part of brainstem) of pigs fed a blend of grains naturally contaminated with *Fusarium* mycotoxins. Similarly, the serotonin concentration of rats orally dosed with DON and T-2 toxin were significantly increased in all brain regions. Although the mechanisms involved in the emetic action of trichothecenes are poorly understood, 5-HT_3 receptors (serotonin-3 receptors) have reasonably been found to be associated with emesis and the control of gastric motility (Fioramonti et al., 1993; Rotter, 1996). The possibility of feed refusal and vomiting in animals can thus be described that DON inhibits gastric emptying by acting on small intestine motility and that effect is mediated through 5-HT_3 receptors in the gastrointestinal tract of rats (Fioramonti et al., 1993). Similarly, Swamy et al. (2002) reported that altered brain neurochemistry in pigs provided a potential mechanistic basis for the feed refusal effect of *Fusarium* mycotoxins.

2.6.1.1 Deoxynivalenol (DON) and its toxicity

Deoxynivalenol (DON), which is also known as Vomitoxin (Figure 7), has a C-9,10 double bond and a 12, 13-epoxide ring at C-12 and C-13

positions which acts toxic in the structure is considered as type B trichothecene. DON consists of hydroxyl group at C-3, C-7 and C-15 positions and is produced by *F. graminearum* and *F. culmorum*, which frequently occur in corn, wheat, barley, rice, soybean and other grains in the field where most commonly detected often at the ppm level (Pestka, 2007; Pestka and Smolinski, 2005; Rotter, 1996). The two *Fusarium* species are the plant pathogens and cause outbreaks of *Fusarium* head blight (also called wheat scab). The most serious outbreaks of the disease occur in years with heavy rainfall during the flowering season. *Fusarium* infections of cereals lead to severe yield loss and reduced kernel quality in addition to the occurrence of toxins in cereals (CAST, 2003; Placinta et al., 1999; Scott, 1989). DON inhibits the synthesis of DNA, RNA and protein synthesis at the molecular level by binding to the 60S ribosomal subunit (Feinberg and McLaughlin, 1989; Rotter, 1996). The most common symptoms resulting from consumption of feeds contaminated with DON in farm animals are reduced feed intake, feed refusal and/or vomiting. Other toxic effects of DON include diarrhea, intestinal inflammation, gastrointestinal hemorrhage and either immunosuppression or immunostimulation depending on dose and duration of exposure (Pestka, 2007; Pestka et al., 2004).

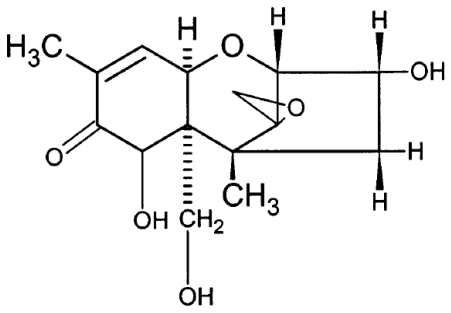

Figure 7 Chemical structure of Deoxynivalenol (Hussein and Brasel, 2001)

1) DON toxicity on terrestrial animals

Terrestrial animal species are susceptible to DON with differential sensitivity because of differences in metabolism, absorption, distribution and elimination of DON (Eriksen, 2003). According to Rotter (1996) and Pestka

(2007), the order of decreasing sensitivity to DON of animals was summarized as following: pigs > mice > rats > poultry ≈ ruminants.

In pigs, there are several studies, in most cases, on feeding of diets contained with DON from naturally or artificially contaminated grains, it is stated that the exposure to DON as low as 1 ppm DON significantly affects growth performance of pigs resulted in the reduction of growth rate, weight gain, feed take and feed conversion efficiency (Friend et al., 1982; ØVernes et al., 1997; Pollmann et al., 1985; Young et al., 1983). Additionally, the symptoms of oral and intraperitoneal exposure to high concentrations 12 and 20 ppm DON include complete feed refusal and vomiting, respectively (Forsyth et al., 1977; Young et al., 1983). In cases of using wheat naturally contaminated with *Fusarium* mycotoxins as a toxin source, feeding of diets containing graded concentrations of DON ranging from 0.13 to 3.06 ppm from wheat naturally contaminated with *Fusarium* mycotoxins for three weeks significantly resulted in a dose-dependent decrease in weight gain and feed intake of young pigs (initial body weight = 21 kg each; Friend et al., 1982). Similarly, feeding of dietary wheat containing graded concentration of DON (0, 1.2, 2.4, and 3.6 ppm) for three weeks linearly reduced feed intake, weight gain and feed conversion efficiency of pigs (initial body weight = 7.7 kg each). In this study, traces of DON (8-28 ppb) were found in the liver and kidney, but no vomiting and no lesion related to DON ingestion were observed. The author suggested that intake of diet exceeded 1 ppm DON could cause negative effects in pigs (Pollmann et al., 1985).

In addition to diets containing DON from wheat naturally contaminated with *Fusarium* mycotoxins, corn naturally contaminated with DON as a toxin source was used to investigate the performance of pigs. Piglets (initial body weight = 7.3 kg each) fed diets containing 1.3 ppm DON or higher (ranging between 0.14 and 11.9 ppm) from naturally contaminated corn caused a linear reduction in weight gain and both of the linear and quadratic reduction in feed intake and feed conversion efficiency. Nonetheless, vomiting and lesion related to DON digestion did not occur in pigs fed with high concentrations of DON (Young et al., 1983). This is consistent with the finding of Lun et al. (1985) which a significant depression of feed intake, weigh gain and feed conversion efficiency in pigs (initial body weight = 8.4 kg each) were related to feeding of 10.5 ppm DON for three weeks.

According to the author, the poor performance resulted from the depression of a mineral absorption and metabolism in pigs might be associated with the consumption of dietary DON (Lun et al., 1985). Based on previous studies, both of the naturally sources (wheat and corn) of DON are causative and adversely affected on performance of pigs. Some reports indicate that the natural form of DON is greater toxic than purified form of DON in pigs exposed both forms with the equal concentrations (Forsyth et al., 1977; Trenholm et al., 1994).

In poultry, weight gain and feed intake of chickens fed contaminated grains linearly decreased with the inclusion of grains containing 9.5 ppm DON during 21-42 days (8-week feeding trial), but the diets did not significantly affect on body weight gain and feed intake at the end of the experiment (Swamy et al., 2004). As well as, feed conversion efficiency was not affected by experimental diets. It is harmonious with the studies of Hamilton et al. (1985) and Yegani et al. (2006) conducted the experiments by feeding diets containing 4.9 and 12.6 ppm DON from naturally contaminated grains in hens and roosters, respectively. No significant effects on those parameters were observed. However, there are some conflicting results from the study of Awad et al. (2011) which high concentrations above 5 ppm DON diet depressed the productivity of laying hens and chickens, but there was no clear evidence of a dose-response relationship. The different results might be due to toxin source or age. There is also some evidence of sex differences of response to toxin. Eriksen and Pettersson (2004) reports that male chickens are more tolerant to naturally contaminated diets with DON than female chickens.

In ruminant, consumption of diets naturally contaminated with 3.2 ppm DON for eight weeks did not affect body weight gain, feed intake, milk production or milk composition in dairy cows (Korosteleva et al., 2007). The other chronic studies demonstrated that diets naturally contaminated with 6 to 8.5 ppm DON did not appear to affect the performance and milk production of dairy cows (Charmley et al., 1993; Ingalls, 1996; Trenholm et al., 1985). At a high concentration of DON, feeding of 66 ppm, to dairy cows for five days did not depress the performance (Côté et al., 1986).

The prolonged dietary exposure to DON in animals resulted in seriously deleterious effects on animal health indicators includes

haematological, biochemical and histopathological parameters. In a 12-week and 3-week trials, hemoglobin and haematocrit of pigs were reduced by 3.5 ppm DON (from naturally contaminated oats) and 10.5 ppm DON (from naturally contaminated corn) reported by Lun et al. (1985) and Bergsjø et al. (1993), respectively. Furthermore, there are some evidences of the relationship between organ weights (e.g. liver, spleen, intestines and kidneys) to dietary DON concentration. In the finding of Bergsjø et al. (1993) and Trenholm et al. (1994), feeding of naturally contaminated diets containing with DON at up to 3.9 ppm to growing pigs for seven weeks or at 3.5 ppm for 12 weeks resulted in the increase in weights of liver, stomach and kidney, but no alteration in histological changes, carcass composition and biochemical parameters was reported. In contrast, Drochner et al. (2006) reported that piglet fed on 0.3 to 1.2 ppm DON for eight weeks resulted in a dose-dependent increase of plasma aspartate aminotransferase activity. Similarly, Tiemann et al. (2006) reported that oral exposure to dietary DON from naturally contaminated wheat was associated with hepatotoxicity in pigs. Histopathological and ultrastructural examination in liver were observed including increases in hemosiderin, number of vacuoles, fatty droplets in cytoplasm, autophogosomes and thickness of interlobular connective tissue with increase collagen fibrils; and also the loss of bounds ribosomes from endoplasmic reticulum membrane was founded in pigs fed naturally contaminated diets with 6.1 and 9.3 ppm.

2) DON toxicity on fish

The effects of DON contamination in animal feed are not only associated with the reduction of growth performance and health of terrestrial animals but also fish. Few reports on reduction of growth performance and health, feed intake and feed conversion efficiency are associated with exposure to DON (Hooft et al., 2011; Woodward et al., 1983). Nonetheless, these observed signs are similar to clinical signs in terrestrial animals exposed to dietary DON. The vomiting do not appear in fish when fed with high concentrations of dietary DON (Hooft et al., 2011; Manning, 2005; Manning et al., 2013; Woodward et al., 1983). Woodward et al. (1983) observed that rainbow trout refused or spited out dietary pellets containing very high concentrations of DON.

Only few studies were reported on adverse impacts of dietary DON on aquatic species, such as rainbow trout (*Oncorhynchus mykiss*) and

channel catfish (*Ictalurus punctatus*) (Woodward et al., 1983; Hooft et al., 2011; Manning, 2005; Manning et al., 2013). As results of the previous studies of fish fed on dietary DON, these demonstrate that each fish species is differently sensitive to dietary DON. The first study on effects of DON on fish was carried out by Woodward et al. (1983). An artificially infected crop as DON source of corn were used to formulate six experimental diets for feeding juvenile rainbow trout (initial body weight = 50 g/fish) in the 8-week feeding trials, resulting in graded concentrations of 19.4 to 109.6 ppm DON for the first feeding trial. The second trial, the experimental diets used in the second trial was similar to the first one but contents of the contaminated corn was less included, resulting in graded concentrations of 1 to 12.9 ppm DON for the second feeding trial. At the end of the 8-week trial, results of the first trial, juvenile rainbow trout fed with dietary DON up to 20 ppm exhibited feed refusal in the first four weeks. However, some of DON-treated fish groups showed the recovery of feeding behavior from exposure to dietary DON when contaminated diets were switched with control diet (with no DON) to feed those fish through eight weeks. The results of the second trial, feeding of diets containing graded concentrations of DON (ranging from 1.0 to 13.0 ppm) to rainbow trout caused the significant reductions in feed intake, growth rate and feed conversion efficiency (Woodward et al., 1983).

The study on the effects of dietary DON on rainbow trout was confirmed by the study of Hooft et al. (2011). Three sources of DON from naturally contaminated corn which were sorted into three groups, including clean corn (0.6 ppm DON), and moderately (10.5 ppm DON) and highly (20.9 ppm DON) contaminated corn, were used to prepare seven experimental diets. Diets with increasing concentration of DON (0.3, 0.8, 1.4, 1.9, 2.0 and 2.6 ppm) fed to rainbow trout (initial boweight = 24 g/fish) for eight weeks. The results of 8-week feeding trial showed the significant linear or quadratic decreases in growth rate, weight gain, feed intake, feed conversion efficiency, retained nitrogen, recovered energy, energy retention efficiency and nitrogen retention efficiency. Apparent digestibility coefficients of crude protein and gross energy were not affected by feeding diets containing DON ranging from 0.3 to 2.0 ppm. Histopathological examination, liver of fish fed dietary

concentration of 1.4 and 2.6 ppm DON exhibited alterations of liver, such as fatty infiltration and, pyknosis and karyolysis in hepatocytes (Hooft et al., 2011).

A comparison of the results of two studies between Hooft et al. (2011) and Woodward et al. (1983) using the dietary DON as low as 1 ppm to feed rainbow trout for weight weeks showed similar deleterious effects, in terms of the significant reduction in weight gain, feed intake and feed conversion efficiency. Although the same fish species used in the studies of Hooft et al. (2011) and Woodward et al. (1983), in terms of a dose-response relationship, Hooft et al. (2011) suggested that rainbow trout used in this study were more sensitive to low diet concentrations of DON than the study of Woodward et al. (1983). Differences in the performance of fish in the study of Hooft et al. (2011) and Woodward et al. (1983) may be due to many factors such as differences in nutritional and health status of fish prior to DON exposure, differences in environmental conditions and differences in source of DON (Hooft et al., 2011; Rotter, 1996).

There were the other studies on effects of DON on channel catfish reported by Manning et al. (2013) and Manning (2005). Two feeding trials of channel catfish were conducted by Manning (2005). In the first trial, juvenile catfish (initial body weight = 6.3 g/fish) did not exhibit any negative effects on feed intake, growth rate, hematocrit values or liver weight when fed diets contained purified form of DON at 0, 1.25, 2.5, 5.0, and 10.0 ppm. In the second trial, wheat naturally contaminated with 37.5 ppm DON used as toxin source was prepared for six experimental diets containing 0, 2.5, 5.0, 10.0 15.0 and 17.5 ppm DON. The reduction in growth rate or feed intake of juvenile catfish (initial body weight = 5.0 g/fish) was not caused by feeding of 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. This corresponds to the finding of Manning et al. (2013), which 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 body weigh = 5.9 g/fish). Based on those studies, it appears that channel catfish are more resistant to dietary DON than rainbow trout.

For DON metabolism in fish, *in vitro* studies of DON on fish were investigated the ability of fish livers and intestinal microorganisms to metabolize DON (Guan et al., 2009; Maul et al., 2012). The finding of Guan et al. (2009) indicated that detoxification of DON by microorganisms in fish alimentary tract was associated with transformation of DON to be DOM-1 (deepoxy-deoxynivalenol) in the de-epoxidation. Base on results of the ability of microorganisms collected from gastrointestinal tracts of nine species (e.g. catfish, pike, bass, sucker, perch, sunfish, crappie, trout and salmon) to transform DON to DOM-1, it was found that the microorganisms of catfish (*Ameiurus nebulosus*) had the high ability to transform some amount of DON to DOM-1 in abroad range of temperatures (ranging between 4 and 25˚C) and pH value from 4.5 to 10.4. However, the complete transformation of DON to DOM-1 of microbial culture C133 was at 15 ˚C in full medium after 96 h incubation (Guan et al., 2009). In case of the other *in vitro* study on hepatic metabolism of DON by liver of carp and liver (Maul et al., 2012), hepatic microsomes were able to transform DON to DON-3-glucuronide (none toxic metabolite) in the glucuronidation activity.

2.6.1.2 T-2 toxin

T-2 toxin, is one of the trichothecenes and classified as type A of the trichothecenes, produced by *Fusarium sporotrichioides* and *F.poae* which normally occur in feeds and feed ingredients, such as wheat, soybean meal, finished feed and straw (Binder et al., 2007; Ueno et al., 1973). Its structure (Figure 8) is 4β,15-diacetoxy-3α-hydroxy-8α-[3-methylbutyryloxy]-12,13-epoxytrichothec-9-ene that is soluble in non-polar solvents, like ethyl acetate and diethyl ether (Hussein and Brasel, 2001; Ueno et al., 1973). The effects of T-2 toxins at the cellular level are to inhibit protein synthesis in mitochondria and electron transport action (Mostrom, 2011). Generally, negative effects of exposure to T-2 toxin are concerned in irritation of skin and gastrointestinal tract, induced immunosuppression and mortality (Hasan and Halwart, 2009; Hsu et al., 1972; Mostrom, 2011). However, according to Sokolović et al. (2008), reductions of feed intake and weight gain are the first visual signs of exposure T-2 toxin.

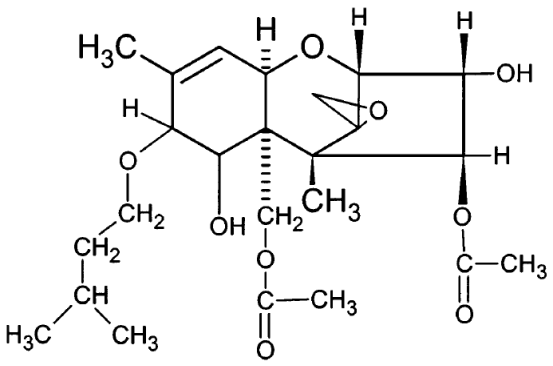

Figure 8 Chemical structure of T-2 toxin (Hussein and Brasel, 2001)

1) T-2 toxin toxicity on terrestrial animals

The negative effects of exposure to T-2 toxin in animals are considered being similar to DON that is associated with alterations of protein synthesis, neurotransmitter, immune, cell membrane function and lipid peroxidation (Mostrom and Raisbeck, 2012). In case studies of poultry, particularly laying hens, exposure to a low concentration of T-2 toxin (1 ppm) was reported to cause decreases in a thinness of egg shell, egg production, low hatchability, and changed feather quality. Moreover, server necrotic lesion in the mouth, crop, gizzard intestinal mucosa and liver were found based on histopathological examination (CAST, 2003; Sokolović et al., 2008). *In vivo* study, one-day male broiler chickens were fed graded concentration of dietary T-2 toxin (0, 1, 2, 4, 8, and 16 ppm purified T-2 toxin) for three weeks. The growth rate of chickens was significantly reduced by feeding of 4, 8 and 16 ppm T-2 toxin, whereas feed conversion ratio was unaffected by any concentration of T-2 toxin. The relative weights of organs, such as spleen and bursa of fabricius, were reduced while the relative weight of the crop was increased, but the liver weight was not affected by any concentration of T-2 toxin. Furthermore, alterations of hematological and biochemical parameters (e.g. hemoglobin, serum protein, serum cholesterol, serum total lipid and plasma uric acid) were not observed (Wyatt et al., 1973). In cows, Hsu et al. (1972) reported that 20% of lactating Holstein cows died after prolonged consumption of a diet containing 2 ppm T-2 toxin from

naturally contaminated in corn for five months and the postmortem examination exhibited a hemorrhagic syndrome on the serosal surface of all internal viscera.

In a case study at high concentration of purified T-2 toxin, female pigs (approximately initial body weight = 31-43 kg/each) exposed to 15 ppm of purified T-2 toxin showed anorexia and lethargy during the first week after exposed to the toxin. The adverse development of the morphological alteration (e.g. swollen skin and necrotizing dermatitis) appeared at dorsal skin of pig after applied with a dose of 15 ppm purified T-2 during 14 days. Moreover, necrosis of internal organ cell, especially lymphoid tissue and pancreas, was observed after 14-day dosing. Only trace of T-2 toxin was detected in dorsal skin, but not in plasma, bile or urine (Pang, Swanson, et al., 1987). In the other study of Pang, Felsburg, et al. (1987), male pigs (approximately initial body weight = 17.5-23.5 kg/each) showed the similar signs of intoxication as female pigs during the first three day after exposure to 15 ppm purified T-2 toxin. Likewise, a group of T-2 toxin treated pigs had lower weight gain than a control group (0 ppm T-2 toxin).

2) T-2 toxin toxicity on fish

One of the important toxins in trichothecenes is T-2 toxin, which occurs in grains used in the production of animal feeds. Although T-2 toxin is not found as frequent as DON in the feedstuff, T-2 toxin is reportedly the most toxic among any other toxins in trichothecene mycotoxins (Mostrom and Raisbeck, 2012). The deleterious effects of T-2 toxin in rainbow trout (*Oncorhynchus mykiss*) have been investigated in both acute and chronic toxicities (Marasas et al., 1969). In a case of chronic trial, fingerling rainbow trout (initial body weight = 50 g/fish) fed on diets containing 0.2 and 0.4 ppm purified T-2 toxin for 12 months had significantly greater reduction in fish fed with the diet control; nonetheless, no evidence of the carcinogen was caused by the long-term feeding of dietary T-2 toxin. For the acute trial, rainbow trout was orally exposed to a single feeding of dietary T-2 toxin at 2, 4 or 8 ppm for one day. The result showed that no significant difference in the mortality was observed even in the highest concentration of dietary T-2 toxin (8 ppm; Marasas et al., 1969). Besides, Poston et al. (1982) reported on significant decreases in growth rate, feed intake, feed conversion efficiency, hematocrit value and blood hemoglobin concentration in rainbow trout (initial body weight = 1 g/fish) were

caused by feeding graded concentration of dietary semi-purified T-2 toxin at 0, 0.1, 2.5, 5.0 10.0 and 15.0 ppm for 16 weeks. Additionally, exposure to the purified T-2 toxin at 15 ppm for four days induced hemorrhaging in the intestines of adult rainbow trout (initial weight = 300 g); anyhow, the use of diet measured as an apparent metabolizable energy and nitrogen digestibility, and activity of intestinal lumen chymotrypsin or trypsin in adult rainbow trout (initial body weight = 300 g/fish) were not significantly affected by the exposure to T-2 toxin at 15 ppm (Poston and Coffin, 1982).

In channel catfish, Manning et al. (2003) studied the influence of feeding a semi-purified T-2 toxin at 0, 0.625, 1.25, 2.5 and 5.0 ppm to channel catfish **(**initial body weight = 8.7 g/fish**)** for eight weeks. The growth rate of fish fed control diet was significantly higher than other groups fed with the dietary T-2 toxin. The fish receiving the dietary T-2 toxin up to 2.5 ppm showed poor feed conversion ratio. Hematocrit value was deleteriously affected in fish fed diets with inclusion of 1.25, 2.5 and 5.0 ppm T-2 toxin. In the pair-feeding trial, the comparison of control diet with three diets containing the highest concentration of T-2 toxin at 1.25, 2.5 and 5.0 ppm. The other trial, similar size of channel catfish was used for the pair-feeding trial to compare differences in weight gain, feed conversion ration hematocrit and survivability of fish fed the highest concentrations of three dietary T-2 toxin (at 1.25, 2.5 and 5.0 ppm) or control diet (with no T-2 toxin). An 8-week interval, weight gain, hematocrit value and survivability of fish from control group were higher than fish fed the dietary T-2 toxin. Moreover, the oral exposure to the three highest concentrations of dietary T-2 toxin in fish significantly altered the stomach, and head and truck kidneys of fish (Manning et al., 2003).

Another study in channel catfish was to prove that the disease resistance of juvenile channel catfish (initial body weight = 6.4 g/fish) could be impaired by oral exposure to T-2 toxin. Feeding feedborne T-2 toxin (1.0 and 2.0 ppm semi-purified T-2 toxin) to fish when challenged with *Edwardsiella ictaluri* for six weeks had significantly greater mortality of fish without the challenge of *E ictaluri* (Manning et al., 2005).

2.6.2 Fumonisins and their toxicity

Fumonisins are ones of the *Fusarium* mycotoxins produced by *Fusarium verticillioides* and *F. proliferatum* (Voss et al., 2007). They commonly occur in animal feeds, worldwide (Binder et al., 2007; Placinta et al., 1999; Rodrigues and Nährer, 2012b). Though several fuminisin mycotoxins (e.g., Fumonisin B_1, Fumonisin B_2, Fumonisin B_3, Fumonisin B_4) commonly produced by *F. verticillioides*, *F. proliferatum* and other *Fusarium* fungi, Fumonisin B_1 (FB_1, Figure 9) is the most plenty fumonisin (approximately 70% of total fumonisin content in fumonisin-contaminated grains), which is associated with mycotoxicoses of animals (Manning and Abbas, 2012; Voss et al., 2007). Owing to its chemical structure is similar to the sphingoid bases; it disrupts the sphingolipid metabolism in many kinds of animal cell, namely hepatocytes, neuronal and renal cells. Wang et al. (1991) stated that FB_1 inhibited the process of sphingolipid biosynthesis resulting in accumulation of free sphinganine in liver, kidney and serum.

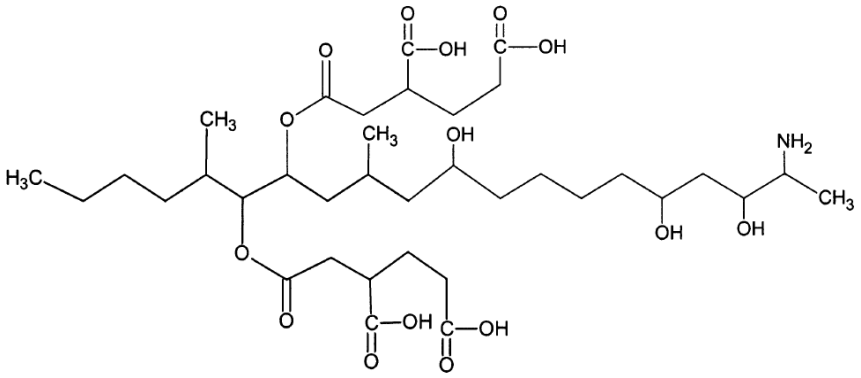

Figure 9 Chemical structure of Fumonisin B_1 (Hussein and Brasel, 2001)

2.6.2.1 Fumonisin B_1 toxicity on terrestrial animals

In animals, pathological effects of exposure to FB_1 include immunological alteration, hepatotoxicity, nephrotoxicity, neurotoxicity and carcinogenicity; furthermore, FB_1 is pronounced to be the causative agent of equine leukoencephalomalacia in horses and porcine pulmonary edema in pigs (Hussein and Brasel, 2001; Voss et al., 2011). Pig is the animal species which have

been extensively studies for influence of exposure to FB_1 because of its unique symptom, especially porcine pulmonary edema, which causes the death of pigs after exposing to high concentration of FB_1 (Hussein and Brasel, 2001; Voss et al., 2007). Nevertheless, decrease in feed intake is usually the first sign of exposure to FB_1 in pigs. In cows, appearing different sensitivity of cows to exposure to FB_1 depending upon difference in ages is reported by Voss et al. (2007). According to the short-term study of Mathur et al. (2001), FB_1 is reported to be hepatoxic and nephrotoxic to claves. The intravenous injection of 1 ppm FB_1 to the claves for seven days induced to increase concentration of SO and SA in the liver, lung, kidney, heart and skeleton muscle; besides, the hepatic and renal damage displayed after two to four days of FB_1 treatment (Mathur et al., 2001). Unlike cows are tolerant to FB_1, the claves appeared to be more sensitive to FB_1 (Voss et al., 2007). Plasma concentration of SA/SO was not observed in cows were orally exposed to 5.0 ppm FB_1 body weight (Prelusky et al., 1995). In poultry, chickens are pronounced to be as tolerant to FB_1 as cows. One-day-old chicks fed diets contaminated with FB_1 isolated from culture material for 21 days significantly decreased in weight gain and increased in liver weight with increasing concentration of FB_1 ranging from 100-400 ppm; in addition to the histopathological evidence, feeding of diets containing ≥100, 200 and 300 ppm FB_1 caused thymic cortical atrophy, hepatic necrosis and biliary hyperplasia (Ledoux et al., 1992). Furthermore, concentration of serum SA/SO ration in chicks was significantly altered by ≥75 ppm FB_1 (Weibking et al., 1993).

2.6.2.2 Fumonisin B_1 toxicity on fish

In fish, effects of consuming diets contaminated with FB_1 have been investigated in channel catfish (*Ictalurus punctatus*), Nile tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*) (Kovačić et al., 2009; Tuan et al., 2003; Yildirim et al., 2000). The finding of Kovačić et al. (2009) demonstrated that FB_1 caused the neurotoxicity to in fish as well as in horses due to its ability to cross the blood-brain barrier of fish. In the 42-day chronic experiment on neurotoxicosis, the brains of one-year-old carp fed diets containing with FB_1 (0, 10, 100 ppm FB_1) isolated from a corn sample contaminated with *F. verticiloides* were evidently damaged: vacuolated, degenerated and necrotic cells. The result showed the significant differences in the mean number (according to the grading of histopathological

alteration) of apoptosis, lesion and inflammation in brain carp among groups fed control diet and diets containing 10 and 100 ppm FB_1.

In channel catfish, one-year-old and two-year-old catfish fed diets containing FB_1 (0.3, 20, 30, 80, 320 and 720 ppm FB_1) originally isolated from corn contaminated with *Fusarium moniliforme* for 10 and 14 days, respectively. In case of one-year-old catfish, weight gain significantly reduced by feeding of 20 FB_1. Catfish fed 80 ppm FB_1 had lower hematocrit value, red blood cell and white blood cell than those of catfish fed control diet and diets containing less 80 ppm FB_1. On the hand, two-year-old catfish were more tolerant to FB_1 than younger catfish. The weight gain of two-year-old catfish was significantly reduced by feeding of 80 FB_1 and hematocrit value, red blood cell and white blood cell were significantly affected by feed of 320 ppm FB_1. In addition, the microscopic examination in liver of both one-year-old and two-year-old catfish obviously exhibited swollen hepatocytes, lymphocyte inflitratation and scattered around necrotic hepatocytes when orally exposured to 20 ppm FB_1 or higher this concentration of FB_1 (Lumlertdacha et al., 1995). It is similarly to the finding of Yildirim et al. (2000), channel catfish fingerling (initial body weight = 1.5 g/fish) fed diets contaminated with FB_1 (0, 20 and 40 ppm FB_1) isolated from *F. moniliforme* culture material for 10 weeks. Catfish fed with 20 and 40 ppm FB_1 significantly had lower weight gain and feed intake, poorer feed conversion efficiency than those of catfish fed with control diet (0 ppm FB_1). Moreover, feeding of dietary concentration of 20 and 40 ppm FB_1 caused a significant decrease in hematocrit and a significant increase in ratio of free sphinganine and free sphingosine in the liver of catfish. Anyhow, the liver and heart of catfish did not exhibit any lesion related to consumption of FB_1 (Yildirim et al., 2000). In the other warm water fish, Nile tilapia fingerling (initial body weight = 2.7 g/fish) was investigated for deleterious effects of subchronic exposure to FB_1 on growth performance, histological anomalies and biochemical changes. Feeding of diets containing with 0, 10, 40, 70 and 150 ppm FB_1 to Nile tilapia showed that the suppressed weight gain and poor FCR of Nile tilapia significantly caused by feeding of containing with 40 or higher 40 ppm FB_1. Additionally, Nile tilapia fed with dietary concentration of 150 ppm ZON significantly had lower value of hematocrit and higher ratio of free sphinganine and free sphingosine than those of Nile tilapia

from control group and the other groups. However, histological alteration of organs, such as liver, spleen, stomach, pyloric intestine, heart and anterior kidney, was not reported in this study (Tuan et al., 2003).

2.6.3 Zearalenone (ZON) and its toxicity

Zearalenone, is a phytoestrogenic compound produced mainly by *Fusarium* fungi including *F. culmorum, F. graminearum, F. cerealis, F. crookwellense* and *F. semitectum,* is found to contaminate in cereal grains worldwide (e.g. corn, wheat, soybean, barley, straw, oat and rice) and animal feed ingredients (Placinta et al., 1999; Rodrigues and Nährer, 2012a; Zinedine et al., 2007). ZON is chemically described (Figure 10) as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid μ-lactone (Hussein and Brasel, 2001). Furthermore, two sterioisomeric metabolites of ZON, such as alpha zearalenol and beta zearalenol (α-ZON and β-ZON), which have no the keto group at C-8, are much less toxic than ZON (Zinedine et al., 2007). *Fusarium* fungi are the plant pathogens that infect cereal grains in the field causing head blight in wheat, barley and ear rot in maize. The toxin begins to contaminate in fields prior to harvest and it is stable during storage, milling, processing, and cooking because of thermo-stable capacity (CAST, 2003; Döll and Dänicke, 2011). Several surveys of worldwide contamination with mycotoxins have been reported that ZON occurs normally with DON in cereal grains and feed ingredients (Binder et al., 2007; Placinta et al., 1999; Rodrigues and Nährer, 2012b).

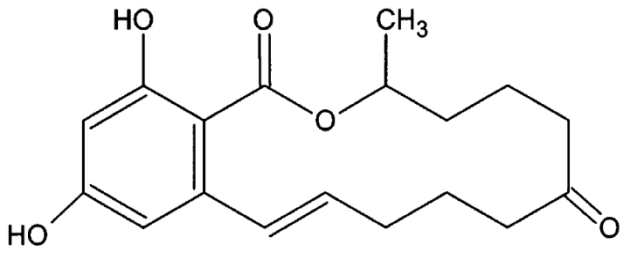

Figure 10 Chemical structure of zearalenone (Hussein and Brasel, 2001)

ZON is an estrogenic mycotoxin, of which the structure is an oestrogen-like structure, competes with body estrogens to bind to sites of oestrogen receptors; thus, effects of ZON toxicity on animals are mainly associated with

alterations of the urogenital system (Döll and Dänicke, 2011; Zinedine et al., 2007). Generally, clinical symptoms resulting from exposure to low levels of ZON (approximately 1 ppm) in female pigs, these are: hyproestrogenism, and swelling of the vulva and enlargement of mammary glands in prepubescent gilts. The symptoms of male pigs resulted from consuming diets containing ZON, testicular atrophy, swollen prepuce and mammary gland enlargement appear in young males. In case of adult male pigs, decreases in libido and spermatogenesis are reported (CAST, 2003; Mostrom, 2012).

2.6.3.1 ZON toxicity in terrestrial animals

Most of the researches on effects of ZON on animals mainly focus on pigs because this animal is considered as the most sensitive species to ZON, especially prepubertal swine (Mostrom, 2012). The relative binding affinity of ZON for estrogenic receptor is greater in pigs than in chicken and rats (Chi et al., 1980; Fitzpatrick et al., 1988). Exposure to ZON causes hyperestrogenism in terrestrial animals, especially pigs (CAST, 2003). *In vivo* long-term experiment (48 days) of immature female pigs, concentrations of 0, 20 and 40 μg/kg body weight were orally administered to pigs (12-month old, initial body weight = 40 kg/each). The decreases in granuloso cells proliferation of the ovarian follicle walls and connective tissue in ovarian stroma of pigs was associated with exposure to 20 and 40 μg/kg body weight; whereas, hormonal concentrations of testosterone and 17β-estradiol in blood plasma were not observed in this study (Gajęcka et al., 2011). In case of *in vitro* study of deleterious effects of ZON metabolites on the maturation rate and early embryonic development of pig oocytes, the rate of oocyte maturation at higher concentration of 7.5 μM α-ZON and the zygotes developing to blastocytes at higher concentration of 7.5 μM α-ZON were significantly different from the control groups. These results indicate that ZON metabolites have a direct effect on the maturation of porcine oocytes and an indirect effect on early embryonic development (Alm et al., 2002). Similarly, the effects of exposure to ZON in the cattle have been reported to possess an estrogenic potency as well as in pigs. Zinedine et al. (2007) reviewed that the exposure to 250 mg of purified ZON for a day resulted in the infertility and reduced milk production; besides, degeneration of the germinal

epithelium in male cattle was influenced by feeding of 20 ppm ZON for 72 days (Vanyi et al., 1980; Weaver et al., 1986) reported by Zinedine et al. (2007).

In contrast to pigs and cattle, chickens are stated to be more tolerant that pigs. Acute toxic effects of purified ZON on growing female White Leghorn chickens were studied by Chi et al. (1980). Chickens which were orally administered to a single dose of 15.0 g ZON per kg body weight (ZON in gelatin capsules) and allowed to survive for 10 consecutive days had lower level of serum calcium and higher level of serum phosphorus than chickens of a control group (empty gelatin capsules). Nevertheless, there were no differences between groups of ZON-untreated chickens and ZON-treated chickens in weight gain, oviduct, weights of comb and liver, hematological parameters, and serum cholesterol. Additionally, oviduct, comb and liver weights of chickens injected intramuscularly with ZON and estradiol 3,17-dipropionate as a standard exhibited the same effects, namely increased weights of liver and oviduct, and decreased comb weight. Based on these negative effects, particularly oviduct growth response, are interpreted as the relative estrogenic biopotency of ZON (Chi et al., 1980). Interestingly, certain reports show that ZON and its metabolites (α-ZON and β-ZON) can accumulate in muscle tissues, adipose tissues and reproductive tissues, and they can be also transmitted into milk and eggs, reviewed by Mostrom (2012), Hagler et al. (1980) and Mirocha et al. (1981).

2.6.3.2 ZON toxicity in fish

Influences of ZON toxicity were investigated in some fish species, including rainbow trout (*Oncorhynchus mykiss*), salmon (*Salemo salar*) and zebrafish (Danio rerio). ZON has low acute toxicity in animals as well as in fish; the effects of ZON exposure are mainly concerned the disturbance of reproductive activities. The *in vitro* study of rainbow trout was compared the competition of the binging affinities of ZON, its metabolites (α-ZON and β-ZON) and estradiol-17β to 17β-estrogenic receptors, the results showed that the binging affinities of α-ZON and ZON were approximately 1/150 and 1/300 to that of 17β-estradiol (Arukwe et al., 1999). However, Arukwe et al. (1999) reported that ZON and α-ZON possess estrogenic potencies that were approximately 50% compared to that of 17β-estradiol. A result of this *in vitro* estrogenic potency of ZON indicated that α-ZON was more toxic to rainbow trout than ZON. Besides, *in vivo* study of estrogenic potency of ZON

and its metabolites on juvenile salmon, increased amount of vitellogenin and *zona radiate* protein in plasma induced by ZON and α-ZON (1 and 10 mg/kg body weight) were found in groups of rainbow trout treated with 17β-estradiol, ZON and α-ZON but not in control group (untreated rainbow trout) (Arukwe et al., 1999). Similarly, the finding of Schwartz et al. (2011) could be confirmed the induction of plasma vitellogenin was associated with an exposure to ZON in zebrafish. A long-term experiment of exposure to 1000 ng/L ZON for 140 days caused a 1.5 fold induction of plasma vitellogenin in female zebrafish. Interestingly, ZON does not only disrupt reproductive activity of fish but also the accumulation in organs is reported. The accumulation of ZON in organs of rainbow trout, which were fed with commercial fish feeds, collected from three farms in north-eastern Poland was investigated by Woźny et al. (2013). The detection of ZON contamination in surface water, commercial fish feeds and selected organs (e.g. intestines, liver, ovary and dorsal muscle) showed that fish feeds from two of three farms contaminated with 0.08 and 0.1 ppm ZON; moreover, ovary was found to contain low concentration of ZON but neither in muscle, intestine nor liver (Woźny et al., 2013).

2.6.4 Masked mycotoxins and other *Fusarium* mycotoxins/metabolites

2.6.4.1 Masked mycotoxin

Deoxynivalenol-3-β-D-glucoside (DON-3-Glu, Figure 11) is the plant metabolite, formed from DON in infected plants with *Fusarium* mycotoxins deoxynivalenol (DON). The structure of DON is conjugated with glucose at C-3 to be a more polar molecule resulting in DON-3-Glu that is the response of plant metabolism to DON. Furthermore, DON-3-Glu is known as "masked mycotoxins" because it is not able to be screened for in foodstuffs or feedstuffs by routinely analytical methods (Berthiller et al., 2005). Occurrence of DON-3-Glu in naturally contaminated wheat and corn had been reported by Berthiller et al. (2005) for the first time and was later found in beer and other brewing intermediates reported by Kostelanska et al. (2009).

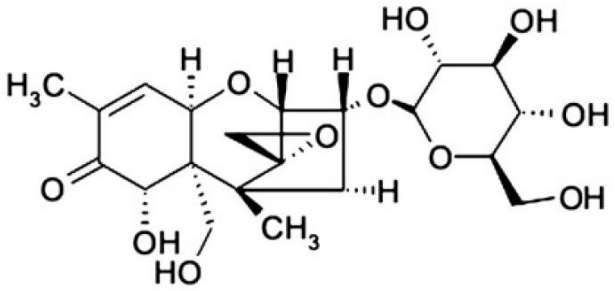

Figure 11 Chemical structure of deoxynivalenol-3-glucoside (Berthiller et al., 2005)

Even if known information of DON-3-Glu toxicity on animals lacks, *in vivo* study of Nagl et al. (2012) indicates that DON-3-Glu is less toxic to rats than DON. Recently, based on the *in vitro* and *in vivo* finding, the majority of DON-3-Glu intake was recovered as DON and DOM-1 in feces of rats. In similarity to *in vitro* study, most portion of DON-3-Glu was hydrolyzed by gut bacteria to be DON; however, DON-3-Glu was not hydrolyzed in acidic condition (Berthiller et al., 2011). DON-3-Glu is converted to DON in the digestive tract of mammals during digestion; a consequence of the gut bacteria activity may remain DON to affect animals depending on a difference in the ability of gut bacteria to hydrolyze DON-3-Glu (Nagl et al., 2012).

2.6.4.2 Other *Fusarium* metabolites

Since a newly analytical method, LC-MS/MS has been used for screening mycotoxins and other fungal metabolites in foodstuffs and feedstuffs, more than 80 metabolites, including well-known mycotoxins and unwell-known fungal metabolites, have been detected in cereal grains (Sulyok et al., 2006; Sulyok et al., 2007, 2010). According to a survey of mycotoxins contamination in samples of feed and feedstuffs from 2010 to 2012 using LC/MS-MS illustrated that mycotoxins co-occurred with other fungal metabolites in total 139 metabolites, such as *Aspergillus* metabolites, *Penicillium* metabolites and *Fusarium* metabolites (Streit et al., 2013). Unfortunately, there is no known knowledge available on the effects of those fungal metabolites.

Aurofusarin, a dimeric naphthoquinone, is the *Fusarium* metabolite mainly produced by numeral *Fusarium* fungi, particularly *F.graminearum*.

It is revealed that grains were contaminated with aurofusarin in Ukraine (Kotyl, 1999 reviewed in Dvorska et al., 2002). Little information on aurofusarin toxicity is available (Dvorska and Surai, 2001; Dvorska et al., 2002), researches were carried out the effects of aurofusarin on Japanese quails (*Coturnix japonica*). Feeding of diet contaminated with 26.4 ppm aurafusarin from *F.graminearum* cultured material for eight weeks significantly changed the fatty acid profile resulting in decreasing in the nutritional quality of egg yolk (Dvorska and Surai, 2001). Additionally, the development of embryo quails was exerted by 26.4 ppm aurafusarin measured as decreased contents of total caroteniod, vitamin E, lutein, and zeaxanthin in the livers (Dvorska et al., 2002). Besides, culmorin is the *Fusarium* metabolite mainly produced by *F.culmorum*, it was found to contaminate with other *Fusarium* mycotoxins in *Fusarium*-infected cereals (Ghebremeskel & Langseth, 2001; Langseth, 1998; Streit et al., 2013). Nevertheless, there is no available report on the effects of culmorin on animals.