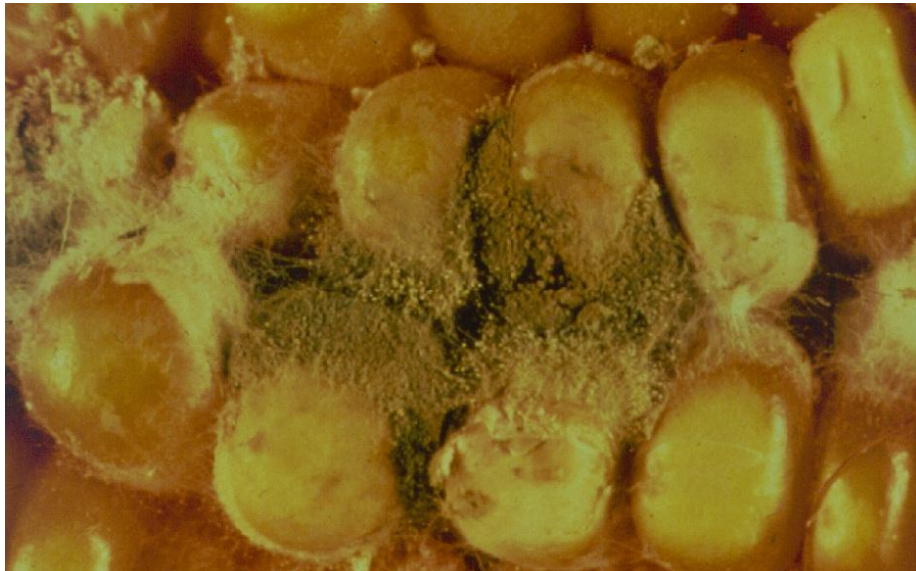




**MYCOTOXINS CONTAMINATION IN MAIZE  
KERNELS IN VIETNAM AND EFFECTS OF FEED  
ADDITIVES ON REDUCING FUMONISIN IMPACTS  
IN PIGS**

by

**Nguyen Hieu Phuong**



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**Institutionen för husdjurens utfodring och vård**

**MSc. Thesis**

**Swedish University of Agricultural Sciences  
Department of Animal Nutrition and Management**

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## **Dedication**

To my passed away grandfather

My father (Van Dung) and my mother (Minh Hieu)

# **Mycotoxins contamination in maize kernels in Vietnam and effects of feed additives on reducing fumonisin impacts in pigs**

Nguyen Hieu Phuong

*Nong Lam University, Department of Animal Nutrition, Ho Chi Minh City, Vietnam*

## **Abstract**

This thesis includes two papers related to mycotoxin problems in Vietnam. The aim of Paper I was to evaluate fumonisins, aflatoxins and zearalenone contamination in maize kernels in the Southeastern provinces and Central Highlands provinces of Vietnam. Paper II tested the detoxifying effects of a commercial additive and *Phyllanthus amarus* extract in pigs fed fumonisins contaminated feed.

A survey on the contamination of maize with aflatoxins, fumonisins and zearalenone was carried out in the Southeastern provinces and Central Highlands provinces in Vietnam, on 97 maize kernel samples. Four provinces were chosen for sampling maize: Dong Nai (22), Binh Phuoc (25), Dak Lak (30) and Dak Nong (20). Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), B<sub>2</sub> (AFB<sub>2</sub>), G<sub>1</sub> (AFG<sub>1</sub>), G<sub>2</sub> (AFG<sub>2</sub>), fumonisin B<sub>1</sub> (FB<sub>1</sub>), fumonisin B<sub>2</sub> (FB<sub>2</sub>) and zearalenone (ZEA) were detected by an HPLC method (Romer Labs, Singapore). Fumonisins was the most common type of toxin found in all samples (67.0%), followed by aflatoxins (55.7%) and zearalenone (27.8%). The incidence of aflatoxin positive samples in the Southeastern provinces (61.7%) was higher than in the Central Highlands (50%), while fumonisins and zearalenone incidences were contrary. The level of fumonisin B<sub>1</sub> in samples from the Central Highlands provinces (1757 ppb) was significantly different from that of the Southeastern provinces (740 ppb) (P<0.05). Moreover, the mean value of AFB<sub>2</sub> in the Highlands samples (20.7 ppb) tended to be higher than in the Southeastern provinces (10.5 ppb) (P=0.071). The co-occurrence of the three kinds of mycotoxin in the 4 provinces was very high, at 77.3, 80.0, 86.7 and 90.0%, respectively, in Dong Nai, Binh Phuoc, Dak Lak and Dak Nong. The incidence of AFB<sub>1</sub> found in Binh Phuoc, Dong Nai, Dak Nong, Dak Lak was 72, 50, 50 and 50%, with mean levels of 135, 55.7, 124, 201 ppb, respectively. AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub> had lower incidences and mean values, particularly AFG<sub>2</sub>, which was not found in samples from Dong Nai and Dak Nong. FB<sub>1</sub> incidences in Binh Phuoc, Dong Nai, Dak Nong and Dak Lak were 44, 68, 85 and 73%, respectively, with average concentrations of 642, 812, 1155 and 2223 ppb, respectively, and FB<sub>2</sub> also had lower incidences and mean values. Furthermore, ZEA concentrations and percentages of positive samples were low in Binh Phuoc and Dong Nai (40, 62 ppb and 12 and 9%, respectively) and were higher in Dak Nong and Dak Lak (456 and 210 ppb and 40 and 47%, respectively). The incidence of positive samples with high concentrations of aflatoxins was about 70%, while fumonisins and zearalenone had more positive samples with low concentrations (90 and 63%, respectively).

Although, aflatoxins are still the most important toxins in Vietnam, fumonisins also had high incidence and some samples had very high levels and they affect the liver of animals. The effects of a *Phyllanthus amarus* extract and a commercial detoxifying additive product were evaluated in protecting pigs from fumonisins with respect to growth performance, pathology and blood biochemistry. Forty eight crossbred (Landrace × Yorkshire × Duroc) weanling pigs were randomly assigned in a completely randomized design (CRD) to six diets containing: 1) low fumonisin B<sub>1</sub> and no feed additive (LFNA); 2) low fumonisin B<sub>1</sub> and commercial detoxicant additive at 1g/kg of feed (LFCA); 3) low fumonisin B<sub>1</sub> and *Phyllanthus amarus* extract at 10g/kg of feed (LFPE); 4) high fumonisin B<sub>1</sub> and no feed additive (HFNA); 5) high fumonisin B<sub>1</sub> and commercial detoxicant additive at 1g/kg of feed (HFCA); 6) high fumonisin B<sub>1</sub> and *Phyllanthus amarus* extract at 10g/kg of feed (HFPE). Fumonisin levels, detoxicants and their combination did not have any effect on final weight, average daily weight gain, average daily feed intake, and feed conversion ratio in pigs. Including 10 mg fumonisin B<sub>1</sub> in the diet decreased the total cholesterol significantly compared with the low fumonisin groups (2.19 mmol/L < 2.42 mmol/L) (P<0.05). The aspartate aminotransferase (AST) blood levels of pigs given the commercial additive were higher than in the no feed additive group (110 U/L > 83.1 U/L, P=0.067) and the *Phyllanthus amarus* group also had a high AST blood level (99.6 U/L). Moreover, fumonisin B<sub>1</sub> thickened the alveolar walls of the lungs, while the commercial and *Phyllanthus amarus* additives partly reduce the thickened alveolar wall lesions. Liver cells also had more severe fatty degeneration and necrosis in the fumonisin and no additive group than in the commercial and *P. amarus* groups. However *P. amarus* extract made the liver tender.

*Key words:* AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>, Binh Phuoc, Dak Lak, mycotoxins, Dak Nong, Dong Nai, fumonisin B<sub>1</sub>, blood biochemistry, aspartate aminotransferase, AST, lung, liver

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## **Appendix**

This thesis is based on the following papers, which are referred to by Roman numerals:

- I. Nguyen Hieu Phuong, Ogle B, Pettersson H, Nguyen Quang Thieu, 2010: Fumonisin, aflatoxins and zearalenone contamination in maize kernels in the Southeastern provinces and Central Highlands provinces of Vietnam
  
- II. Nguyen Hieu Phuong, Ogle B, Pettersson H, Nguyen Quang Thieu, 2010: Detoxifying effects of a commercial additive and *Phyllanthus amarus* extract in pigs fed fumonisin contaminated feed



## List of abbreviations

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2-AAF	2-acetaminofluorene
ADFI	Average daily feed intake
ADG	Average daily gain
AF	Aflatoxin
ALP	Alkaline phosphatase
ALT (GPT)	Alanine amino transferase (Glutamine Pyruvic Transaminase)
APP	Actinobacillus Pleuropneumonia
AST (GOT)	Aspartate Aminotransferase (Glutamic Oxaloacetic Transaminase)
Bilirubin D	Bilirubin direct
Bilirubin I	Bilirubin indirect
Bilirubin T	Bilirubin total
BW	Body weight
CAT	Catalase
CREA	Creatinine
DM	Dry matter
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
EE	Ether extract
ELEM	Equine leukoencephalomalacia
FB <sub>1</sub>	Fumonisin B <sub>1</sub>
FB <sub>2</sub>	Fumonisin B <sub>2</sub>
FCR	Feed conversion ratio
FUM	Fumonisin
GGT	Gamma Glutamyl Transpeptidase
GPx	Glutathione peroxidase
GST	Glutathione-S-transferase
Hb	Hemoglobin
HL-60	Human myeloid leukemia
LD	Lactatedehydrogenase
LW	Live weight
MCV	Mean corpuscular volume
MNNG	N-methyl N'-nitro-N-nitrosoguanidine
OTA	Ochratoxin A
PDA	Potato Dextrose Agar
PLC	Primary liver cancer
ppb	Part per billion
ppm	Part per million
SOD	Superoxide dismutase
ZEA	Zearalenone

---

## 1. Introduction

Maize is a food and feed crop that is commonly contaminated with mycotoxins, which are chemical products of molds, such as aflatoxins, fumonisins and zearalenone. The toxin producing molds infest and cause many diseases in the maize plant (Ritchie, 2002; Arora and Khachatourians, 2004; Osweiler, 1996). With its tropical monsoon climate, Vietnam has favourable conditions (high humidity and temperature) for the development of these fungi and for the production of mycotoxins (CAST, 2003). Maize in Vietnam plays an important role, as in many countries in the world (Thanh Ha et al., 2004; [http://www.fao.org/inpho/content/compend/text/ch23\\_01.htm](http://www.fao.org/inpho/content/compend/text/ch23_01.htm)). Although, maize is the source of several products for humans and animals, and maize production has risen sharply since 1990 in Vietnam, there are still many constraints in production; for example, drought, insect damage and flooding provide suitable conditions for mycotoxin formation (Thanh Ha et al., 2004; CAST, 2003).

In addition, mycotoxins are harmful to animal and human health and cause economic losses worldwide ([http://www.fao.org/ag/agn/agns/chemicals\\_mycotoxins\\_en.asp](http://www.fao.org/ag/agn/agns/chemicals_mycotoxins_en.asp)). Aflatoxins (AF), are well known for their toxicity and occurrence in food and feed, and are produced by *Aspergillus flavus*, *A. parasiticus*, *A. nomius* and *A. niger* molds (Ritchie, 2002). These mycotoxins have negative impacts on growth performance, liver function, and the immune system of poultry and pigs (Ledoux et al., 1998; Quezada et al., 2000; Hale et al., 1979; Schell et al., 1993; Silvotti et al., 1997). Moreover, they are liver cancer risk factor in humans (Wogan, 1992). Fumonisins are also a cause of outbreaks of equine leukoencephalomalacia disease in horses and porcine pulmonary edema in pigs (CAST, 2003). Besides, they induce hepatotoxicosis, changes of some serum enzymes for liver function, changes of sphinganine to sphingosine ratio, are a carcinogenic factor and have cardiovascular effects in animals (Osweiler et al., 1992; Casteel et al., 1993; Zomborszky et al., 2002; Tran et al., 2003; Smith et al., 1996a; Voss et al., 2001). There was found to be a relationship between fumonisins consumption and human esophageal cancer in China and Southern Africa (CAST, 2003). Zearalenone (ZEA), the second most frequently found mycotoxin in maize after deoxynivalenol (DON), is produced mainly by *Fusarium roseum* (*F. graminearum*), *F. culmorum* and *F. Sacchari* species. ZEA has an influence on the reproductive system of animals, especially swine, owing to its estrogenic toxicity (Nollet, 2000). It causes hyperestrogenism in females, lengthens the weaning to estrus interval and causes feminization in young male pigs (CAST, 2003; Young et al., 1989). Furthermore elevated blood parameters and serum enzymes in rabbits and rats revealed the effect on liver function (Maaroufi et al., 1996; Conkova et al., 2001). However, there are few studies and surveys about the contamination of mycotoxins in maize in Vietnam (Trung et al., 2007; Wang, 1995; Thieu, 2008) and this does not reflect the mycotoxin situation in the country. In addition, to reduce the detrimental effects of mycotoxins in Vietnamese animal production, farmers and feed factories use some decontamination methods, particularly mixing in feed additives to adsorb or to degrade mycotoxins. Almost all these feed additives come from foreign companies and are expensive. Thieu et al. (2008) tested the efficiency of a cheap local adsorbent, bentonite, as feed additive in preventing aflatoxicosis in piglets, but it did not show any positive effects. On the other hand, the Vietnamese usually use herbs as traditional medicine in treating intoxication of the human liver. *Phyllanthus amarus* is a popular traditional plant in Vietnam for detoxifying the liver (National Institute of Medical Materials, 2004). Today, we are focusing on

using traditional plants to treat animal diseases. Therefore, *Phyllanthus amarus* can be a suitable detoxicant for mycotoxicosis in animals.

For these reasons, the objectives of the study were:

- To determine the extent and level of mycotoxin contamination in maize in some Southeastern and Central Highlands provinces of Vietnam.
- To evaluate the efficacy of *Phyllanthus amarus* extract in reducing fumonisin effects in piglets.

## 2. General discussion

### 2.1. Occurrence of mycotoxins in maize

Mycotoxins are toxic secondary metabolites produced by molds, especially *Aspergillus*, *Penicillium* and *Fusarium* genera and have negative effects on both humans and animals. The molds produce toxins in a wide range of agricultural conditions throughout the world ([http://www.fao.org/ag/agn/agns/chemicals\\_mycotoxins\\_en.asp](http://www.fao.org/ag/agn/agns/chemicals_mycotoxins_en.asp)). Some types of mold can produce more than one mycotoxin, and one kind of mycotoxin can be produced by many species of mold (Hussein and Brasel, 2001). These natural toxins threaten human and animal lives because their accumulation in foods and feeds causes serious health problems. Moreover, due to their negative impacts on performance, mycotoxins and the mold reduce the economic profits in agriculture. FAO estimated that toxic fungi invade 25% the world's food crops with many important foods, and in the world around 1,000 million tonnes of foodstuffs per year are lost because of mycotoxins ([http://www.fao.org/ag/agn/agns/chemicals\\_mycotoxins\\_en.asp](http://www.fao.org/ag/agn/agns/chemicals_mycotoxins_en.asp)).

Maize is an important food crop that is easily contaminated with mycotoxins. Maize is grown widely around the tropical world owing to its good adaptation to climate and its popularity. It is one of the three cereal crops that have the highest production. Besides being distributed widely, maize can be used for many purposes, such as animal feed, industrial uses, and is even the staple food in many developing countries. It also makes a large contribution to the economies of developed and developing countries ([http://www.fao.org/inpho/content/compend/text/ch23\\_01.htm](http://www.fao.org/inpho/content/compend/text/ch23_01.htm)). Maize is widely used and makes up 24% of the ingredients in commercial feed in Asia (Chin and Tan, 2005). However, the percentage of raw maize samples contaminated with mycotoxins and their levels are very high, particularly some important toxins such as aflatoxins, deoxynivalenol, zearalenone and fumonisins (Biomin Newsletter, 2008; Solovey et al., 1999). In 2005, 68% of maize samples from Asia contained fumonisin B<sub>1</sub>, while DON had 67% positive samples, ZEA 40% and AF 19% (Chin and Tan, 2005). According to Biomin Newsletter (2008) fumonisins are mostly found in this matrix, with 71% positive samples, followed by DON, AF, ZEA and ochratoxin A (OTA) with 59, 40, 37 and 15%, respectively. Furthermore, the highest levels of those toxins were 2483 ppb for AF, ZEA (3 112 ppb), fumonisin (FUM) (9 481 ppb) and OTA (197 ppb) (Biomin Newsletter, 2008). A survey conducted in Vietnam on maize intended for both human and animal consumption revealed that the *Aspergillus* genus developed in almost all maize samples (90%), and 68% of tested samples was contaminated with aflatoxin B<sub>1</sub>. Fumonisin B<sub>1</sub> was also detected in 32% samples, with a range from 0.4 to 3.3 mg/kg (Trung et al., 2007).

## **2.2. Information about some maize growing zones in Vietnam**

### *2.2.1. Dong Nai*

Dong Nai is a southeastern province of Vietnam and has an area of 5,894.73 km<sup>2</sup>, of which 3,028.45 km<sup>2</sup> is agricultural land. In 2006, its population was 2,254,676 with a density of 380 people / km<sup>2</sup>. Dong Nai's topography includes plain and flat land with rare, scattered mountains and a gradual declivity in the southward direction. Although the use of land in Dong Nai has changed over the past years, this province still possesses the largest area of agricultural land in the eastern region of South Viet Nam. Dong Nai lies in the monsoon tropical zone and the climate is divided in two distinct seasons. The rainy season lasts from March or April to November and the dry season from December to March or April of the following year. Average temperature ranges between 23.9-29<sup>0</sup> C. Rainfall is quite high, with 1,500mm – 2,700mm per annum. The average humidity is around 80 - 82% and humidity in the dry season is 10 - 12% lower than that of the rainy season. Dong Nai province's weather, with regular sunshine, rain and high humidity, found in all the localities, facilitates agricultural production and the development of industry and cultural and tourism activities. ([http://www.dongnai.gov.vn/dongnai/tongquan\\_KT-XH/?set\\_language=en&cl=en](http://www.dongnai.gov.vn/dongnai/tongquan_KT-XH/?set_language=en&cl=en) )

### *2.2.2. Binh Phuoc*

Binh Phuoc is also located in the southeast of Vietnam with an area of 6,857.4 km<sup>2</sup>. Its population is over 800 000, and comprises many ethnic groups with maize as their staple crop, and population density is about 78 people/km<sup>2</sup>. Dong Xoai town is the provincial capital. Binh Phuoc's terrain is hilly, sloping from the northeast to the southwest due to its transitional position between the highlands and the plain. This province is in the tropical monsoon region, with two distinct seasons, the rainy season which is from May to October, while the dry season includes the other months. The average temperature is around 28<sup>0</sup>C and annual humidity is from 77.8% to 84.2%.

(<http://www.aseconnectvietnam.gov.vn/LocalGovernment/Local.aspx?ProvinceId=49&Langid=2&MenuID=8>)

### *2.2.3. Dak Lak*

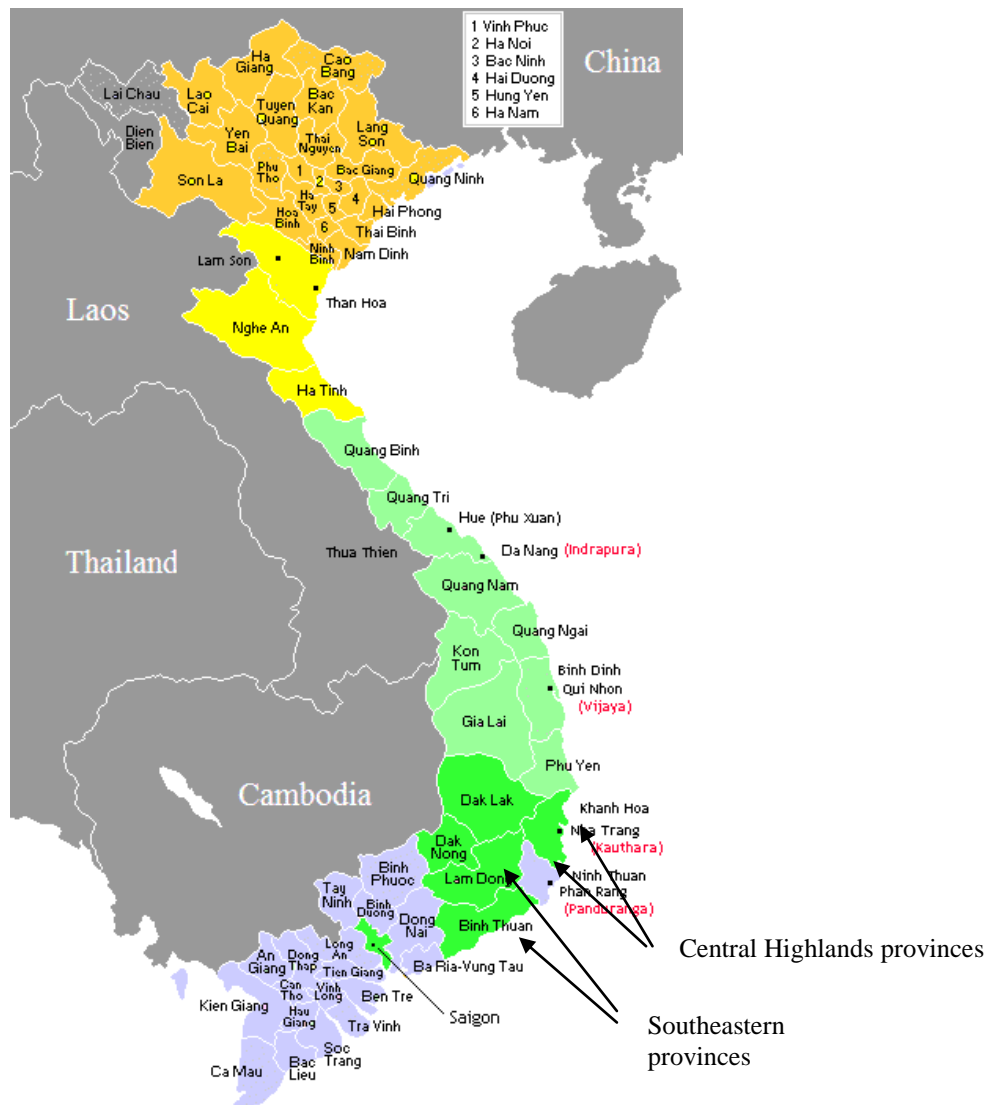
Dak Lak is in the south central region of Vietnam and covers 13,125 km<sup>2</sup>, with about 1,737,000 people, including various ethnic minorities. The province is divided into 14 administrative districts. Like the other provinces in the south of Vietnam, its climate also has a rainy and a dry season. Since the altitude ranges from 500 to 800 meters above sea level, the climate is characterized by both tropical monsoon and highland weather. Annual average temperature is from 23 to 24<sup>0</sup>C and average rainfall is 1600-1800 mm, with about 82% humidity ([http://clv-triangle.vn/portal/page/portal/clv\\_vn/825586?p\\_page\\_id=1&p\\_cateid=866437&item\\_id=1305459&article\\_details=1](http://clv-triangle.vn/portal/page/portal/clv_vn/825586?p_page_id=1&p_cateid=866437&item_id=1305459&article_details=1)). This kind of weather is suitable for a wide range of perennial crops, including coffee, pepper, rubber, cashew and cotton.

([http://www.rddl-daklak.org/publications/rddl\\_mandatory\\_eng\\_2586031.html](http://www.rddl-daklak.org/publications/rddl_mandatory_eng_2586031.html))

### 2.2.4. Dak Nong

Dak Nong is located in the south of Dak Lak province, with an area of 6,510 km<sup>2</sup> with only 400,000 people. Gia Nghia townlet is the provincial capital of Dak Nong. This province includes the end of Truong Son mountain range, resulting in alternative areas between valleys, high lands, and high mountains. The east side is higher than the west. The average height is over 800 meters above sea level. Because of the highland terrain, Dak Nong has a humid tropical highlands climate, with dry and rainy seasons. Like Dak Lak, the mean annual temperature is 22-23<sup>0</sup>C. The rainfall is about 2,200-2,400 mm per year with an average humidity of 84%

(<http://www.aseconnectvietnam.gov.vn/Localgovernment/Local.aspx?ProvinceId=72&Langid=2&MenuID=8>).



**Figure 1.** Location of Dong Nai, Binh Phuoc, Dak Lak and Dak Nong

[http://upload.wikimedia.org/wikipedia/commons/5/56/Vietnam\\_Expand1.gif](http://upload.wikimedia.org/wikipedia/commons/5/56/Vietnam_Expand1.gif)

### 2.3. Aflatoxins

Aflatoxins are mycotoxins produced by many strains of *Aspergillus flavus*, *A. parasiticus*, *A. nominus* and *A. niger* molds. They are a group of chemicals with four common types, B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, and aflatoxin B<sub>1</sub> is the most prevalent and toxic. The letter B was named because of its exhibition of blue fluorescence under ultraviolet light, while the letter G represents the yellow-green exhibition. Besides these four toxins, aflatoxin M<sub>1</sub> and M<sub>2</sub> are derivatives of B<sub>1</sub> and B<sub>2</sub>, and are found in milk and meat of B<sub>1</sub> and B<sub>2</sub> consuming animals. Aflatoxins are produced in food crops in the field prior to harvest and storage. Depending on the mold, host organic material and environment, the amount of toxin produced is determined. Aflatoxins prefer high temperature and high humidity postharvest. Maize contamination with aflatoxins is worldwide due to its widespread cultivation and its position as staple food in many countries. Over the last 30 years, these compounds have caused concern owing to significant economic losses and public health problems. The first target organ in the animal and human bodies attacked by aflatoxins is the liver (Ritchie, 2002). In poultry, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) significantly reduced feed intake, body weight gain and increased FCR, liver, heart, kidney, proventriculus and pancreas weight of broiler chickens (Ledoux et al., 1998; Quezada et al., 2000). Moreover, a high concentration of AFB<sub>1</sub> also markedly decreased plasma proteins, albumin, and renal and hepatic protein content (Quezada et al., 2000). Although a low AF level in the diet did not change serum chemistry in broiler chickens, the serum Na and the aspartate amino transferase (AST) and alanine amino transferase (ALT) enzyme activities were significantly elevated (Guz et al., 2002). Increases in some serum enzymes, such as sorbitol dehydrogenase, alanine aminotransferase and aspartate aminotransferase revealed the effect of AFB<sub>1</sub> on liver function of rats, quail and chickens (Gawai et al., 1991). It was also reported that AF might affect the reproductive system of roosters by totally or partly suppressing spermatogenesis, causing spermatozoa abnormality and testes atrophy (Ortatatli et al., 2002). Furthermore, aflatoxins not only increased FCR, but also lowered digestibility coefficients for dry matter, ether extract, nitrogen and nitrogen balance of pigs (Hale et al., 1979). In weanling pigs, an aflatoxin contaminated diet markedly reduced average daily feed intake and average daily weight gain, and affected liver function by raising  $\gamma$ -glutamyltransferase and alkaline phosphatase in serum (Schell et al., 1993). Aflatoxin B<sub>1</sub> and G<sub>1</sub> appeared in milk of sows fed a high AF diet in the form of AFB<sub>1</sub>, G<sub>1</sub> and M<sub>1</sub>, resulting in the reduction of some immunological measurements in their piglets (Silvotti et al., 1997). After one day of consuming 100ppb aflatoxins in the feed, AFM<sub>1</sub> was detected in cow milk, and then reached the highest level after three days. It took four days after the contaminated feed was removed for the absence of AFM<sub>1</sub> in milk (Diaz et al., 2004). On the other hand, in lambs, a high aflatoxin diet (2ppm) did not significantly decrease body weight, while average daily gain decreased clearly in the clearance period. However, the animals were more sensitive to infectious disease due to the altered immune response (Fernandez et al., 1999). In human health, aflatoxins were emphasized as a risk factor in primary liver cancer (PLC), a very prevalent form of cancer, because of their frequency of occurrence in food and their liver carcinogenic ability in experimental animals, such as subhuman primates. Besides, many surveys reported the relationship between AF ingestion and PLC incidence in Asia and Africa (Wogan, 1992).

### 2.4. Fumonisin

Fumonisin are products of the genus *Fusarium* fungi. More than ten species produce these toxins, although only *F. verticillioides* (or *F. moniliforme*) and *F. proliferatum* can produce significant amounts of fumonisins. *F. verticillioides* causes ear and stalk rot in maize worldwide, and together with *F. proliferatum*, they are the most common pathologies of this plant, and fumonisins are found frequently in maize (Arora et al., 2004; Joint FAO/WHO Expert Committee on Food Additives, 2001). There are four types of fumonisins known: fumonisin B<sub>1</sub> (FB<sub>1</sub>), B<sub>2</sub>, B<sub>3</sub> and B<sub>4</sub>, and among them FB<sub>1</sub> is found in the highest levels in maize. Insect damage, temperature stress and high water activities play an important role in fumonisin production. Except in extreme conditions, fumonisins formation happens only before harvest or during the early stage of drying, but not in the storage stage (Arora and Khachatourians, 2004). Besides in maize, fumonisins are also detected in other foods, such as sorghum, asparagus, rice, beans and mung beans. Fumonisin are poorly absorbed in the digestive tract and are quickly removed from the body of experimental animals. However, they mainly remain in liver and kidney (Joint FAO/WHO Expert Committee on Food Additives, 2001). In animals, fumonisins are known to cause the equine leukoencephalomalacia (ELEM) in the horse and porcine pulmonary edema (CAST, 2003; Piva et al., 2005). There was an association between human esophageal cancer found in Southern Africa and China after the consumption of contaminated corn (CAST, 2003). These toxins caused interstitial pulmonary edema, hydrothorax and death in pigs in an outbreak in 1989, and in experimental weanling pigs with from 5 to 50% affected and a high fatality rate (50 to 90%). Moreover, fumonisin B<sub>1</sub> also caused subacute hepatotoxicosis with individual hepatocellular necrosis and hepatomegalocytosis (Osweiler et al., 1992). Chronic toxicity of fumonisin B<sub>1</sub> showed a development of nodular hyperplasia in liver and changes in the distal esophageal mucosa of pigs (Casteel et al., 1993). These mycotoxins tended to decrease the lean yield of growing-finishing pig carcasses when fumonisin B<sub>1</sub> increased (Rotter et al., 1997). Furthermore, a high concentration of fumonisin B<sub>1</sub> in the diet (30 ppm) significantly increased FCR of weanling piglets (Piva et al., 2005). Liver function of pigs fed fumonisins in high doses was affected, shown by the elevation of some serum enzymes, such as AST, ALT, alkaline phosphatase (ALP) and CREA (Creatinine) and the abnormality of liver histopathology (Zomborszky et al., 2002; Piva et al., 2005). In ducks and rats, the sphinganine to sphingosine ratio was found to be changed significantly when they consumed fumonisin B<sub>1</sub> (Tran et al., 2003; Voss et al., 2001). Some more findings on the cardiovascular effects of fumonisins in swine were reported in 1996 by Smith et al. (1996 a,b) and revealed a significant increase in mean pulmonary artery pressure, accompanied by decreased heart rate, cardiac output, and mixed venous oxygen tension. These changes suggest the reason for pulmonary edema is pulmonary hypertension caused by hypoxic vasoconstriction. It was also found that fumonisin B<sub>1</sub> was carcinogenic to rodents (Voss et al., 2001).

## 2.5. Zearalenone

Zearalenone (ZEA), a nonsteroidal, estrogenic toxin, is produced by the *Fusarium* genus, and *Fusarium roseum* (*F. graminearum*), *F. culmorum* and *F. Sacchari* are the main producing species (Conkova ´ et al., 2001; Nollet, 2000). These fungi cause pink ear rot and scab diseases in maize and wheat. Maize, wheat, barley and milo are commonly contaminated with zearalenone, and sometimes oats (Conkova ´ et al., 2001). Among these food crops, zearalenone is the second most frequently found in maize (Nollet, 2000). The high moisture content of grain and alternating high and low temperature during the maturing and harvesting stages are

favourable conditions for the development of zearalenone. In the animal body, this toxin is absorbed easily from the gastrointestinal tract and excreted in bile, feces, and urine under metabolized forms ( $\alpha$  and  $\beta$  zearalenol) (Conkova ´ et al., 2001). ZEA mostly affects the reproductive system of animals, especially in swine (Nollet, 2000). It causes hyperestrogenism in female pigs with clinical signs such as swollen vulva and enlargement of the mammary glands or rectal and vaginal prolapsed in severe cases (CAST, 2003). Moreover it lengthened the weaning to estrus interval of sows at a dose of 10ppm, decreased fetuses per sow and tended to increase numbers of bred sows that were non-pregnant (Young et al., 1989). In the male pig, zearalenone induces young male feminization, with clinical signs such as testicular atrophy, swelling of prepuce, and mammary gland enlargement or libido reduction in boars (CAST, 2003). A in-vitro study also showed the negative impact of zearalenone and its metabolized form ( $\alpha$ -zearalenol) at doses of 60 and 80  $\mu\text{g}/\text{ml}$  of semen on the binding ability of boar spermatozoa to the zona pellucid (Tsakmakidis et al., 2007). In addition, ZEA changed some blood parameters as well as the biochemical index in female rats, such as hematocrit, mean corpuscular volume (MCV), the number of platelets and white blood cell, ALT, AST, alkaline phosphatase (ALP), serum creatinine and bilirubin, indicating liver toxicity (Maaroufi et al., 1996). Changes in serum enzymes also were reported in rabbits fed low and high doses of zearalenone. It increased significantly ALP activity of rabbit serum when consumed in low doses and elevated more enzymes including AST, ALT, ALP, gamma-glutamyltransferase (GGT) and total lactatedehydrogenase (LD) in the high dose group (Conkova ´ et al., 2001). However, chickens are highly tolerant to ZEA, and this toxin only had small effects on chicken calcium and phosphorus. Very high doses of ZEA increased oviduct weight, liver weight and reduced comb weight of chickens (Chi et al., 1980).

## **2.6. Mycotoxins analysis methods**

There are several methods for mycotoxin determination, divided into two main groups: testing for the presence of toxins and quantifying the toxin amount. They are known as rapid (screening) methods, reference method and research method. The rapid method is used for quick detection and quantification of mycotoxins, such as immunological methods using sensitive fluorometers, ELISA, to detect toxins. The reference method quantitates more accurately the amount of mycotoxins and involves a chromatographic technique such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), or thin layer chromatography (TLC). The third method has limited application, or has not yet been used widely owing to its novelty (CAST, 2003). Therefore, in the present study, HPLC was used for analyzing aflatoxins, fumonisins and zearalenone.

## **2.7. Mycotoxin prevention and use of feed additives**

Since mycotoxins are harmful in many ways, people try to prevent their occurrence and toxicity. According to FAO (Semple et al., 1989) there are many ways to prevent fungi contamination and mycotoxin production in agriculture. Prevention starts in the field and continues to the finished products. Even after harvesting, the storage stage also needs an effective decontamination of mycotoxins. There are many methods for controlling toxins in storage, such as physical, chemical and biological decontamination and removing mycotoxins by using solvent extraction



(Magan and Olsen, 2004). Using feed additives is a method that is gradually becoming more interesting and is considered to be cheaper than removing or degrading contaminants (Pettersson, 2004; Leslie and Visconti, 2008). They are categorized into three groups based on their action mechanism. Adsorbents limit the absorption of mycotoxins in the intestinal tract. Antioxidants and vitamins act on liver, tissue or cells in order to reduce toxic effects, and enzymes and bacteria have the ability to degrade mycotoxins in the digestive tract before being absorbed (Pettersson, 2004). Some commercial feed additives are now available in Vietnam, such as Mycosorb, Novasil and Mycofixplus are used widely in commercial feeds.

## **2.8. *Phyllanthus amarus* and *Phyllanthus niruri* or Chanca piedra (Stone Breaker)**

Chanca piedra is categorized in Euphorbiaceae, *Phyllanthus* genus and *niruri*, *amarus* species. It is a small, erect, annual herb that can reach 30 to 40 cm high. Chanca piedra is found in the Amazon rainforest and tropical areas all over the world, such as Bahamas, southern India and China. The *Phyllanthus* genus has over 600 species of shrubs, trees, and annual or biennial herbs in both hemispheres (Taylor, 2003). They have green flowers, and are small pantropical herbs, common in gardens. Their leaves are in one plane, with stipules of  $8 \times 3.5$  mm. Flowers are pendants hanging from one side of the branch, with separate males and females. The fruit is globular-depressed, and split into 3, ribbed seeds. ([http://cms.jcu.edu.au/discovernature/weedscommon/JCUDEV\\_012289](http://cms.jcu.edu.au/discovernature/weedscommon/JCUDEV_012289)). In Vietnam, this plant grows widely in areas lower than 800m above sea level. It develops from seed, and the growing period is around 3 to 4 months (National Institute of Medical Materials, 2004).

In this experiment, *Phyllanthus amarus* was selected because it is easy to find in the surrounding area and recently several studies have shown the protective effect of *Phyllanthus amarus* extract against mycotoxins both in vitro and in vivo. In 2002, Raphael et al. found out that 0.25-2 mg methanolic extract of *Phyllanthus amarus* per plate could inhibit the mutagenicity of 2-acetaminofluorene (2-AAF) and aflatoxin B<sub>1</sub> in *Salmonella typhimurium* strains TA1535, TA100, and TA102. Moreover, the ethanolic extract also prevented liver damage in mice given aflatoxin B<sub>1</sub> ( $66.6 \mu\text{gkg}^{-1} \text{BW}0.2\text{ml}^{-1} \text{day}^{-1}$ ) orally at a dose of  $0.3 \text{ g kg}^{-1} \text{BW} 0.2\text{ml}^{-1} \text{day}^{-1}$ .

Through the biochemical parameters and histopathological evaluation, the hepatoprotectiveness of this extract was found to be due to its strong capability in enhancing both enzymatic and non-enzymatic antioxidant levels, such as glutathione, glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) (Naaz et al., 2007). Not only the extract but also the powder of *Phyllanthus niruri* fed at 1% with 100 ppb aflatoxin B<sub>1</sub> in the diet might effectively reverse the damage of aflatoxin in broiler chickens. While the aflatoxin diet had negative effects on production and blood parameters in chickens, the diet supplemented with *Phyllanthus niruri* powder maintained body weight gain, feed efficiency, packed cell volume (PCV), Hb, ALT, AST, uric acid level and nitrogen balance and clearly protected the birds from the negative effects of aflatoxin (Sundaesan et al., 2007).

Furthermore, the hepatoprotective effect of this plant was proven clearly in many studies. For example Kodakandla et al. (1985) extracted *Phyllanthus niruri* by hexane and isolated four substances, phyllanthin, hypophyllanthin, triacontanal and tricontanol, and tested their protective abilities in primary cultured rat hepatocytes affected by carbon tetrachloride and

galactosamine. Among these substances, phyllanthin and hypophyllanthin, the major lignans in *Phyllanthus amarus* ([http://www.allianceingredients.com/pdfdocs/PHYLLANTHUS\\_AMARUS.PDF](http://www.allianceingredients.com/pdfdocs/PHYLLANTHUS_AMARUS.PDF)), showed their antihepatotoxicity. This potential also was evaluated in rats by Wongnawa et al. (2005). Aqueous extracts at doses of 1.6 and 3.2 g/kg reduced AST, ALT and bilirubin levels and histopathological score when rats were treated orally with paracetamol (3 g/kg) (Wongnawa et al., 2005). In addition, other studies which tested the aqueous extract with mice treated with nimesulide and albino rats showed that the extract decreased levels of AST, ALT, ALP, cholesterol and urea in serum at 100mg extract/kg body weight (James, 2009; Chatterjee and Sil, 2007). However, a dose of 200mg/kg body weight increased the ALT level of albino rats (James, 2009). With partially hepatectomised albino rat liver cells injured by alcohol, *P. amarus* extract helped liver regenerate at 24 hours by increasing the activities of thymidine kinase, which induces DNA synthesis (Chattopadhyay et al., 2006). Besides the hepatoprotective effect, *Phyllanthus amarus* extract also showed its anticancer activity. It decreased by 44% the incidence of gastric neoplasms in rats caused by N-methyl N'-nitro-N-nitrosoguanidine (MNNG) and reduced the elevated levels of some enzymes in the stomach to normal levels, such as  $\gamma$ -glutamyl transpeptidase, glutathione S-transferase, and glutathione reductase (Raphael, 2006). Another study in Swiss albino mice administered with Ehrlich Ascites Carcinoma,  $2 \times 10^6$  cells/mouse, after treating with a mixture (1:1) of Phyllanthin and Hypophyllanthin from *P. amarus* showed antitumor activities through the survival time, normal peritoneal cell count and hematological parameters (Islam et al., 2008).



**Figure 2.** *Phyllanthus amarus* ([duochanoi.com/diendan/showthread.php?t=3666](http://duochanoi.com/diendan/showthread.php?t=3666))



**Figure 3.** *Phyllanthus amarus* ([duochanoi.com/diendan/showthread.php?t=3666](http://duochanoi.com/diendan/showthread.php?t=3666))



**Figure 4.** *Phyllanthus niruri* (<http://www.jnkvv.nic.in/IPM%20Project/weed-management.html>)



**Figure 5.** *Phyllanthus niruri* leaves ([http://www.ruhr-uni-bochum.de/boga/html/Phyllanthus\\_niruri\\_Foto.html](http://www.ruhr-uni-bochum.de/boga/html/Phyllanthus_niruri_Foto.html))

### 3. Conclusions

Concentration and incidence of aflatoxins and fumonisins in maize kernels in Southeastern and Central Highlands of Vietnam were higher than of zearalenone. However fumonisins should be noted more in Vietnam due to their high concentration and incidence in the Central Highlands areas.

Aflatoxins should still be carefully monitored because of their high and dangerous levels in positive samples.

The concentration of fumonisins and the addition of detoxicants in the experiment did not result in any changes in the growth performance of pigs.

However, a high fumonisin level in the diet caused a decrease in serum total cholesterol to under the normal limit, and the commercial additive and *Phyllanthus amarus* extract increased this concentration and also reduced the histopathology in lungs and liver, although the reduction was not so clear.

The *Phyllanthus amarus* extract concentration in feed was too high and could have induced the tenderness of the liver that was noted.

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# Fumonisin, aflatoxin and zearalenone contamination in maize kernels in the Southeastern provinces and Central Highlands provinces of Vietnam

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## Abstract

A survey on the contamination of maize with aflatoxins, fumonisins and zearalenone was carried out in the Southeastern provinces and Central Highlands provinces in Vietnam, on 97 maize kernel samples. Four provinces were chosen for sampling maize: Dong Nai (22), Binh Phuoc (25), Dak Lak (30) and Dak Nong (20). Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), B<sub>2</sub> (AFB<sub>2</sub>), G<sub>1</sub> (AFG<sub>1</sub>), G<sub>2</sub> (AFG<sub>2</sub>), fumonisin B<sub>1</sub> (FB<sub>1</sub>), fumonisin B<sub>2</sub> (FB<sub>2</sub>) and zearalenone (ZEA) were detected by an HPLC method (Romer Labs, Singapore). Fumonisin was the most common type of toxin found in all samples (67%), followed by aflatoxins (55.7%) and zearalenone (27.8%). The incidence of aflatoxin positive samples in the Southeastern provinces (61.7%) was higher than in the Central Highlands (50%), while fumonisins and zearalenone incidences were contrary. The level of fumonisin B<sub>1</sub> in samples from the Central Highlands provinces (1757 ppb) was significantly different from that of Southeastern provinces (740 ppb) ( $P < 0.05$ ). Moreover, the mean value of AFB<sub>2</sub> in the Highlands samples (20.7 ppb) tended to be higher than in the Southeastern provinces (10.5 ppb) ( $P = 0.071$ ). The co-occurrence of the three kinds of mycotoxin in the 4 provinces was very high, at 77.3, 80.0, 86.7 and 90.0%, respectively, in Dong Nai, Binh Phuoc, Dak Lak and Dak Nong. The incidence of AFB<sub>1</sub> found in Binh Phuoc, Dong Nai, Dak Nong, and Dak Lak was 72, 50, 50 and 50%, with mean levels of 135, 55.7, 124, 201 ppb respectively. AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub> had lower incidences and mean values, particularly AFG<sub>2</sub>, which was not found in samples from Dong Nai and Dak Nong. FB<sub>1</sub> incidences in Binh Phuoc, Dong Nai, Dak Nong and Dak Lak were 44, 68, 85 and 73%, respectively, with average concentrations of 642, 812, 1155 and 2223 ppb, respectively, and FB<sub>2</sub> also had lower incidences and mean values. Furthermore, ZEA concentrations and percentages of positive samples were low in Binh Phuoc and Dong Nai (40, 62 ppb and 12 and 9%, respectively) and were higher in Dak Nong and Dak Lak (456 and 210 ppb and 40 and 47%, respectively). The incidence of positive samples with high concentrations of aflatoxins was about 70%, while fumonisins and zearalenone had more positive samples with low concentrations (90 and 63%, respectively).

**Key words:** AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>, Binh Phuoc, Dak Lak, mycotoxins, Dak Nong, Dong Nai

## 1. Introduction

Since the 1960s, effects of mycotoxins have been noted with respect to animal and human disease and death. They are fungal metabolites which have low molecular weight and that are not recognized by the body's immune system and are insidious poisons (Pitt, 1989). Moreover, the mycotoxin-producing fungi are also pathogens in plants, leading to economic losses. These toxins can be formed in many stages of plant production, from growing to harvest, drying, and storage (CAST, 2003). Among the many kinds of mycotoxins, aflatoxins, fumonisins and zearalenone are well known owing to their occurrence and toxicities. Aflatoxins are produced by *Aspergillus spp.* including *A. flavus*, *A. parasiticus*, *A. nomius*, and *A. pseudotamarii*, and there are four major types of aflatoxins: B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> (CAST, 2003; Rustom, 1996). Maize is another important crop, besides peanuts, that is often contaminated with aflatoxins (Rustom, 1996). Its negative effects, called aflatoxicosis in animals, include reduced growth performance of livestock, changes in some liver enzymes, resulting in liver damage and cancer, decreased milk and egg production and reduced immune response (CAST, 2003; Marin et al., 2002; Schell et al., 1993; Quezada et al., 1999). Fumonisins are also an important kind of mycotoxin, produced by *Fusarium verticillioides* (previously known as *F. moniliforme*) with four main types, B<sub>1</sub>, B<sub>2</sub>, A<sub>1</sub>, and A<sub>2</sub>, of which fumonisin B<sub>1</sub> is produced in the largest amount and has the highest toxicity (Gelderblom et al., 1988). The fumonisins cause equine leukoencephalomalacia, pulmonary edema syndrome in pigs and were thought to induce esophageal cancer in humans in South Africa and China (Gelderblom et al., 1988; Yoshizawa et al., 1994). Furthermore, fumonisin B<sub>1</sub> lowered apparent digestibility of ether extract in pigs (Gbore and Egbunike, 2007). According to GASGA (1997), fumonisin B<sub>1</sub> is produced commonly in maize-based food and feed in Africa, China, France, Indonesia, Italy, the Philippines, South America, Thailand, and the USA. In Southeast Asia, fumonisins are the most prevalent mycotoxin detected, with 58% positive samples, and maize is the most contaminated feed, with 71% positive samples (Biomin Newsletter, 2008). Not only fumonisins but also many species and subspecies of the *Fusarium* genus can produce zearalenone with estrogenic characteristics (Chekowski, 1987). In this genus, *Fusarium graminearum* is the most widely distributed species and infests wheat and maize world-wide (IARC, 1993). Zearalenone toxicity mostly affects reproductive function of animals, such as inducing feminization at high dietary concentrations (CAST, 2003), decreasing the number of fetuses per sow and reducing sow fertility (Young, 1989).

In terms of mycotoxin contamination, maize is one of the most affected crops and is a good medium for the development of these toxins. In Vietnam, maize is also considered as the staple food after rice, particularly in the rural and mountainous areas. Secondly, it is the main energy feed source for Vietnam's livestock industry. The Southeast region—Mekong Delta Upland is an agroecological zone with the second largest maize area in Vietnam, followed by the Central Highlands—central coast Upland (Thanh Ha et al., 2004). The Southeastern provinces and Central Highlands provinces are in the tropical monsoon region where the weather conditions are favourable for the production of many mycotoxins. However, the contamination and occurrence of some important mycotoxins in these regions in maize have not been examined in detail. Therefore, a survey of fumonisins, aflatoxins and zearalenone contamination in maize kernels in the Southeastern provinces and Central Highlands Vietnam was conducted to evaluate the real situation of mycotoxins in these areas.

## **Objectives were to:**

- Collect maize kernel samples from local retail traders and small farmers in 4 provinces: Binh Phuoc, Dong Nai, Daklak and Dak Nong
- Analyze the concentration of aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, fumonisin B<sub>1</sub> and B<sub>2</sub>, and zearalenone in maize kernels by the HPLC method

## **2. Materials and methods**

### **2.1. Site description**

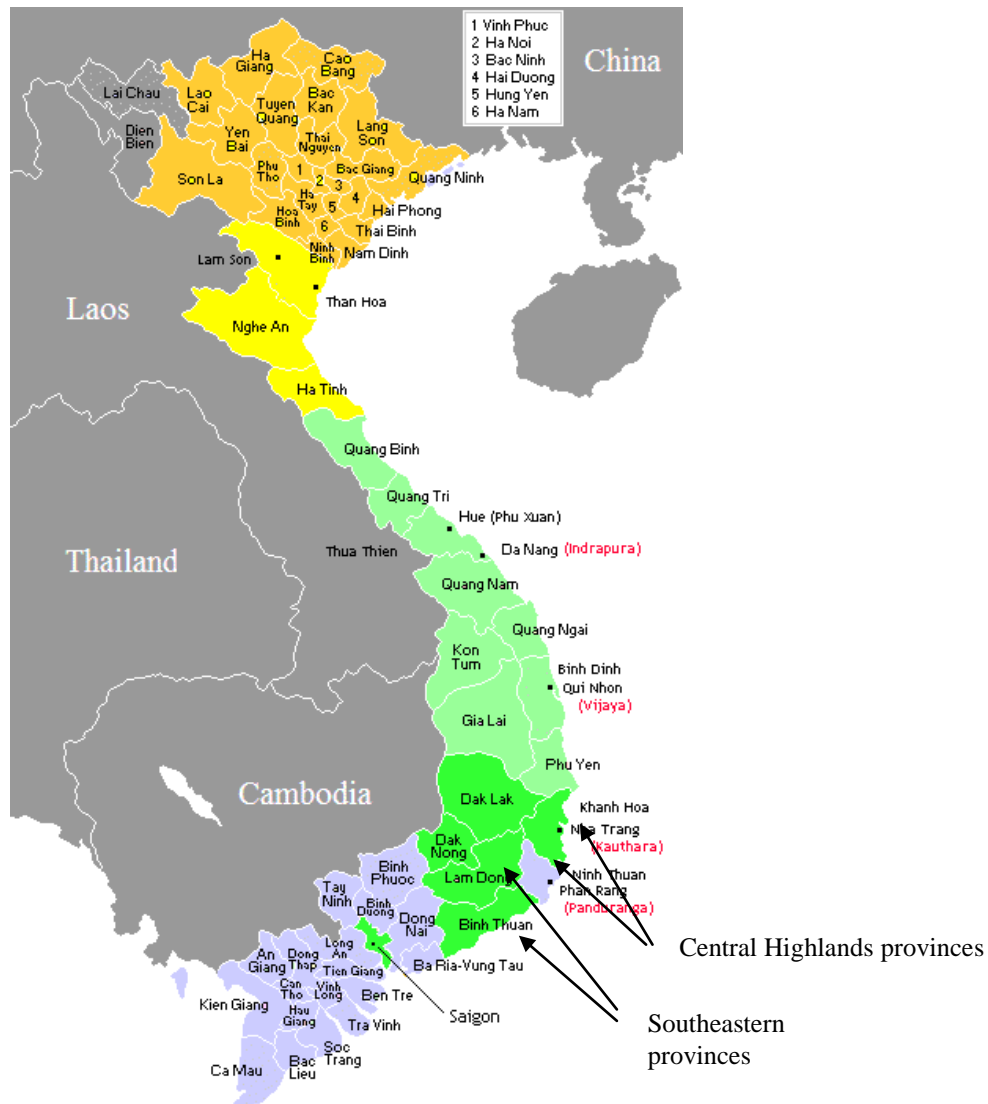
Two provinces in Southeastern Vietnam, Binh Phuoc and Dong Nai, and two in the Central Highlands, Dak Lak and Dak Nong (Figure 1), were chosen for the survey, since their maize production is well developed and is used for livestock in the area.

### **2.2. Dong Nai and Binh Phuoc**

These provinces are located in the tropical monsoon area; the weather is hot and humid, with two seasons, the rainy season from May to November and dry season from December to April. The average rainfall is from 1 800 to 2 000 mm per year and average temperature is about 27<sup>0</sup>C. Humidity is 80 to 90%. They have the second largest planted maize area in Vietnam. This area has an elevation ranging from 100 to 200 metres above sea level. There are two rainfed maize seasons in one year, the summer-autumn and autumn-winter. Maize grown in these provinces is mainly for commercial production, owing to the good transportation system and the proximity to feed factories. The maize farm size is usually around 1.0 ha and is an important source of income for the farmers (Thanh Ha et al., 2004).

### **2.3. Dak Lak and Dak Nong**

These provinces are located in the Central Highlands region of Vietnam, are at 500 to 800m above sea level, and the main sources of income are perennial industrial plants, forestry, mine ores, tourism and animal production. The provinces are in the tropical monsoon area, with two seasons, the rainy season (from May to November) and the dry season (from December to April). The average temperature is from 18 to 25<sup>0</sup>C, so it is cool throughout the year. The average rainfall is from 1 750 to 3 150 mm per year and humidity is around 85 to 87%. There is one rainfed maize season, the summer-autumn season, and maize is mainly found in semi-commercial maize production systems on sloping land. The infrastructure in many places is still poor. Although the farmers own on average 1.3 ha for maize cultivation, there are still many poor families in the rural areas (Thanh Ha et al., 2004).



**Figure 1.** Location of Dong Nai, Binh Phuoc, Dak Lak and Dak Nong  
[http://upload.wikimedia.org/wikipedia/commons/5/56/Vietnam\\_Expand1.gif](http://upload.wikimedia.org/wikipedia/commons/5/56/Vietnam_Expand1.gif)

## 2.4. Sampling method

In total 97 representative maize kernel samples (about 1kg per sample) were purchased from local retail traders and small farmers in four provinces in the Southeastern and Central highlands of Vietnam from the end of August to the beginning of October 2009, the maize harvesting season. The number was 22, 25, 20 and 30 samples from Dong Nai, Binh Phuoc, Dak Nong and Dak Lak, respectively. Samples were dried in an oven at 60°C for 8 hours then stored in plastic bags with desiccators (small silica gel bags) in a cool place before analysis, to stop the activity of molds.

## 2.5. Mycotoxin analysis methods

Aflatoxins, fumonisins and zearalenone analyses were done by the HPLC method (Romer Labs, Singapore). All the samples were cleaned up by MycoSep® columns to remove analytical interferences from food and feed extracts by pushing the column into a test tube (containing the extract), and then forcing the extract to filter upwards through the packing material of the column. The mycotoxins adhere to the chemical packing in the column and the purified extract passes through a membrane (frit) to the surface of the column. Then the columns are used to detect mycotoxins by the HPLC method.

For aflatoxins, samples were cleaned up by MycoSep® 226 AflaZon and detected by fluorescence detector using the electrochemical derivatization method. Limit of detection is 1 µg/kg.

In the case of Zearalenone, a MycoSep® 226 column was used to clean-up for purification prior to HPLC-FLD analysis. Limit of detection is 32 µg/kg.

Fumonisins samples were cleaned up by Multisep® 211 column and also detected by HPLC-FLD using the NDA (Naphthalene-2,3-dicarboxaldehyde) derivatization method. Limit of detection is 100 µg/kg.

## 2.6. Data analysis

The data were calculated and analyzed using the Excel program, with the incidence of positive aflatoxin, fumonisin and zearalenone samples in the total, range, mean and median values. The distribution of aflatoxin, fumonisin and zearalenone levels in maize kernels was also calculated by Excel. The differences in aflatoxin, fumonisin and zearalenone concentrations in maize between the Southeastern provinces and Central Highlands were compared by ANOVA in Minitab 13. Sources of variance were area and error.

## 3. Results and discussion

The occurrence of mycotoxins between the two study areas in Vietnam is shown in Table 1, and is different among toxins. The Southeastern provinces had about 10% higher incidence of aflatoxin contaminated samples than the Central Highlands. However, the incidence of zearalenone and fumonisin positive samples in the Highlands provinces was much higher than in the Southeast. In general, the percentage of samples with fumonisins was highest in all samples (67%), followed by aflatoxins and zearalenone (50% and 27.8%, respectively). According to Biomin Newsletter (2008), these results are similar to the situation in Southeast Asia which had 52% of aflatoxin containing samples, 58% of fumonisin samples and 39% of zearalenone samples. Moreover, fumonisins were also the most detected toxin in maize samples (71%), followed by aflatoxins (40%) and zearalenone (37%) (Biomin Newsletter 2008). In contrast, Trung et al. (2007) conducted a survey by collecting samples from North, Central and South Vietnam that showed 68% of tested samples were contaminated with AFB<sub>1</sub>, while the percentage of fumonisin B<sub>1</sub> positive samples was only 32%. Dong Nai and Binh Phuoc provinces have higher mean temperatures than Dak Nong and Dak Lak, which provide good conditions for the development of aflatoxins

(<http://www.ansci.cornell.edu/plants/toxicagents/aflatoxin/aflatoxin.html#Factors>). Moreover, most of the samples taken in Binh Phuoc were not dried well and stored in poor conditions in the small farmers' houses. Possibly because of these reasons, the percentage of AF positive samples

in the Southeastern provinces was higher than that in the Central Highlands. On the other hand, fumonisins and zearalenone develop rapidly in high air humidity and moderate temperature environments (DeVries, 1996; Munkvold and Desjardins, 1997). With a cooler climate and higher rainfall compared to Dong Nai and Binh Phuoc, Dak Nong and Dak Lak thus had considerably more fumonisin and zearalenone positive samples than the Southeastern provinces.

The distribution of aflatoxins, fumonisins and zearalenone was different between the Southeast Uplands and Central Highlands (Table 2). The results show very clearly that the mean FB<sub>1</sub> level in DN+DL was significantly higher than in DN+BP (P<0.05). Moreover the median and concentration range of FB<sub>1</sub> in DN+DL were also much higher and wider than in DN+BP. Although aflatoxin B<sub>1</sub> concentration in DN+DL samples was not significantly higher than in DN+BP, the aflatoxin B<sub>2</sub> mean value in DN+DL tended to be higher than in DN+BP (P=0.071), and one sample from DL had 810 ppb of AFG<sub>1</sub>. The means of aflatoxin B<sub>1</sub> in the two areas were much higher than the maximum tolerated AFB<sub>1</sub> level of the European Union and U.S. Food and Drug Administration (FDA) Compliance Policy Guides for maize (20µg/kg). Furthermore, the concentrations of toxins in DN+DL had wider ranges than in DN+BP. These ranges are also wider than the fumonisin B<sub>1</sub> range in samples of North, Central and South Vietnam (400-3300 ppb), and aflatoxin B<sub>1</sub> range (7-126.5 ppb) of Trung et al (2007). Although there was no significant difference between zearalenone contents in samples from the Southeast Upland and Central Highlands, the mean and range of this toxin in DN+DL was much higher than in DN+BP. The co-occurrence of three mycotoxin types in DN+DL positive maize samples was much higher than in DN+BP.

The incidence of co-occurrence of mycotoxins was very high, and was highest in Dak Nong, followed by Dak Lak, Binh Phuoc and Dong Nai. Among the four provinces, Dak Lak had the highest mean value of aflatoxin B<sub>1</sub>, followed by Binh Phuoc, Dak Nong and Dong Nai. The contamination ranges of AFB<sub>1</sub> positive samples in Dak Nong and Dak Lak were wider than in the other provinces. However, Binh Phuoc had the highest AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> incidences (18/25; 14/25; 4/25; 1/25). This suggests a synergistic effect of mycotoxins which is more serious than the effect of only one toxin in human and animal health. Meanwhile, the percentages, mean and the range in values of FB<sub>1</sub>, FB<sub>2</sub> and zearalenone positive samples were highest in Dak Lak and Dak Nong. In particular, the mean concentrations of zearalenone in samples from Dak Lak and Dak Nong were much higher than in those from Dong Nai and Binh Phuoc. The levels of toxins in this survey were much higher than in a survey of Thieu et al. (2007) conducted in 8 provinces of southern Vietnam from January to February 2005, the dry season. Lower Aflatoxin B<sub>1</sub> and zearalenone concentrations and ranges were found in the study of Thieu et al. (2007) (77.5 ppb, 164 ppb and 0.4 – 555 ppb, 38 – 254 ppb, respectively).

Possibly, in the present survey, because the rainy season was favorable for toxin production, the content of these toxins in maize kernels was higher. Moreover, small farmers stored the maize in their houses under poor conditions, which also resulted in the maize kernels containing high concentrations of aflatoxin and fumonisins. This is very concerning, because these small farmers feed their poultry and pigs with contaminated maize.

The percentage of positive samples that had over 20 ppb aflatoxin was very high (about 70%) (Table 4). Moreover, the incidence of samples with over 300 ppb was higher than the incidence of samples with from 98.4 to 126.5 ppb in the survey of Trung et al. (2007) (14.8% > 11.8%).

However, Table 5 shows that more than 90% of the positive samples had fumonisin concentrations less than 4000 ppb (4ppm). The incidence of samples with over 12000 ppb was low (3.1%).

The number of Zearalenone positive samples that had less than 100 ppb (0.1 ppm) was high (63%) and samples over 500 ppb was about 11% (Table 6). However, only about 17% positive samples with less than 100 ppb, and 6.7% samples with over 500 ppb were found in the survey of Silva and Vargas (2000) in Brazil.

#### **4. Conclusions**

Maize kernel samples from the Southeastern and Central Highlands of Vietnam had very high concentrations and incidence of aflatoxins and fumonisins. The aflatoxin B<sub>1</sub> level was much higher than the maximum tolerated AFB<sub>1</sub> level of the European Union and U.S. Food and Drug Administration (FDA) Compliance Policy Guides for maize (20 µg/kg).

In the Central Highlands, the percentages and contents of positive maize samples contaminated with fumonisins and zearalenone were higher than in the Southeastern provinces.

Although the incidence of aflatoxin contamination was relatively low compared to fumonisins and zearalenone, the aflatoxins should still be carefully monitored because of their high concentration in positive samples.

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**Table 1.** Incidence of zearalenone, aflatoxin and fumonisin contaminated samples in Southeastern and Central Highlands provinces

	Aflatoxins			Fumonisin			Zearalenone		
	DN+BP*	DN+DL*	Total	DN+BP*	DN+DL*	Total	DN+BP*	DN+DL*	Total
Analyzed samples	47	50	97	47	50	97	47	50	97
Positive samples	29	25	54	26	39	65	5	22	27
Percentage (%)	61.7	50.0	55.7	55.3	78.0	67.0	10.6	44.0	27.8

\*DN+BP: Dong Nai + Binh Phuoc; DN+DL: Dak Nong + Dak Lak

**Table 2.** Aflatoxins, fumonisins and zearalenone levels ( $\mu\text{g}/\text{kg}$ ) in the Southeast Uplands and Central Highlands

	Mycotoxins	Region	DN+BP*	DN+DL*	Prob.
Analyzed samples			47	50	
Positive samples			37	44	
Percentage (%)			78.7	88	
AFB <sub>1</sub> **	n		29	25	
	Mean		104.7	170.2	0.221
	Median		81	28	
	Range		1-450	2-844	
AFB <sub>2</sub> **	n		25	15	
	Mean		10.5	20.7	0.071
	Median		5.5	11	
	Range		1-33	2-86	
AFG <sub>1</sub> **	n		5	4	
	Mean		12.8	208	0.306
	Median		2	9.5	
	Range		2-54	3-810	
FB <sub>1</sub> **	n		26	39	
	Mean		740	1757	0.042
	Median		390.5	871	
	Range		102-2747	160-10799	
FB <sub>2</sub> **	n		20	35	
	Mean		357.9	751	0.102
	Median		229.5	367	
	Range		108-834	102-5051	
ZEA**	n		5	22	
	Mean		48.8	299.5	0.367
	Median		44	98	
	Range		35-80	36-2409	

\*DN+BP: Dong Nai + Binh Phuoc; DN+DL: Dak Nong + Dak Lak

\*\*AFB<sub>1</sub>: Aflatoxin B<sub>1</sub>; AFB<sub>2</sub>: Aflatoxin B<sub>2</sub>; AFG<sub>1</sub>: Aflatoxin G<sub>1</sub>; FB<sub>1</sub>: Fumonisin B<sub>1</sub>;

FB<sub>2</sub>: Fumonisin B<sub>2</sub>; ZEA: Zearalenone

**Table 3.** Occurrence of aflatoxins, fumonisins and zearalenone in maize kernel samples in Southeastern Vietnam

	N	Positive samples		Number, mean ( $\mu\text{g}/\text{kg}$ ), median and range of samples contaminated by aflatoxins, fumonisins and zearalenone						
		n	%	Aflatoxins				Fumonisin		Zearalenone
				B <sub>1</sub>	B <sub>2</sub>	G <sub>1</sub>	G <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>	
<b>Binh Phuoc</b>	25	20	80	18	14	4	1	11	7	3
Mean				134.7	15.4	15.5	11	641.6	346.1	40
Median				122	15.5	3		308	247	40
Range				1-450	2-33	2-54		102-2669	108-766	35-45
<b>Dong Nai</b>	22	17	77.3	11	10	1	0	15	13	2
Mean				55.7	3.6	2	ND*	811.7	364.2	62
Median				53	3			458	212	62
Range				3-126	1-8			134-2747	149-834	44-80
<b>Dak Nong</b>	20	18	90	10	5	1	0	17	15	8
Mean				123.5	22.2	9	ND*	1154.9	468.6	455.9
Median				25.5	4			631	367	99
Range				4-844	2-86			232-5793	138-1867	43-2409
<b>Dak Lak</b>	30	26	86.7	15	10	3	1	22	20	14
Mean				201.3	19.9	274.3	45	2222.5	962.8	210.1
Median				33	19	10		1132	385	97.5
Range				2-713	2-48	3-810		160-10799	102-5051	36-1788

ND: Not detected

**Table 4.** Distribution of total aflatoxin levels in maize kernel samples

Aflatoxin ( $\mu\text{g}/\text{kg}$ )	Frequency	% of samples
$0 \leq 20$	17	31.5
21-100	14	25.9
101-300	15	27.8
$> 300$	8	14.8

**Table 5.** Distribution of total fumonisins levels in maize kernel samples

Fumonisin ( $\mu\text{g}/\text{kg}$ )	Frequency	% of samples
$0 \leq 4000$	59	90.8
4001-8000	3	4.6
8001-12000	1	1.5
$> 12000$	2	3.1

**Table 6.** Distribution of zearalenone levels in maize kernel samples

Zearalenone ( $\mu\text{g}/\text{kg}$ )	Frequency	% of samples
$0 \leq 100$	17	63.0
$<100-500$	7	25.9
500 - 1000	1	3.7
$>1000$	2	7.4

# Detoxifying effects of a commercial additive and *Phyllanthus amarus* extract in pigs fed fumonisins contaminated feed

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## Abstract

Reduction of the negative effects fumonisins in pigs of a *Phyllanthus amarus* extract and a commercial detoxifying additive product was evaluated with respect to growth performance, pathology and blood biochemistry. Forty eight crossbred (Landrace × Yorkshire × Duroc) weanling pigs were randomly assigned in a completely randomized design (CRD) to six diets containing: 1) low fumonisin B<sub>1</sub> and no feed additive (LFNA); 2) low fumonisin B<sub>1</sub> and a commercial detoxicant additive at 1g/kg of feed (LFCA); 3) low fumonisin B<sub>1</sub> and *Phyllanthus amarus* extract at 10g/kg of feed (LFPE); 4) high fumonisin B<sub>1</sub> and no feed additive (HFNA); 5) high fumonisin B<sub>1</sub> and commercial detoxicant additive at 1g/kg of feed (HFCA); 6) high fumonisin B<sub>1</sub> and *Phyllanthus amarus* extract at 10g/kg of feed (HFPE). Fumonisin levels, detoxicants and their combination did not have any effect on final weight, average daily weight gain, average daily feed intake, and feed conversion ratio in pigs. Including 10 mg fumonisin B<sub>1</sub> in the diet decreased the total cholesterol significantly compared with the low fumonisin groups (2.19 mmol/L < 2.42 mmol/L) (P<0.05). The aspartate aminotransferase (AST) blood levels of pigs given the commercial additive were higher than in the no feed additive group (110 U/L > 83.1 U/L, P=0.067) and the *Phyllanthus amarus* group also had a high AST blood level (99.6 U/L). Moreover, fumonisin B<sub>1</sub> thickened the alveolar walls of the lungs, while the commercial and *Phyllanthus amarus* additives partly reduce the thickened alveolar wall lesions. Liver cells also had more severe fatty degeneration and necrosis in the fumonisin and no additive group than in the commercial and *P. amarus* groups. However *P. amarus* extract made the liver tender.

*Key words: Fumonisin B<sub>1</sub>, blood biochemistry, aspartate aminotransferase, AST, lung, liver*

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## 1. Introduction

Fumonisin presents one of the greatest potential mycotoxin risks to human and animal health, as a food and feed contaminant, along with aflatoxins, trichothecenes, zearalenone, ochratoxin A and ergot alkaloids (CAST, 2003). There are four main types of fumonisin, B<sub>1</sub>, B<sub>2</sub>, A<sub>1</sub> and A<sub>2</sub>, and among these fumonisin B<sub>1</sub> is produced in the largest amount and has highest toxicity. These toxins are produced by *Fusarium verticillioides* (previously known as *F. moniliforme*)

(Gelderblom et al., 1988). Fumonisin B<sub>1</sub> causes equine leukoencephalomalacia and pulmonary edema syndrome in pigs and horses (CAST, 2003). Raviprakash et al. (1997) revealed that fumonisin B<sub>1</sub> affected kidney weight and induced apoptosis in mouse liver. It is also reported that fumonisins could cause hepatocarcinoma in rats (Gelderblom et al., 2001). Fumonisin elevated some serum enzymes, such as AST, ALT, ALP and CREA (Creatinine) and caused an abnormality of liver histopathology (Zomborszky et al., 2002; Piva et al., 2005). Galvano et al. (2001) indicated that the prolonged exposure of FB<sub>1</sub> at high concentration caused DNA damage of apoptotic type in human fibroblasts. In Vietnam, in earlier studies of Nguyen Quang Thieu et al. (2008) and Phuong et al. (2010), the occurrence of aflatoxins, fumonisins and zearalenone was widespread and in high concentrations. Especially, fumonisin incidence was about 70% in tested samples, and the highest fumonisin B<sub>1</sub> level was around 10.8 ppm in the Southeastern and Highlands provinces (Phuong et al., 2010).

Since the occurrence of fumonisins is harmful to animal and human health, there are many methods to reduce their production or their negative impacts, such as using resistant corn varieties, controlling insects, heating, chemical detoxificants like Ca (OH)<sub>2</sub> and ammonia, or degradation of fumonisins by ozone. Nevertheless, some of these methods are not very effective; some are successful but need high temperatures to hydrolyze fumonisins and other toxins (Maja Šegvić and Stjepan Pepeljnjak, 2001). Using adsorbents to adsorb mycotoxins, especially aflatoxin, is also one of the methods that can effectively reduce the harmful effects. However, they do not really show any effects on fumonisins, and adsorbents such as activated carbon seem to worsen the toxic effect of fumonisin B<sub>1</sub> (Piva et al., 2005), while cholestyramin also did not have a specific effect in adsorption of fumonisins in vivo (Solfrizzo et al., 2001). Some commercial products have adsorptive effects on fumonisins, but their costs are too high, particularly for small farmers. Besides, fumonisins after being absorbed through the intestine, will go to the liver first, and exert effects on it, and then on other organs.

According to Taylor (2003), indigenous peoples use plants from *Phyllanthus sp.*, a small annual herb that is distributed throughout the tropical regions of the world, to cure many diseases including hepatitis and other liver diseases. In clinical research, this plant also demonstrated its anti-hepatotoxic function. A study by Huang et al. (2004) showed that the water extract of *Phyllanthus urinaria*, the same family and the same use as *Phyllanthus amarus*, could reduce the cell viability of HL-60 cells (human myeloid leukemia), which is additional evidence for its anticancer effect. This plant had the ability to reverse the negative impacts of aflatoxicosis of broiler chickens (Sundaresan et al., 2007). Phyllanthin, hypophyllanthin and niranthin are the main substances in *P. niruri* that have a hepatoprotective effect (Kodakandla et al., 1985). *Phyllanthus urinaria* or *P. amarus* is a weed that is easy to cultivate and is widespread. In Vietnam, *Phyllanthus amarus* grows widely in nature and has been used as a medicinal plant for a long time to treat hepatic disease, eye infection, snakebites and acnes (Do Tat Loi, 2004). Today, many farmers in the poor Central provinces of Vietnam are earning money from growing this plant for use as human medicine (Hung Phien, 2009). For these reasons, we can hope that adding *Phyllanthus amarus* or *P. urinaria* extract to the feed of pigs can reduce the effects of fumonisins on liver, and thus liver, kidney and lung functions will not be affected and the pigs will retain good production performance.

## 2. Materials and methods



## 2.1. Site description

The experiment was conducted in the experimental farm of the Animal Husbandry and Veterinary Medicine Faculty of Nong Lam University, Ho Chi Minh City, Vietnam. This area is in Southeastern Vietnam, and has a tropical monsoonal climate, with a rainy season from May to October and a dry season from November to April. The average temperature is 27.5°C with high humidity. The duration of this study was 37 days, from 22 December 2009 to 31 January 2010.

## 2.2. Pigs, treatments and experimental design

The experiment had a completely randomized design (CRD), with a 2 × 3 factorial arrangement and 4 replicates. Each replication had 2 pigs (one male castrate and one female) in one pen. In total 48 crossbred (Landrace × Yorkshire × Duroc) piglets (10.9 ± 1.21 kg initial body weight) were used in the experiment. The pigs had been vaccinated against mycoplasma and swine fever at one week of age and five weeks of age. After 4 days of acclimatization, the pigs were randomly assigned to the treatments and pens according to the design show in Table 1 and Figure 1. The treatments were:

### *Fumonisin factor:*

**HF:** 10 mg fermented fumonisin B<sub>1</sub> per kg of feed

**LF:** Low fumonisin B<sub>1</sub> in feed

### *Additive factor:*

**NA:** No detoxicant additive

**CA:** Commercial detoxicant additive (1 g/kg feed)

**PE:** *Phyllanthus amarus* extract (10g extract/kg feed)

## 2.3. Experimental diets and feeding

### **Fumonisin B<sub>1</sub> production**

Fumonisin B<sub>1</sub> (FB<sub>1</sub>) was from two sources: fumonisin B<sub>1</sub> produced by *Fusarium proliferatum* cultured on maize grains was purchased from the National I-Lan University Laboratory, Taiwan, and production by *Fusarium proliferatum* isolates that was assessed in cultures grown on autoclaved rice. In brief, 500 g of ground maize was placed in a jar, was moistened for 1 h in distilled water and then autoclaved at 121°C for 60 minutes.

The maize medium was inoculated with mycelia from seven-day-old cultures of *F. proliferatum* grown on PDA (Potato Dextrose Agar) and incubated at 26°C for 15 days. Samples (three replicates) of each isolate were dried in a forced-air draft oven at 55°C for 48 h, crushed with a mortar and pestle, and extracted with 1 ml of CH<sub>3</sub>CN:H<sub>2</sub>O (1: 1, v/v) for 1h. The extracts were centrifuged at 1500g for 10 min, and the supernatants were cleaned on a Sep-Pak C18 cartridge column (Waters, Milford, MA, USA) with CH<sub>3</sub>CN:H<sub>2</sub>O (1: 1, v/v) as the solvent. The level of FB<sub>1</sub> was determined from the extracts using HPLC. The concentration of fumonisin B<sub>1</sub> in the

substrate was 882 ppm. Pure fumonsin B<sub>1</sub> was supplied by Biomin Company with in total 5514 mg fumonisin B<sub>1</sub> in 280.9g powder.

### **Detoxicant**

A commercial detoxicant additive was provided by Biomin Company, Austria. *Phyllanthus amarus* extract was purchased from Hong Dai Viet Company, Vietnam, and its components (phyllanthin, hypophyllanthin and niranthin) were analyzed at the Institute of Chemical Technology, Vietnam.

### **Diets**

The diet was formulated to meet or exceed the nutrient requirement for pigs (NRC, 1998) (Table 3) and was then mixed with fumonisin B<sub>1</sub> to produce the diet that contained 10 mg FB<sub>1</sub> per kg feed. Finally, detoxicant additives were mixed with feed according to treatments. Feed was analyzed for gross composition, including crude protein, ether extract, crude fibre, ash, Ca and P at the Department of Animal Nutrition, Nong Lam University (Table 4).

Feed and drinking water were offered *ad libitum* and hygienic conditions maintained throughout the experiment. Fresh feed was provided at 08:00 h and 15:30 h, when remaining feed was removed and weighed.

## **2.4. Data collection**

The animals were weighed at the beginning and the end of the trial to calculate average daily weight gain (ADG) and then feed conversion ratio (FCR) was calculated based on feed consumption. Blood samples of all pigs were collected at the end of the experiment and sent to MEDIC Medical Center, Ho Chi Minh City for analysis of bilirubin T, bilirubin D, bilirubin I, albumin, total cholesterol, glucose, AST (Aspartate Aminotransferase or GOT: Glutamic Oxaloacetic Transaminase), ALT (GPT: Glutamine Pyruvic Transaminase), GGT (Gamma Glutamyl Transpeptidase) and ALP (Alkaline Phosphatase).

At the end of the 37-day experiment, two pigs per treatment were slaughtered and necropsies performed. The lungs, liver and kidneys were sectioned to observe lesions and biopsies carried out to examine the histopathology.

## **2.5. Chemical analysis**

The formulated feed was sampled and the composition of the basal diet was determined. The feed also was checked with fumonisins and aflatoxins concentrations.

Serum analysis was done by MEDIC Medical Center, Ho Chi Minh City.

Histopathology examination was carried out by the Veterinary Hospital, Nong Lam University, Ho Chi Minh City.

## 2.6. Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using the CRD procedure of Minitab software (version 13.3). Sources of variance were treatments and errors.

## 3. Results

The chemical composition of the basal diet is shown in Table 4.

The concentrations of aflatoxins in the feed were 6.3 and 13.5  $\mu\text{g}/\text{kg}$  for LF and HF groups, respectively. Although the expected fumonisin B<sub>1</sub> level in the diet was 10 ppm, the actual level was 3980  $\mu\text{g}/\text{kg}$  in the HF group. Moreover, the feed of LF group contained 1132  $\mu\text{g}/\text{kg}$  feed.

In order to ensure that the data gave exact results, the performance data of two pens from two treatments (LFCA and LFNA) in the experiment were not used in data analysis because these pigs were infected by *Actinobacillus Pleuropneumonia* (APP) and depressed for a long period. Furthermore, the blood profiles of pigs from five pens from four treatments (two from LFPE, three from LFCA, HFNA, HFCA) were also not used in data analysis owing to identification mistakes in the laboratory.

High fumonisins and adding detoxicants in the diets did not affect the growth performance (Table 5). Final weight, average daily weight gain, average daily feed intake, and feed conversion ratio were not significantly different among treatments ( $P>0.05$ ) (Table 6).

Table 7 shows the differences in blood bilirubin T, D, I, albumin and total cholesterol among pigs given diets with different toxin levels and detoxicants. High fumonisins in the diet decreased the total cholesterol significantly (2.19 mmol/L) compared with the low fumonisin groups (2.42 mmol/L) ( $P<0.05$ ). The differences in these parameters among the no feed additive, commercial additive and *Phyllanthus amarus* extract treatments were not significant ( $P>0.05$ ). Moreover, the combination of detoxicant type and fumonisin level did not significantly affect bilirubin T, D, I, albumin and total cholesterol levels in pig blood ( $P>0.05$ ) (Table 8). However, blood albumin in the high fumonisin - no feed additive group tended to be lower than in the other treatments (3.00 g/100mL).

Serum enzymes levels are presented in Table 9 and Table 10. Although there was no significant difference in blood GGT, ALP, AST and ALT levels between toxin levels and detoxicants and their combination, some trends were noted among treatment groups. For example, AST blood levels of pigs given the commercial additive were higher than in the no feed additive group (110 U/L > 83.1 U/L,  $P=0.067$ ) and the *Phyllanthus amarus* group also had a high AST blood level (99.6 U/L). Furthermore, pigs given the high fumonisins only diet (HFNA) tended to have lower blood levels of GGT, AST and ALT.

The results of the microscopic and macroscopic pathology of the experimental pigs are summarized in Table 11. The lesions found indicated mycoplasma infection in pigs, and there were some distinct points in the lungs and liver of pigs consuming fumonisin. Microscopic examination showed that fumonisin thickened the alveolar walls of the lungs. However, the

groups that were treated with commercial and *Phyllanthus amarus* additives had less thickened alveolar walls than the no additive group. Moreover, in the pigs given fumonisins fatty degeneration and necrosis of the liver cells was observed and more severe degeneration of the hepatocytes was noted, especially in the groups given diets without additives. In addition, macroscopic observation seemed to show tenderness and swelling of the liver in the *P. amarus* groups.

## **4. Discussion**

### **Growth performance**

The results in this study are in contrast with the results from Piva et al. (2005), which showed a significant decrease of ADG and a significant increase FCR of piglets fed diets that contained 30 ppm fumonisins given for 42 days. However, there was no difference in the ADFI of these pigs among treatments. Rotter et al. (1996) found out that average daily weight gain of male pigs fed a 10 ppm pure fumonisin B<sub>1</sub> diet through 8 weeks decreased by 11% compared to a 0 ppm diet. Furthermore in the first 4 weeks of the experiment, a general increase of feed consumption was observed. Nevertheless, in agreement with growth performance results in the present study, Osweiler et al. (1992), and Zomborszky et al. (2002) did not find any difference in the feed intake, body weight gain, and feed conversion ratio of pigs fed a 10 ppm fumonisins diet and a 17 ppm fumonisin B<sub>1</sub> diet. Possibly, the concentration of fumonisin B<sub>1</sub> was not high enough and time on experiment sufficiently long to cause any changes in the growth performance of pigs. In addition, the fumonisin B<sub>1</sub> in this study was a mixture of pure and cultured FB<sub>1</sub>, and therefore the results were not as clear as those found by Rotter et al. (1996).

### **Blood parameters**

While high fumonisins in the diet lowered total serum cholesterol in the present experiment, Rotter et al. (1996) demonstrated an increase in cholesterol serum after 2 weeks. When fumonisins were fed at 30 ppm, cholesterol concentration was significantly higher than in control pigs (Piva et al., 2005). On the other hand, the cholesterol and serum enzyme levels were within the normal ranges in the study of Zomborszky et al. (2002), and only some pigs had histopathological changes (fumonisins dose 1 and 5 ppm) and showed an elevation outside the normal ranges of AST, ALT and ALP in serum (ALP >500 U/l, AST >100 U/l and/or ALT >70 U/l). Furthermore, a dose of 17 ppm fumonisin B<sub>1</sub> in the diet did not show clearly any increase in AST, GGT and total bilirubin of pigs (Osweiler et al., 1992). High fumonisins concentration in the diet in the present study decreased the total cholesterol concentration (2.19 mmol/L) to values under the normal range of total cholesterol of from 2.36 to 3.72 mmol/L (Tumbleson and Kalish, 1971). It was reported that the apparent digestibility of the ether extract (EE) of 10 mg FB<sub>1</sub>/kg feed was significantly reduced during the weanling phase, and then increased to the level in the control group after this phase (Gbore and Egbunike, 2007). Because fumonisins are mentioned in the disruption of lipid metabolism action in animals, this suggests that a 10 ppm mixture of cultured and pure fumonisin B<sub>1</sub> decreased the cholesterol concentration in pig serum, and that this may be related to the lower digestibility of the ether extract over the 37 days of experiment. This mixture level and the length of experiment may not have been enough to increase the total cholesterol serum, as reported in some studies. However, the commercial

additive and *Phyllanthus amarus* extract could have increased the cholesterol level (Table 7 and 8).

In terms of enzyme activity, the commercial additive and *Phyllanthus amarus* extract elevated the concentration of AST to higher levels than the safe limit (AST >100 U/L) (Zomborszky et al., 2002), especially, the commercial additive.

## **Histopathology**

The observed microscopic pathology of lung is in the present study consistent with Osweiler et al. (1992) and Zomborszky et al. (2002), who found thickening of alveolar walls. Moreover, the liver histopathology is also in agreement with Gelderblom et al. (1988) concerning the degeneration of hepatocytes. It can be concluded that the commercial additive and *Phyllanthus amarus* extract had a mild effect in reducing the negative impacts of fumonisins. However, possibly because of the high dose, *P. amarus* extract caused tenderness of liver and AST elevation. Furthermore, the commercial additive also showed an AST level increase over the limit which can cause liver damage.

## **5. Conclusions**

The concentration of fumonisin and the addition of detoxicants in this study did not cause any changes in the growth performance of pigs

However, high fumonisin in the diet caused a decrease in serum total cholesterol to under the normal level, and the commercial additive and *Phyllanthus amarus* extract increased this concentration and reduced the histopathology in lungs and liver, although the reduction was not so clear.

The *Phyllanthus amarus* extract concentration in feed may have been too high, and therefore could have induced tenderness and swelling of the liver.

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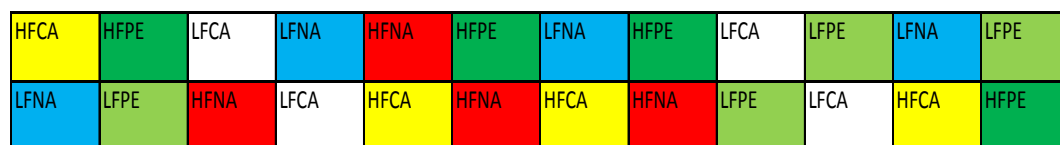
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**Table 1.** Individual treatments

	<b>NA</b>	<b>CA</b>	<b>PE</b>
<b>HF</b>	HFNA	HFCA	HFPE
<b>LF</b>	LFNA	LFCA	LFPE



**Figure 1.** Experimental layout

**Table 2.** Composition of *Phyllanthus amarus* extract

Composition	Niranthin	Hypophyllanthin	Phyllanthin
Concentration (mg/g dry matter)	2.71	1.71	7.98



**Table 3.** Ingredient composition of the basal diet, as fed

Ingredients	Amount (%)
Extruded maize	48.4
Extruded soybean	20.6
Rice bran	8.01
Soybean meal	7.01
Fish meal 60%	7.01
Lactose	5.01
Dicalciumphosphate	1.03
Fat powder	0.54
Whey powder	0.42
Lactic acid	0.40
Threonine	0.39
Vitamin and mineral premix	0.30
Lysine HCl	0.25
Choline 60%	0.20
Methionine	0.16
Salt	0.15
Colistin 10%	0.10
Total	100

**Table 4.** Chemical composition of the basal diet, analysed values (% of DM)

Dry matter	Crude protein	Ether extract	Crude fibre	Ash	Ca	P
89.7	21.4	6.37	3.56	5.94	1.07	0.73

**Table 5.** Effects of fumonisin level and commercial additive or *Phyllanthus amarus* extract on growth performance of weaned pigs

	Fumonisin			Detoxicant					
	Low	High	SEM	Prob.	NA*	CA*	PE*	SEM	Prob.
<i>LW**</i> , kg									
<i>Initial</i>	11.1	10.8	0.223	0.374	10.9	10.7	11.3	0.288	0.456
<i>Final</i>	25.3	25.7	0.775	0.737	26.0	24.9	25.6	0.950	0.697
<i>ADG**</i> , kg	0.384	0.402	0.018	0.482	0.409	0.382	0.388	0.022	0.656
<i>ADFI**</i> , kg	0.735	0.741	0.028	0.883	0.744	0.735	0.735	0.035	0.979
<i>FCR**</i>	1.93	1.85	0.038	0.193	1.83	1.93	1.91	0.047	0.276

\*NA: No detoxicant additive; CA: Commercial detoxicant additive; PE: *Phyllanthus amarus* extract

\*\*LW: live weight; ADG: average daily weight gain; ADFI: average daily feed intake; FCR: feed conversion ratio

**Table 6.** Combination effects on piglet performance of commercial detoxifying additive or *Phyllanthus amarus* extract with two levels of fumonisin in the diets

	Low fumonisins			High fumonisins			SEM	Prob.
	NA*	CA*	PE*	NA*	CA*	PE*		
<i>LW**</i> , kg								
<i>Initial</i>	11.1	11.2	11.0	10.6	10.3	11.5	0.407	0.282
<i>Final</i>	26.4	25.4	24.1	25.6	24.4	27.1	1.34	0.426
<i>ADG**</i> , kg	0.413	0.384	0.354	0.405	0.380	0.421	0.031	0.290
<i>ADFI**</i> , kg	0.788	0.733	0.683	0.699	0.736	0.788	0.049	0.164
<i>FCR**</i>	1.92	1.92	1.94	1.74	1.94	1.88	0.066	0.308

\*NA: No detoxicant additive; CA: Commercial detoxicant additive; PE: *Phyllanthus amarus* extract

**Table 7.** Effects of fumonisin level and commercial additive or *Phyllanthus amarus* extract on serum blood parameters of piglets

	Fumonisin				Detoxicants				
	Low	High	SEM	Prob	NA*	CA*	PE*	SEM	Prob
<i>Bilirubin T, mg/100mL</i>	0.381	0.430	0.032	0.298	0.466	0.380	0.370	0.039	0.179
<i>Bilirubin D, mg/100mL</i>	0.198	0.219	0.015	0.349	0.230	0.177	0.219	0.018	0.124
<i>Bilirubin I, mg/100mL</i>	0.183	0.197	0.025	0.685	0.216	0.203	0.150	0.032	0.309
<i>Albumin, g/100mL</i>	3.29	3.23	0.081	0.631	3.17	3.27	3.33	0.100	0.518
<i>Tot Chol**</i> , mmol/L	2.42	2.19	0.049	0.007	2.24	2.34	2.33	0.060	0.407

\*NA: No detoxicant additive; CA: Commercial detoxicant additive; PE: *Phyllanthus amarus* extract

\*\*Tot Chol: Total cholesterol

**Table 8.** Combination effects of commercial additive and *Phyllanthus amarus* extract on serum blood parameters of piglets fed fumonisin contaminated diets

	Low fumonisins			High fumonisins				
	NA*	CA*	PE*	NA*	CA*	PE*	SEM	Prob.
<i>Bilirubin T, mg/100mL</i>	0.418	0.335	0.390	0.515	0.425	0.349	0.055	0.412
<i>Bilirubin D, mg/100mL</i>	0.218	0.160	0.218	0.242	0.193	0.221	0.026	0.852
<i>Bilirubin I, mg/100mL</i>	0.200	0.175	0.173	0.232	0.232	0.128	0.042	0.506
<i>Albumin, g/100mL</i>	3.34	3.14	3.38	3.00	3.40	3.29	0.140	0.129
<i>Tot Chol**</i> , mmol/L	2.40	2.45	2.41	2.08	2.23	2.26	0.084	0.602

\*NA: No detoxicant additive; CA: Commercial detoxicant additive; PE: *Phyllanthus amarus* extract

\*\*Tot Chol: Total cholesterol

**Table 9.** Effects of fumonisin level and commercial additive or *Phyllanthus amarus* extract on serum enzyme activities of piglets

	Fumonisin				Detoxicants				
	Low	High	SEM	Prob	NA*	CA*	PE*	SEM	Prob
<i>GGT**</i> , U/L	66.1	60.1	7.23	0.569	56.5	67.8	65.0	8.85	0.631
<i>ALP**</i> , U/L	322	291	26.5	0.118	326	318	275	32.5	0.888
<i>AST**</i> , U/L	99.2	94.1	6.56	0.593	81.9	110	97.7	8.03	0.067
<i>ALT**</i> , U/L	65.6	63.6	3.96	0.733	58.3	68.7	66.7	4.85	0.285

\*NA: No detoxicant additive; CA: Commercial detoxicant additive; PE: *Phyllanthus amarus* extract

\*\*GGT: Gamma Glutamyl Transpeptidase; ALP: Alkaline Phosphatase; AST: Aspartate Aminotransferase (GOT: Glutamic Oxaloacetic Transaminase); ALT: Alanine Aminotransferase (GPT: Glutamine Pyruvic Transaminase)

**Table 10.** Combination effects of commercial additive and *Phyllanthus amarus* extract on serum enzyme activities in piglets fed fumonisin contaminated diets

	Low fumonisin			High fumonisin			SEM	Prob.
	NA*	CA*	PE*	NA*	CA*	PE*		
GGT**, U/L	64.3	65.3	68.7	48.7	70.3	61.4	12.4	0.703
ALP**, U/L	373	288	304	279	349	246	45.7	0.238
AST**, U/L	90.1	115.4	92.1	73.7	105.1	103.4	11.3	0.476
ALT**, U/L	62.8	65.4	68.5	53.8	72.0	65.0	6.82	0.514

\*NA: No detoxicant additive; CA: Commercial detoxicant additive; PE: *Phyllanthus amarus* extract

\*\*GGT: Gamma Glutamyl Transpeptidase; ALP: Alkaline Phosphatase; AST: Aspartate Aminotransferase (GOT: Glutamic Oxaloacetic Transaminase); ALT: Alanine Aminotransferase (GPT: Glutamine Pyruvic Transaminase)

**Table 11.** Pathology in organs of pigs fed high and low fumonisin contaminated feed with or without detoxicants

Treatments	Organs		
	Lung	Liver	Kidney
LFNA	<i>Macroscopically:</i> interstitial pneumonia <i>Microscopically:</i> mild Mycoplasma	<i>Macroscopically:</i> signs of cirrhosis <i>Microscopically:</i> mild hyperemia, but normal	<i>Macroscopically:</i> pale <i>Microscopically:</i> mild hyperemia, mild hemorrhage at the renal cortex
LFCA	<i>Macroscopically:</i> signs of <i>Actinobacillus pleuropneumoniae</i> (APP) <i>Microscopically:</i> mild mycoplasma	<i>Macroscopically:</i> signs of cirrhosis <i>Microscopically:</i> normal	<i>Microscopically:</i> normal
LFPE	<i>Macroscopically:</i> Pleuritis, pale, pulmonary edema <i>Microscopically:</i> Mycoplasma (+)	<i>Macroscopically:</i> tenderness of liver, pale <i>Microscopically:</i> normal	<i>Microscopically:</i> normal
HFNA	<i>Macroscopically:</i> focal inflammation on the right lung <i>Microscopically:</i> thickening of alveolar walls, hemorrhage, Mycoplasma (+), red hepatization of many lobules, pneumonitis	<i>Microscopically:</i> fatty degeneration of hepatocytes in many lobules	<i>Macroscopically:</i> degeneration <i>Microscopically:</i> inflammation, hemorrhage in renal cortex and renal medulla
HFCA	<i>Macroscopically:</i> pulmonary edema, moderate interstitial pneumonia, hydrothorax, inflammation of apical lobe <i>Microscopically:</i> thickening of alveolar walls but mild and no pneumonitis, Mycoplasma (+)	<i>Macroscopically:</i> mild swollen, pale <i>Microscopically:</i> normal, hyperemia in some lobules, mild fatty degeneration	<i>Macroscopically:</i> swollen, pale <i>Microscopically:</i> normal
HFPE	<i>Macroscopically:</i> Pulmonary necrosis, atelectasis - carnification, Mycoplasma <i>Microscopically:</i> thickening of alveolar walls but mild- serofibrinous pneumonia, Mycoplasma (+), many leukocytes	<i>Macroscopically:</i> tenderness of liver, necrosis <i>Microscopically:</i> normal	<i>Microscopically:</i> normal

