Aflatoxins and Their Metabolites Residues in Swine Tissues from 7 Regions of Bangkok and Near-by, Thailand

สารพิษอะฟลาทอกซินและเมแทบอไลท์ตกค้างในเนื้อเยื่ออวัยวะของสุกร จาก 7 เขตของกรุงเทพมหานครและปริมณฑล

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ระหว่างเดือนมีนาคม พ.ศ. 2541 ถึงเดือนกุมภาพันธ์ พ.ศ. 2542 เก็บตัวอย่างตับและกล้ามเนื้อสุกรจำนวน 630 ตัวอย่าง โดยการสุ่มจาก 7 เขตของกรุงเทพมหานครและปริมณฑล มาตรวจวิเคราะห์หาสารพิษอะฟลาทอกซินและเมแทบอไลท์ โดยนำแต่ละตัวอย่างหนัก 50 กรัม มาสกัด ทำให้บริสุทธิ์ด้วยเซปแผคฟลอริซิลคาร์ทริด และวัดหาปริมาณอะฟลาทอกซินโดยใช้วิธีเอชพีแอลซี พบว่าร้อยละ 20.3 ของตัวอย่างตับ (64 จาก 315 ตัวอย่างตับ) และร้อยละ 10.2 ของตัวอย่างกล้ามเนื้อ (32 จาก 315 ตัวอย่างกล้ามเนื้อ) ตรวจพบว่ามีอะฟลาทอกซินชนิดบี1 บี2 จี1 เอ็ม1 และอาร์ศูนย์ ระดับอะฟลาทอกซินบี1 ตกค้างพบในตับด้วยค่าเฉลี่ย 0.518 ± 0.084 พีพีบี สูงกว่าในกล้ามเนื้อซึ่งพบค่าเฉลี่ย 0.036 ± 0.011 พีพีบี ระดับอะฟลาทอกซินบี1 ตกค้างมีค่าต่ำกว่า 5 พีพีบี ซึ่งเป็นระดับที่อนุญาตให้มีได้สูงสุดในอาหารที่มนุษย์บริโภค ตัวอย่างจากฤดูหนาวพบอะฟลาทอกซินตกค้างมากที่สุด (ร้อยละ 21.4) รองลงไปคือฤดูฝน (ร้อยละ 19.0) และฤดูร้อน (ร้อยละ 5.2) ตามลำดับ ซึ่งทั้งฤดูหนาวและฤดูฝนมีการเกิดสารพิษจากเชื้อราได้สูง ตัวอย่างตับและกล้ามเนื้อจากเขตบางซื่อพบอะฟลาทอกซินตกค้างมากที่สุด คือพบอะฟลาทอกซินบี1 ด้วยค่าเฉลี่ย 0.854 ± 0.137 พีพีบี และอะฟลาทอกซินเอ็ม1 ด้วยค่าเฉลี่ย 0.315 ± 0.083 พีพีบี และพบตกค้างในจำนวนสูงที่สุดคือ ร้อยละ 29.2 (28 จาก 96 ตัวอย่างที่ตรวจพบ) ที่พบรองลงไปคือเขตธนบุรีด้วยค่าเฉลี่ยชนิดบี1 และ เอ็ม1 0.631 ± 0.126 พีพีบี และ 0.210 ± 0.026 พีพีบี ตามลำดับ รองลงไปอีกคือจังหวัดนนทบุรี เขตสัมพันธวงศ์ ดุสิต บางกอกน้อยและจตุจักรของกรุงเทพมหานครตามลำดับ แสดงว่าเขตบางซื่อและธนบุรีของกรุงเทพมหานครมีความเสี่ยงอันตรายสูงต่อผู้บริโภคเนื้อเยื่อสุกรที่มีสารพิษอะฟลาทอกซินตกค้างอยู่ ดังนั้นระดับของสารพิษอะฟลาทอกซินในอาหารสุกรควรมีมาตรการควบคุมให้มีค่าต่ำที่สุด เพื่อลดอัตราการเสี่ยงของการเกิดมะเร็งลงได้

คำสำคัญ : อะฟลาทอกซิน เมแทบอไลท์ สารตกค้าง สุกร ตับ กล้ามเนื้อ

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Abstract : Anong Bintvihok and Danis Davitiyananda. 2003. Aflatoxins and their metabolites residues in swine tissues from 7 regions of Bangkok and near-by, Thailand. Thai J Health Res 17 (1) : 11 – 21.

Six hundred and thirty samples of swine livers and muscles were collected from seven regions of Bangkok and near-by, Thailand during March 1998 to February 1999 by means of multistage random sampling for the detection of aflatoxins and their metabolites. Samples of 50 grams each were extracted and cleaned up by sep-pak florisil cartridge and quantitatively analysed by HPLC (High Performance Liquid Chromatography). 20.3% of the liver samples (64 out of 315) and 10.2% of the muscle samples (32 out of 315) were found to contain aflatoxin B1, B2, G1, M1 and R0. The increasing residual levels of aflatoxin B1 were detected in the livers with mean value of 0.518 ± 0.084 ppb whereas the decreasing residual levels of aflatoxin B1 were detected in the muscles with mean value of 0.036 ± 0.011 ppb. The levels of aflatoxin B1 residues in both tissues were lower than tolerance level (5 ppb) in human food. Samples collected in winter contained the highest aflatoxin residues with the highest frequency (21.4%) followed by samples collected in rainy season (19.0%) and summer (5.2%), respectively. Both winter and rainy season were related with the high incidence of aflatoxin producing moulds. Both liver and muscle samples collected from Bangsua region contained the highest aflatoxin B1 residues (0.854 ± 0.137 ppb) and the highest aflatoxin M1 residues (0.315 ± 0.083 ppb) with the highest frequency 29.2% (28 out of 96). The decreasing residual levels of aflatoxin B1 and aflatoxin M1 were detected from Thonburi region with mean value of 0.631 ± 0.126 ppb and 0.210 ± 0.026 ppb, respectively. The decreasing residual levels of aflatoxins were detected from Nonthaburi, Sumpuntawong, Dusit, Bangkoknoi and Chathuchak region, respectively. It can be estimated that Bangsua and Thonburi region of Bangkok could pose a high risk to human health through ingestion of aflatoxins residues in swine tissues. Therefore, the policy to decrease levels of aflatoxins and their metabolites in the swine feeds could be set up to avoid the risk of cancer.

Key words : aflatoxins, metabolites, residue, swine, liver, muscle

Introduction

Thailand is a humid tropical country thus many kinds of foods and feedstuffs such as peanuts, maize, rice and other cereals are frequently contaminated with aflatoxins, mycotoxin produced by *Aspergillus flavus* and *A. parasiticus* (Bhat, 1991). Aflatoxin producing strains of these molds are now known to occur worldwide and widespread in nature, and also to grow well and produce the toxin on various kinds of crops in the field or in the storeroom under suitable conditions (Glinsukon *et al.,* 1980; Siriacha *et al.,* 1988). Conditions favoring the growth of the fungus and toxin production are high moisture content (18-30%) and high temperature (optimal 75-95 °F) (Anonymous, 1977; Christensen and Kaufman, 1965). Therefore, Thailand is one of high risk are as for aflatoxin contamination.

Major metabolites which are produced by the fungi are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). AFB1 is the most abundant and most potent hepatotoxic and carcinogenic metabolite of *A. flavus*. However, conversion of aflatoxin B1 to aflatoxin M1 (AFM1), aflatoxin P1 (AFP1), aflatoxin Q1 (AFQ1), aflatoxin B2a (AFB2a) and aflatoxicol (AFR0) are regarded to be detoxification steps, because the toxicity or carcinogenicity of these metabolites are lower than those of aflatoxin B1 (Campbell and Hayes, 1976; Shank, 1981; Larsson and Tjalve, 1992; Patterson, 1973; Kumagai *et al*., 1995).

Aflatoxin contaminated in feed trends to cause economical loss of animal production and subsequently create public health problems in human (Pier *et al.,* 1977; Stoloff *et al.,* 1977; Angsubhakorn, 1983a). Hepatocarcinogenicity of aflatoxin has been observed in several experimental animals. The importance of aflatoxin in human hepatic cancer as an etiological agent has also been demonstrated in some areas of Asia and Africa by epidemiological studies. Moreover, aflatoxin in feeds could pose a risk to human health through ingestion of aflatoxin containing foods derived from the animals fed the toxin contaminated diet (Rodricks and Stoloff, 1977; Angsubhakorn, 1983b; Shank *et al.,* 1972; Bruce, 1990; Bintvihok *et al.,* 1991, 1992b; Jackson and Groopman, 1999; Hollinger and Ekperigin, 1999; Fink-Gremmels, 1999).

Aflatoxin residues in feeds have been studied extensively for cattle, swine, fish and poultry (Bintvihok *et al.,* 1992a; Wyllie and Morehouse, 1978; Rodricks and Stoloff, 1977; Sugano *et al*., 1993; Papp *et al*., 1999; Vanbarneveld, 1999; Ioannou-Kakovri *et al*., 1999; Stroka *et al*., 1999), but little is known about the residue in swine tissues. In the present study, we examined the aflatoxins and their metabolites residues in swine livers and muscles from seven regions of Bangkok and near-by, Thailand.

In order to identify aflatoxins residues capable of causing adverse effects on health and possibly present in a selected food. We examined the residues of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and their metabolites aflatoxin M1 (AFM1) and aflatoxicol (AFR0) in the livers and muscles of swines, five to six months of age which suitably were slaughtered for food.

Materials and Methods

Swine tissues sampling

Six hundred and thirty samples of swine livers and muscles were collected from retail or fresh market of seven regions of Bangkok and near-by, Thailand, by means of multistage random sampling technique. Approximately 50 grams of each sample in the same organ was collected from 50 kilograms of each seller. Fifteen samples of livers and muscles were collected from each region in each season. One hundred and five samples of livers and muscles were collected from seven regions in each season. We collected three hundred and fifteen samples of livers and those of muscles in three seasons, summer (March to June 1998), rainy season (July to October 1998) and winter (November 1998 to February 1999). These samples were kept in -20°C and used within one month.

Analysis of residues of aflatoxins and their metabolites

Aflatoxins (AFB1, AFB2, AFG1, AFG2) and their metabolites (AFM1, AFR0) in swine tissues were determined using a method of Association of Official Analytical Chemistry (AOAC, 1995). Each tissue sample was blended with a blender until being homogeneous and weighed 50 grams mixture into a 500 ml screw-capped Erlenmeyer flask, added 5 ml of 20% citric acid solution and mixed thoroughly with a glass stirring rod. After being mixed with 10 grams diatomaceous earth, tissues were extracted with 100 ml dichloromethane on a shaker for 30 min, then filtered through a fast filter paper into 250 ml Erlenmeyer flask containing 10 grams sodium sulfate anhydrous. Clear filtrate was evaporated to near dryness in a vacuum rotary evaporator. Aflatoxins in dry extract were further purified with Sep-Pak Florisil cartridge (Sugano *et al*., 1993) by sequential elution with chloroform : methanol (9 : 1, v/v, 10 ml), n-hexane (10 ml), ether : n-hexane (6 : 4, v/v, 10 ml) and acetone : water (99 : 1, v/v, 50 ml). Acetone : water fraction was evaporated to dryness in a vacuum rotary evaporator and nitrogen gas, then dissolved in methanol and filtered through millipore membrane filter (0.45 μm). The clear methanol filtrate (20 μl) was injected into HPLC (High Performance Liquid Chromatography) Shimadzu, Japan, LC-6 A pump, RF-535 fluorescence detector with excitation 365 nm and emission 425 nm, CTO-6A oven, SCL-6B system controller, and C-R3A chromatopac). AFB1 , AFB2 , AFG1 and AFG2 were determined by HPLC with a normal phase column (Nucleosil 50-10 porous silica gel stainless steel column, 30 cm. x 4.6 mm.) using water saturated with chloroform : cyclohexane : acetonitrile : ethanol (50 : 15 : 2 : 1, v/v/v/v) as mobile phase at a flow rate of 1 ml/min. AFM1 and AFR0 were determined by HPLC with a reverse phase column (Micro Bondapak C18 stainless steel column, 15 cm. x 4.6 mm.) using 45% methanol as mobile phase at a flow rate of 1 ml/min. Standard aflatoxins for HPLC were purchased from Sigma Chemical. CO., MO., USA. Statistical significance were computed by analysis of variance and Student Newman-Keuls Test.

Results

The positive samples of aflatoxins residues detected in swine livers and muscles are summarised in table 1. Twenty point three percent of the liver samples (64 out of 315) and 10.2% of the muscle samples (32 out of 315) were found to contain aflatoxins B1, B2, G1, M1 and/or R0. Aflatoxins residues in livers and muscles of the swines are summarised in table 2. The increasing residual levels of AFB1 were detected in the livers with mean value of 0.518 ± 0.084 ppb and the decreasing residual levels of AFB1 were detected in the muscles with mean value of 0.036 ± 0.011 ppb. The residual levels of aflatoxins and their metabolites were remarkably higher in the livers than in the muscles. The positive samples were found to be contaminated with AFB1 and their levels were lower than the tolerance level (5 ppb).

Table 1 Positive samples of aflatoxins residues detected in swine livers and muscles.

Tissue	**No. of sample**	**No. of positive**[1]	**Percentage (%)**
Liver	315	64	20.3
Muscle	315	32	10.2
Total	**630**	**96**[2]	**15.2**

[1]Positive samples of aflatoxin residues were found to contain aflatoxin B1, B2, G1, M1 and/or R0.

[2]The levels of AFB1 residues in both tissues were lower than tolerance level (5 ppb).

Table 2 Residual levels of aflatoxins and their metabolites detected in swine livers and muscles.

Aflatoxins	**Residual levels (ppb)**[1]		
	Liver (n=315)	**Muscle** (n=315)	**Total** (n=630)
B1	0.518 ± 0.084*	0.036 ± 0.011	0.277 ± 0.045
B2	0.051 ± 0.034	0.013 ± 0.012	0.032 ± 0.016
G1	0.016 ± 0.003	0.007 ± 0.001	0.012 ± 0.001
G2	ND	ND	ND
M1	0.266 ± 0.017**	0.006 ± 0.001	0.136 ± 0.009
R0	0.038 ± 0.013	0.002 ± 0.001	0.019 ± 0.005
Total	**0.162 ± 0.013***	**0.012 ± 0.003**	**0.087 ± 0.007**
	(n = 1890)	(n = 1890)	(n = 3780)

[1] Residual levels are mean ± SEM (standard error of the mean)

*Significantly difference (*P< 0.05, **P<0.01), aflatoxins residues in livers are higher than muscles

AFB1 residues are higher than AFM1 and others

ND = Non detected,<0.001 ppb., n = number of sample

The influence of sampling season on aflatoxin contamination was summarised in table 3 and 4. Samples collected in winter contained the highest AFB1 residues level (0.583 ± 0.092 ppb) and lower AFM1 level was detected (0.125 ± 0.027 ppb) with the highest frequency (21.4%). Samples collected in rainy season contained lower residual levels in AFB1 (0.325 ± 0.084 ppb) and the highest in AFM1 (0.259 ± 0.028 ppb) and also the lower frequency (19.0%). Both winter and rainy season were related with the high incidence of aflatoxin producing moulds. The residual levels of AFB1 and AFM1 were remarkably higher in winter and rainy season than in summer.

Table 3 The influence of sampling season on aflatoxins contaminated samples detected in swine livers and muscles.

Tissue	Summer	Rainy season	Winter
Liver	6.7% (7/105)[1]	25.7% (27/105)	28.6% (30/105)
Muscle	3.8% (4/105)	12.4% (13/105)	14.3% (15/105)
Total	**5.2% (11/210)**	**19.0% (40/210)****	**21.4% (45/210)***

[1]percentage of positive samples (positive samples/total samples)

* Significantly difference (*P < 0.05, **P<0.01) compared to summer.

Table 4 Residual levels of aflatoxins detected in swine tissues in each season.

Aflatoxins	Residual levels (ppb)[1]		
	Summer (n = 210)	Rainy season (n = 210)	Winter (n = 210)
B1	0.041 ± 0.012	0.325 ± 0.084**	0.583 ± 0.092*
B2	ND	0.015 ± 0.002	0.104 ± 0.055
G1	0.003 ± 0.001	0.004 ± 0.001	0.028 ± 0.004
G2	ND	ND	ND
M1	0.023 ± 0.008	0.259 ± 0.028*	0.125 ± 0.027**
R0	0.003 ± 0.001	0.018 ± 0.005	0.038 ±0.021

[1]Residual levels are mean ± SEM (standard error of the mean)

ND = Non detected, <0.001 ppb., n = number of sample

* Significantly difference (*P<0.05, **P<0.01) compared to summer.

The region of aflatoxin contamination was summarised in table 5 and 6. Both liver and muscle samples collected from Bangsua region contained the highest AFB1 residues (0.854 ± 0.137 ppb) and the highest AFM1 residues (0.315 ± 0.083 ppb) with the highest frequency 29.2% (28 out of 96). The decreasing residual levels of AFB1 and AFM1 were detected from Thonburi region with mean value of 0.631 ± 0.126 ppb and 0.210 ± 0.026

ppb, respectively, with the decreasing frequency 27.1% (26 out of 96). The decreasing residual levels of AFB1 and AFM1 were detected from Nonthaburi (0.236 ± 0.014 ppb and 0.144 ± 0.032 ppb), Sumpuntawong (0.087 ± 0.023 ppb and 0.083 ± 0.024 ppb), Dusit (0.255 ± 0.092 ppb and 0.113 ± 0.075 ppb), Bangkoknoi (0.327 ± 0.191 ppb and 0.043 ± 0.012 ppb), and Chathuchak region (0.125 ± 0.037 ppb and 0.072 ± 0.012 ppb) with the decreasing frequency 13.5%, 11.5%, 9.4%, 7.3% and 2.1%, respectively.

Table 5 Positive samples of aflatoxins residues detected in swine livers and muscles in each region.

Regions	**Number of positive samples**[1]			
	Liver	**Muscle**	**Total**	**Percentage (%)**
Bangsua	15	13	28	29.2
Dusit	7	2	9	9.4
Chathuchak	1	1	2	2.1
Sumpuntawong	8	3	11	11.5
Thonburi	20	6	26	27.1
Bangkoknoi	6	1	7	7.3
Nonthaburi	7	6	13	13.5
Total	**64**	**32**	**96**	**100**

[1]Positive samples of aflatoxin residues were found to contain aflatoxin B1, B2, G1, M1 and/or R0.

Table 6 Residual levels of aflatoxins detected in swine tissues in each region.

Regions	**Residual levels (ppb)**[1] **(n = 96)**					
	AFB1	**AFB2**	**AFG1**	**AFG2**	**AFM1**	**AFR0**
Bangsua	0.854 ± 0.137*	ND	0.012 ± 0.005	ND	0.315 ± 0.083**	0.047 ± 0.016
Dusit	0.255 ± 0.092	0.020 ±0.008	0.004 ± 0.002	ND	0.113 ± 0.075	0.004 ± 0.001
Chathuchak	0.125 ± 0.037	ND	0.002 ± 0.001	ND	0.072 ± 0.012	0.067 ± 0.003
Sumpuntawong	0.087 ± 0.023	0.016 ±0.041	0.013 ± 0.004	ND	0.083 ± 0.024	0.002 ± 0.001
Thonburi	0.631 ± 0.126	ND	0.039 ± 0.012	ND	0.210 ± 0.026	0.004 ± 0.002
Bangkoknoi	0.327 ± 0.191	ND	0.015 ± 0.003	ND	0.043 ± 0.012	0.003 ± 0.001
Nonthaburi	0.236 ± 0.014	0.011 ±0.005	ND	ND	0.144 ± 0.032	0.069 ± 0.021

[1]Residual levels are mean ± SEM (standard error of the mean)

* Significantly difference (*P< 0.05, **P<0.01), compared to others regions and others toxins.

ND = Non detected, <0.001 ppb., n = number of sample

Discussion

From table 1 and 2, the residual levels of aflatoxins and their metabolites were remarkably higher in the livers than in the muscles. The regulatory level of AFB1 in human foods adopted in many countries in the European community and USA is 5-25 ppb (Gilbert, 1991). The range from 5 ppb AFB1 for all foods in Belgium, Ireland, Netherlands and Spain to 25 ppb AFB1 for peanuts in Portugal. Regulatory aspects of AFB1 in human foods adopted in Asia and Africa is 5-50 ppb (Van Egmond, 1991). The level of 5 ppb AFB1 for legumes and fermented foods in China or peanuts, maize, sorghum and cereals in Mauritius and Zimbabwe. The level of 10 ppb AFB1 for all foods in Japan or rice in China. The level of 15 ppb AFB1 for almonds, maize, peanuts, cereals, pistachio nuts, pine nuts and rice in Jordan. The level of 20 ppb AFB1 for maize, peanuts and coconut in China, Hong Kong and Philippines or all foods except infant foods in Nigeria. The level of 30 ppb AFB1 for all foods in India and cereal grains in Nepal. The level of 50 ppb AFB1 for cereals (rice, maize, peanuts, legumes, etc.) in Taiwan.

In this study, the positive samples were found to be contaminated with AFB1 lower than the tolerance level (5 ppb). The AFB1 levels in swine tissues should be considered as an acceptable level of aflatoxins, although it may cause economic losses associated with growth retardation. Moreover, the mean value level of AFB1 in tissues were lower than 1 ppb. The AFB1 less than 1 ppb was also reported in chicken tissues from slaughterhouses and/or farms which used to have an incidence of aflatoxin exposure in/or around Bangkok, Thailand during 1986 to 1987. Contamination of aflatoxins were highest in liver and lowest in muscles. The level of AFB1 in tissues were 0.010-0.068 ppb. The rainy season was the highest season for aflatoxin contamination (Bintvihok *et al*., 1990). However, the AFB1 level higher than 5 ppb was also reported in chicken tissues from 5 parts (10 provinces) of Thailand in 1996-1997. Four point nine percent of the positive samples (17 out of 344) were found to be contaminated with AFB1 higher than the tolerance level (5 ppb) but the mean value level of AFB1 in tissues were lower than 1 ppb (Bintvihok and Davitiyananda, 2002). Furthermore, the tissues might contain other toxic metabolites of AFB1 and policy to decrease levels of AFB1 in the swine feeds could be set up to avoid the risk.

From table 3 and 4, the residual levels of AFB1 was higher in winter (0.583 ± 0.092 ppb) than in rainy season (0.325 ± 0.084 ppb) but the residual levels of AFM1 was higher in rainy season (0.259 ± 0.028 ppb) than in winter (0.125 ± 0.027 ppb). The samples collected in winter contained the aflatoxins with the highest frequency (21.4%) whereas the samples collected in rainy season contained the lowest frequency (19.0%). The residual levels of AFB1 and AFM1 were remarkably higher in winter and rainy season than in summer season. According to our data and others, the seasonal and geographical patterns of aflatoxin contamination have affected the production of aflatoxins from the fungus (Sinchaisri *et al*., 1983; Siriacha *et al*., 1988; Bintvihok *et al*., 1990, 2002). Both winter and rainy season were related with the high incidence of aflatoxin producing moulds.

From table 5 and 6, both liver and muscle samples collected from Bangsua region contained the highest AFB1 and AFM1 residues with the highest frequency. The decreasing residual levels of AFB1 and AFM1 were detected from Thonburi region with the decreasing frequency. From the above data, it can be estimated that Bangsua region of Bangkok and Thonburi region could pose a high risk to human health through ingestion of aflatoxins and their metabolites residues in swine livers and muscles. The mean value level of aflatoxins and their metabolites in tissues were lower than 1 ppb indicated that the most of samples could be safety enough for the consumers. However, the levels of aflatoxins and their metabolites in human foods should be kept as low as possible in order to reduce the incidence rate of cancer in the countries where foods are frequently contaminated with aflatoxins.

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