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Aflatoxins and Their Metabolites Residues in Chicken Tissues from 5 Parts (10 Provinces) of Thailand

สารพิษอะฟลาทอกซินและเมตาโบไลท์ตกค้างในเนื้อเยื่อไก่ จาก 5 ภาค (10 จังหวัด) ของประเทศไทย

Anong Bintvihok Danis Davitiyananda**

บทคัดย่อ: อนงค์ บิณฑวิหค และ ดานิศ ทวีติยานนท์. 2545. สารพิษอะฟลาทอกซินและเมตาโบไลท์ตกค้างในเนื้อเยื่อไก่จาก 5 ภาค (10 จังหวัด) ของประเทศไทย. วารสารวิจัยวิทยาศาสตร์การแพทย์ 16 (1) : 37-50.

ระหว่างเดือนกันยายน พ.ศ. 2539 ถึงเดือนพฤษภาคม พ.ศ. 2540 เก็บตัวอย่างตับและกล้ามเนื้อไก่จำนวน 900 ตัวอย่าง โดยการสุ่ม จาก 5 ภาค (10 จังหวัด) ของประเทศไทย มาตรวจวิเคราะห์หาสารพิษอะฟลาทอกซินและเมตาโบไลท์ โดยนำแต่ละตัวอย่างหนัก 50 กรัม มาสกัดทำให้บริสุทธิ์ด้วยเซปแผคฟลอริซิลคาร์ทริด และวัดหาปริมาณสารพิษโดยใช้วิธีเอชพีแอลซี พบว่าร้อยละ 55.11 ของตัวอย่างตับ (248 จาก 450 ตัวอย่างตับ) และร้อยละ 21.33 ของตัวอย่างกล้ามเนื้อ (96 จาก 450 ตัวอย่างกล้ามเนื้อ) ตรวจพบว่ามีสารพิษอะฟลาทอกซินชนิดบี1 บี2 จี1 เอ็ม1 และอาร์ศูนย์ ระดับอะฟลาทอกซินบี1ตกค้างพบในตับ ด้วยค่าเฉลี่ย 0.6092 ± 0.0994 พีพีบี สูงกว่าในกล้ามเนื้อซึ่งพบ 0.0451 ± 0.0116 พีพีบี ร้อยละ 4.94 ของตัวอย่างที่ตรวจพบสารพิษ (17 จาก 344 ตัวอย่างที่ตรวจพบสารพิษ) มีระดับอะฟลาทอกซินบี1ตกค้างสูงกว่า 5 พีพีบี ซึ่งเป็นระดับที่อนุญาตให้มีได้สูงสุดในอาหารที่มนุษย์บริโภค ตัวอย่างจากฤดูหนาวพบสารพิษตกค้างมากที่สุด (ร้อยละ 51.67) รองลงไปคือฤดูฝน (ร้อยละ 48.67) และฤดูร้อน (ร้อยละ 14.33) ตามลำดับ ตัวอย่างจากเขตวงเวียนใหญ่ กรุงเทพมหานคร (ภาคกลาง) พบสารพิษตกค้างมากที่สุด รองลงไปคือจังหวัดขอนแก่น (ภาคตะวันออกเฉียงเหนือ) จังหวัดพิษณุโลก (ภาคเหนือ) อยุธยาและนครปฐม (ภาคกลาง) สงขลาและสุราษฎร์ธานี(ภาคใต้) เชียงใหม่ (ภาคเหนือ) ชลบุรี (ภาคตะวันออก) และนครราชสีมา (ภาคตะวันออกเฉียงเหนือ) ตามลำดับ แสดงว่าเขตวงเวียนใหญ่ในกรุงเทพมหานคร จังหวัดขอนแก่น และจังหวัดพิษณุโลก มีความเสี่ยงอันตรายสูงต่อผู้บริโภคตับและกล้ามเนื้อไก่ที่มีสารพิษอะฟลาทอกซินและเมตาโบไลท์ตกค้างอยู่ ดังนั้นระดับของสารพิษอะฟลาทอกซินในอาหารไก่ควรมีมาตรการควบคุมให้มีค่าต่ำที่สุด เพื่อลดอัตราการเสี่ยงของการเกิดมะเร็งลงได้

คำสำคัญ : สารพิษอะฟลาทอกซิน เมตาโบไลท์ สารตกค้าง เนื้อเยื่อไก่

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Abstract: Anong Bintvihok and Danis Davitiyananda. 2002. Aflatoxins and their metabolites residues in chicken tissues from 5 parts (10 provinces) of Thailand. Thai J Hlth Resch 16(1) : 37-50.

Nine hundred samples of chicken livers, breast and leg muscles were collected from five parts (ten provinces) of Thailand during September 1996 to May 1997 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). 55.11% of the liver samples (248 out of 450) and 21.33% of the muscle samples (96 out of 450) were found to contain aflatoxin B1, B2, G1, M1 and R0. The higher residual levels of aflatoxin B1 were detected in the livers with mean value of 0.6092 ± 0.0994 ppb whereas the lower residual levels of aflatoxin B1 were detected in the muscles with mean value of 0.0451 ± 0.0116 ppb. The levels of aflatoxin B1 residues in both tissues were only 4.94% of the positive samples (17 out of 344) higher than tolerance level (5 ppb) in human food. Samples collected in winter season contained the highest aflatoxin residues with the highest frequency (51.67%) followed by samples of rainy season (48.67%) and summer season (14.33%), respectively. The samples collected from Wongwienyai region of Bangkok (the central part) contained the highest aflatoxins residues, followed by samples of Khonkaen province (the north-eastern part), Phitsanulok province (the northern part), Ayuthaya and Nakhonpathom (the central part), Songkhla and Suratthani (the southern part), Chiangmai (the northern part), Chonburi (the eastern part) and Nakhonratchasima (the north-eastern part), respectively. It can be estimated that Wongwienyai region of Bangkok, Khonkaen province and Phitsanulok province could pose a high risk to human health through ingestion of aflatoxins and their metabolites residues in chicken livers and muscles. Therefore, the policy to lower levels of aflatoxins in the chicken feeds could be set up to avoid the risk of cancer.

Key words : aflatoxins, metabolites, residue, chicken tissue

Introduction

Aflatoxins are a group of potent toxic and carcinogenic secondary metabolites of some species of *Aspergillus flavus*_and *A. parasiticus*. Aflatoxin-producing strains of these molds are now known to occur worldwide and wildspread 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. The 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). Thailand is a humid tropical country thus many kinds of foodstuffs and feedstuffs such as peanuts, maize, rice and other cereals are frequently contaminated with aflatoxins (Glinsukon et al.,1980; Siriacha et al., 1988). Aflatoxins contaminated in feed trend to cause the economical loss in terms of growth retardation, increased in mortality, decreased in production of meat and egg, depression of egg quality, egg size, egg shell and hatchability. In addition to the economic losses, aflatoxin in feeds could pose a risk to human health because of ingestion of aflatoxin containing foods derived from the animals fed the toxin-contaminated diet (Angsubhakorn, 1983). Residues of aflatoxins and their metabolites could be present in the meat, milk and eggs of animals receiving aflatoxin contaminated feeds. (Gregory and Manley, 1981; Stubblefield and Shotwell, 1981; Trucksess and Stoloff, 1979; Trucksess et al., 1983; Micco et al., 1988; Bintvihok et al., 1987, 1990, 1991, 1993). The close relationship between aflatoxin intake and human liver cancer has also been demonstrated in various areas of Asia and Africa, and the regulatory level for aflatoxin B1 in foods adopted in many countries is 5 ppb (Shank et al., 1972; Wogan, 1973; Bruce, 1990; Trucksess et al., 1983; Gilbert, 1991; Van Egmond, 1991; Bintvihok et al., 1987, 1991; IARC, 1993). However, conversion of aflatoxin B1 to aflatoxin M1, aflatoxin P1, aflatoxin Q1, aflatoxin B2a and aflatoxicol (R0) 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).

The purpose of this study is to identify aflatoxins residues capable of causing adverse effects on health and possibly present in a selected food. This prompted the author to determine residues of aflatoxin B1 (AFB_1) and their metabolites (AFM_1, AFR_0, AFB_{2a}, AFP_1, AFQ_1) in the livers and muscles of chickens, forty to forty-five days of age which suitably were slaughtered for food.

Materials and Methods

Chicken tissues sampling

Nine hundred samples of chicken livers, breast and leg muscles were collected from retail or fresh market of five parts (10 provinces) of Thailand by means of multistage random sampling. Approximately 50 grams of each sample in the same organ was collected from 50 kilograms of each seller. Fifteen samples of livers and those of muscles were collected from each province in each season. We collected three hundred samples in each season, rainy (September to November 1996), winter (December 1996 to February 1997) and summer seasons (March to May 1997). These samples were frozen in -20°C refrigerator until tested within one month.

Analysis of residues of aflatoxins and their metabolites

Aflatoxins and their metabolites in chicken tissues were determined using a method of Association of Official Analytical Chemistry (AOAC, 1995). Each tissue sample was blended with a blender until 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). AFB_1 , AFB_2 , AFG_1 and AFG_2 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. AFM_1 and AFR_0 were determined by HPLC with a reverse phase column (Micro Bondapak C_{18} 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 chicken livers and muscles are summarised in table 1. 55.11% of the liver samples (248 out of 450) and 21.33% of the muscle samples (96 out of 450) were found to contain aflatoxins B1, B2, G1, M1 and/or R0. Aflatoxins residues in livers and muscles of the chickens are summarised in table 2. The higher residual levels of AFB_1 were detected in the livers with mean value of 0.6092 ± 0.0994 ppb and the lower residual levels of AFB_1 were detected in the muscles with mean value of 0.0451 ± 0.0116 ppb. The residual levels of aflatoxins and their metabolites were remarkably higher in the livers than in the muscles. 4.94% of the positive samples (17 out of 344) were found to be contaminated with AFB_1 higher than the tolerance level (5 ppb).

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

Tissue	No. of sample	No. of positive[1]	Percentage (%)
Liver	450	248	55.11
Muscle	450	96	21.33
Total	**900**	**344**[2]	**38.22**

[1]Positive samples of aflatoxin residues were found to contain aflatoxin B_1, B_2, G_1, M_1 and/or R_o.

[2]The levels of AFB_1 residues in both tissues were only 4.94% of the positive samples (17 out of 344) higher than tolerance level (5 ppb).

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

Aflatoxins	Residual levels (ppb)[1]		
	Liver (n=360)	Muscle (n=360)	Total (n=720)
B1	0.6092 ± 0.0994	0.0451 ± 0.0116	0.3271 ± 0.0511*
B2	0.0603 ± 0.0345	0.0140 ± 0.0140	0.0371 ± 0.0186
G1	0.0160 ± 0.0029	0.0057 ± 0.0013	0.0108 ± 0.0016
G2	ND	ND	ND
M1	0.2661 ± 0.0267	0.0057 ± 0.0017	0.1359 ± 0.0142**
R0	0.0382 ± 0.0139	0.0014 ± 0.0005	0.0198 ± 0.0070
Total	0.1650 ± 0.0188	0.0120 ± 0.0031	0.0885 ± 0.0109
	(n = 2160)*	(n = 2160)	(n = 4320)

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

*Significantly different (*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, 4 and 5. Samples collected in winter season contained the highest aflatoxins residues in livers (0.2844 ± 0.0422 ppb) and muscles (0.0183 ± 0.0076 ppb) with the highest frequency (51.67%). Samples of rainy season contained lower residual levels both in livers (0.1912 ± 0.0362 ppb) and muscles (0.0141 ± 0.0047 ppb) and also the lower frequency (48.67%). The residual levels of aflatoxin B1 was remarkably higher in winter and rainy seasons than in summer season.

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

Tissue	Summer	Rainy	Winter
Liver	22.67 % (34/150)[1]	69.33 % (104/150)	73.33 % (110/150)
Muscle	6.00 % (9/150)	28.00 % (42/150)	30.00 % (45/150)
Total	**14.33 % (43/300)**	**48.67 % (146/300)****	**51.67 % (155/300)***

[1]positive sample/total *Significantly different (*P < 0.05, **P < 0.01) compared to summer

Table 4 The influence of sampling season on residual levels of aflatoxins detected in chicken livers and muscles

Season	Residual levels (ppb)[1]		
	Liver (n = 720)	Muscle (n = 720)	Total (n = 1440)
Summer	0.0191 ± 0.0065	0.0034 ± 0.0016	0.0113 ± 0.0034***
Rainy	0.1912 ± 0.0362	0.0141 ± 0.0047	0.1027 ± 0.0184**
Winter	0.2844 ± 0.0422	0.0183 ± 0.0076	0.1514 ± 0.0217*

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

*Significantly different (*P< 0.05, **P < 0.01, ***P < 0.001), n = number of sample

Table 5 Residual levels of aflatoxins detected in chicken tissues in each season

Aflatoxins	Residual levels (ppb)[1]		
	Summer (n = 240)	Rainy (n = 240)	Winter (n = 240)
B1	0.0400 ± 0.0185	0.3375 ± 0.1041	0.6038 ± 0.1082
B2	ND	0.0005 ± 0.0005	0.1109 ± 0.0557
G1	0.0039 ± 0.0014	0.0002 ± 0.0001	0.0284 ± 0.0044
G2	ND	ND	ND
M1	0.0228 ± 0.0083	0.2594 ± 0.0298	0.1255 ± 0.0275
R0	0.0010 ± 0.0006	0.0187 ± 0.0057	0.0396 ± 0.0202

[1]Residual levels are mean ± SE (standard error of the mean) ND = Non detected (<0.001), n = number of sample

Table 6 Residual levels of aflatoxins detected in chicken livers and muscles in each part

Part	Residual levels (ppb)[1]		
	Liver*	Muscle	Total
Northern	0.1581 ± 0.0414 (n = 432)	0.0062 ± 0.0045 (n = 432)	0.0821 ± 0.0210 (n = 864)**
North-eastern	0.1448 ± 0.0454 (n = 432)	0.0268 ± 0.0123 (n = 432)	0.0858 ± 0.0236 (n = 864)**
Eastern	0.0510 ± 0.0137 (n = 216)	0.0076 ± 0.0023 (n = 216)	0.0293 ± 0.0070 (n = 432)***
Central	0.2786 ± 0.0449 (n = 648)	0.0051 ± 0.0017 (n = 648)	0.1419 ± 0.0228 (n = 1296)*
Southern	0.0785 ± 0.0207 (n = 432)	0.0153 ± 0.0074 (n = 432)	0.0439 ± 0.0110 (n = 864)***

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

*Significantly different (*P< 0.05, **P < 0.01, ***P < 0.001), compared to others parts, n = number of sample

Table 7 Residual levels of aflatoxins detected in chicken tissues in each part.

Afla toxins	Residual levels (ppb)[1]				
	Northern (n=144)	North-eastern (n = 144)	Eastern (n = 72)	Central (n = 216)	Southern (n = 144)
B1	0.2830 ± 0.0984*	0.3866 ± 0.1338*	0.0775 ± 0.0333*	0.4781 ± 0.1230*	0.2101 ± 0.0549*
B2	0.0731 ± 0.0731	0.0350 ± 0.0350	ND	0.0518 ± 0.0309	ND
G1	0.0078 ± 0.0029	0.0293 ± 0.0058	0.0129 ± 0.0045	0.0063 ± 0.0026	0.0012 ± 0.0007
G2	ND	ND	ND	ND	ND
M1	0.0940 ± 0.0192	0.0615 ± 0.0141	0.0833 ± 0.0233*	0.2977 ± 0.0414*	0.0357 ± 0.0116
R0	0.0350 ± 0.0110	0.0023 ± 0.0013	0.0020 ± 0.0011	0.0174 ± 0.0061	0.0346 ± 0.0319
Total	0.0820 ± 0.0210 (n = 864)	0.0860 ± 0.0240 (n = 864)	0.0290 ± 0.0070 (n = 432)	0.1420 ± 0.0230 (n = 1296)	0.0470 ± 0.0110 (n = 864)

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

*Significantly different (*P< 0.01), compared to others toxins. ND = Non detected (<0.001), n = number of sample

The part of aflatoxin contamination was summarised in table 6 and 7. Both liver and muscle samples collected from the central part contained the highest aflatoxin B1 residues (0.4781 ± 0.1230 ppb) with the highest aflatoxins residues (0.2786 ± 0.0449 ppb) in the livers and lowest aflatoxins residues (0.0051 ± 0.0017 ppb) in the muscles. The aflatoxin B1 residual levels of the north-eastern part (0.3866 ± 0.1338 ppb), the northern part (0.2830 ± 0.0984 ppb) and the southern part (0.2101 ± 0.0549 ppb) were higher

than the eastern part (0.0775 ± 0.0333 ppb). The aflatoxins residual levels in livers of the northern part (0.1581 ± 0.0414 ppb) and the north-eastern part (0.1448 ± 0.0454 ppb) were higher than the southern part (0.0785 ± 0.0207 ppb) and the eastern part (0.0510 ± 0.0137 ppb). The highest aflatoxins residual levels (0.0268 ± 0.0123 ppb) in muscles were found from the north-eastern part and the lower aflatoxins residual levels in muscles were 0.0153 ± 0.0074 ppb (southern part), 0.0076 ± 0.0023 ppb (eastern part) and 0.0062 ± 0.0045 ppb (northern part), respectively.

Table 8 The influence of sampling season on aflatoxins contaminated samples detected in chicken livers and muscles in each province

Province	Number of positive sample[1]									
	Summer		Rainy		Winter		Total[2]		Percentage (%)	
	L	M	L	M	L	M	L	M	L	M
Chiangmai	5	0	15	2	8	0	28	2	2.22	4.44
Phitsanulok	0	0	9	10	13	1	22	11	48.89	24.44
Khonkaen	5	6	15	0	13	14	33	20	73.33	44.44
Nakhonratchasima	0	0	5	8	15	6	20	14	44.44	31.11
Chonburi	9	3	9	6	9	5	27	14	60.00	31.11
Ayuthaya	4	0	15	10	5	5	24	15	53.33	33.33
Nakhonpathom	2	0	10	1	10	0	22	1	48.89	2.22
Bangkok	7	0	15	1	15	8	37	9	82.22	20.00
Suratthani	2	0	10	4	9	6	21	10	46.67	22.22
Songkhla	0	0	1	0	13	0	14	0	31.11	0
Total	34	9	104	42	110	45	248	96	55.11	21.33

[1]Positive samples of aflatoxin residues were found to contain aflatoxin B_1, B_2, G_1, M_1 and/or R_o

[2]Total samples of each tissue are 45 samples in each province

L = Positive samples of livers, M = Positive samples of muscles, % = Percentage of positive samples

The province of aflatoxin contamination was summarised in table 8, 9 and 10. Both liver and muscle samples collected from Bangkok contained the highest aflatoxins residues (0.2590 ± 0.0607 ppb) with the highest frequency 82.22% (37 out of 45) in the livers and the lower frequency 20.00% (9 out of 45) in the muscles. The aflatoxins residual levels of Khonkaen (0.1476 ± 0.0454 ppb) and Phitsanulok (0.1226 ± 0.0410 ppb) were higher than Ayuthaya (0.0962 ± 0.0246 ppb), Nakhonpathom (0.0704 ± 0.0184 ppb) and the other provinces. The residual levels of aflatoxin B1 was remarkably higher in

Bangkok (1.0594 ± 0.3372 ppb), Khonkaen (0.7319 ± 0.2616 ppb) and Phitsanulok (0.5297 ± 0.1912 ppb) provinces than others provinces.

Table 9 Residual levels of aflatoxins detected in chicken livers and muscles in each province

Province	Residual levels (ppb)[1]		
	Liver (n = 216)	Muscle (n = 216)	Total (n = 432)
Chiangmai	0.0727 ± 0.0141	0.0108 ± 0.0090	0.0417 ± 0.0085
Phitsanulok	0.2434 ± 0.0812	0.0017 ± 0.0006	0.1226 ± 0.0410**
Khonkaen	0.2728 ± 0.0899	0.0225 ± 0.0065	0.1476 ± 0.0454**
Nakhonratchasima	0.0167 ± 0.0039	0.0312 ± 0.0238	0.0239 ± 0.0121
Chonburi	0.0510 ± 0.0137	0.0076 ± 0.0023	0.0293 ± 0.0070
Ayuthaya	0.1797 ± 0.0483	0.0128 ± 0.0049	0.0962 ± 0.0246***
Nakhonpathom	0.1409 ± 0.0362	ND	0.0704 ± 0.0184***
Bangkok	0.5154 ± 0.1191	0.0025 ± 0.0012	0.2590 ± 0.0607*
Suratthani	0.0578 ± 0.0235	0.0307 ± 0.0147	0.0442 ± 0.0139
Songkhla	0.0992 ± 0.0340	ND	0.0496 ± 0.0172

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

*Significantly different (*P<0.05, **P<0.01, ***P<0.001), compared to others provinces.

ND = Non detected (<0.001), n = number of sample

Table 10 Residual levels of aflatoxins detected in chicken tissues in each province

Province	Residual levels (ppb)[1] (n = 72)					
	AFB1	AFB2	AFG1	AFG2	AFM1	AFR0
Chiangmai	0.0363 ± 0.0269	ND	ND	ND	0.1444 ± 0.0349	0.0698 ± 0.0213
Phitsanulok	0.5297 ± 0.1912**	0.1461 ± 0.1461	0.0156 ± 0.0056	ND	0.0436 ± 0.0142	0.0003 ± 0.0003
Khonkaen	0.7319 ± 0.2616**	ND	0.0394 ± 0.0102	ND	0.1100 ± 0.0266	0.0043 ± 0.0026
N. ratchasima	0.0413 ± 0.0173	0.0700 ± 0.0700	0.0191 ± 0.0051	ND	0.0130 ± 0.0052	0.0003 ± 0.0003
Chonburi	0.0775 ± 0.0333	ND	0.0129 ± 0.0045	ND	0.0833 ± 0.0233	0.0020 ± 0.0011
Ayuthaya	0.2553 ± 0.1202	ND	0.0047 ± 0.0022	ND	0.3133 ± 0.0777	0.0042 ± 0.0019
N. pathom	0.1197 ± 0.0453	0.1353 ± 0.0905	0.0033 ± 0.0031	ND	0.1640 ± 0.0411	0.0003 ± 0.0002
Bangkok	1.0594 ± 0.3372*	0.0200 ± 0.0184	0.0109 ± 0.0069	ND	0.4156 ± 0.0858	0.0476 ± 0.0176
Suratthani	0.1246 ± 0.0472	ND	0.0023 ± 0.0015	ND	0.0714 ± 0.0226	0.0671 ± 0.0639
Songkhla	0.2956 ± 0.0985	ND	ND	ND	ND	0.0020 ± 0.0014

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

*Significantly different (*P< 0.05, **P<0.01), compared to others provinces.

ND = Non detected (<0.001), 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 AFB_1 in human foods adopted in the European community and USA is 5-25 ppb (Gilbert, 1991). The range from 5 ppb AFB_1 for all foods in Belgium, Ireland, Netherlands and Spain to 25 ppb AFB_1 for peanuts in Portugal. Regulatory aspects of AFB_1 in human foods adopted in Asia and Africa is 5-50 ppb (Van Egmond, 1991). The level of 5 ppb AFB_1 for legumes and fermented foods in China or peanuts, maize, sorghum and cereals in Mauritius and Zimbabwe. The level of 10 ppb AFB_1 for all foods in Japan or rice in China. The level of 15 ppb AFB_1 for almonds, maize, peanuts, cereals, pistachio nuts, pine nuts and rice in Jordan. The level of 20 ppb AFB_1 for maize, peanuts and coconut in China, Hong Kong and Philippines or all foods except infant foods in Nigeria. The level of 30 ppb AFB_1 for all foods in India and cereal grains in Nepal. The level of 50 ppb AFB_1 for cereals (rice, maize, peanuts, legumes, etc.) in Taiwan.

In this study, 4.94% of the positive samples (17 out of 344) were found to be contaminated with AFB_1 higher than the tolerance level (5 ppb). However, the mean value level of AFB_1 in tissues were lower than 1 ppb. The AFB_1 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 in 1986 - 1987. Contamination of aflatoxins were highest in liver and lowest in muscles. The level of AFB_1 in tissues were 0.010-0.068 ppb. The rainy season was the highest season for aflatoxin contamination. (Bintvihok et al., 1990). The AFB_1 levels in chicken tissues would be anticipated to cause risk to human health. Furthermore, the tissues might contain other toxic metabolites of AFB_1 and policy to lower levels of AFB_1 in the chicken feeds could be set up to avoid the risk.

From table 3, 4 and 5, the residual levels of aflatoxin B1 was remarkably higher in winter and rainy seasons 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). Both winter and rainy season were related with the high incidence of aflatoxin producing moulds.

From table 6, 7, 8, 9 and 10, it can be estimated that most of samples could be safety enough for the consumers except the sample from Wongwienyai region of Bangkok (the central part), Khonkaen province (the north-eastern part) and Phitsanulok province (the

northern part) could avoid a risk to human health through ingestion of aflatoxins and their metabolites residues in chicken livers and muscles. 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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