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Dioxin Concentrations in Body Tissues and Egg of Female Chicken

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To investigate the transfer and accumulation of ingested dioxins, the concentrations of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar-polychlorinated biphenyls (Co-PCBs) in the muscle, abdominal fat, liver, and egg of hens (Dekalb TX-35) at 1, 58, 184, and 462 days of age were measured. The levels of these three components in the four diets—starting, early growing, late growing, and laying—fed to the experimental birds were also measured. Using gas chromatography-mass spectroscopy, the concentration of each isomer of PCDD, PCDF, and Co-PCB was measured. The concentration of each component was multiplied by the individual toxic equivalency factor (TEF), the values for the isomers of each dioxin component were added together, and a toxic equivalency quantity (TEQ) was derived for each component. The total dioxin concentration (sum of TEQs for PCDDs, PCDFs, and Co-PCBs) in the muscle rose 5-fold from 58 to 462 days of age due to an increase in the amount of ether extract from muscle. These dioxin values were within the range reported for domestic chicken meat (0.0007 to 0.265 pg-TEQ/g). However concentrations in the abdominal fat and liver did not increase during the same period, suggesting that the liver is not a leading dioxin accumulation site for fowl. Dioxin concentrations in whole egg did not differ between 184 and 462 days of age and were within the reported range (0.009 to 0.138 pg-TEQ/g). This finding suggests that most of the ingested and stored dioxin did not enter the egg during the early laying period.

Key words : accumulation, body tissues, chicken, dioxins, egg

Introduction

Dioxins are polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar-polychlorinated biphenyls (Co-PCBs) that are very harmful to animal health and may disrupt the endocrine system. These chlorinated organic compounds decompose poorly and have been spread throughout the environment (U.S. EPA, 1994 ; Keith, 1997). The biological half-life of PCDDs in the human is estimated at 2555 days (almost 7 years) (U.S. EPA, 1994). Therefore, ingested dioxins can potentially affect the biological functions of animals for long time.

Because the exposure of humans to dioxins through food is a source of concern, many countries have surveyed the dioxin concentrations of various foods. Animal pro-

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ducts are the primary source of ingested dioxins in North American and European countries and the second greatest source in Japan. The daily intake of dioxin by Japanese adults (average weight ; 50 kg) is estimated as 81.47 pg-TEQ (Ministry of Health and Welfare of Japan and Environment Agency of Japan, 1999), 10.8% of which is from animal products (Ministry of Health and Labor of Japan, 2003).

Although the metabolism of dioxins in experimental animals has been studied extensively, few studies address the transfer and accumulation of dioxins in livestock. Breeders would like to produce safe foods of animal origin by improving feed management, and consumers will seek reliably safe foods for their good health. Therefore, we studied the concentrations of dioxins in the body tissues and egg of domestic chicken.

Materials and Methods

The female chickens (Dekalb TX35) in our study population were monitored until they were 462 days of age. They were maintained under heating battery brooding conditions until 28 days of age, on a flat wire battery until 112 days of age, and in laying cages thereafter. The study population received four commercial diets—starting, early growing, late growing, and laying (Table 1)—*ad libitum* according to their growth stage. Feeding periods of the four diets were 28 days for the starting diet, 28 days for the early growing diet, 75 days for the late growing diet, and 331 days for the laying diet. Individual body weight and total feed intake in a flock were measured every 2 weeks, and laying results were recorded every day after the birds began to lay. We collected samples of muscle, abdominal fat, and liver from each bird. Like samples from 1-day-old chicks (n＝50) were combined, as were those from 58-day-old birds (n

Table 1. Feed composition

Ingredient	(Materials)	Starting Diet	Growing Diet (Early)	Growing Diet (Late)	Laying Diet
		%	%	%	%
Grains	(Corn and Milo)	61	60	65	61
Plant oil cakes	(Soy bean residue and Corn glutein meal)	26	22	15	20
Brans	(Rice and Wheat bran)	3	12	16	2
Fish meal		5	3	2	6
Other	(Sodium carbonate, Alfalfameal, Salt, Papurica and Marygoldextracts and Coco palm residue)	5	3	2	1
Nutrient composition					
Crude protein		20.0	18.0	15.0	17.0
Crude fat		2.0	2.0	2.0	3.0
Crude fiber		6.0	6.0	7.0	6.0
Crude ash		8.0	8.0	9.0	14.5

=25). Individual samples were collected at 184 and 462 days of age (n=3 each). In addition we collected each bird's eggs for a week before the day 184 and 462 time points. The shells of the eggs were broken, and the contents were mixed together by using a blender.

We asked measurement of dioxins in the samples to Japan Food Research Laboratories (Tokyo, Japan). The amounts of the analytical samples were 100 g for muscle, 50～100 g for liver, 10～20 g for abdominal fat, 100 g for whole egg and 100 g for diet, respectively. The each dioxin component in the body tissues, whole egg, and diet was measured through the gas chromatography-mass spectroscopy method recommended by the Ministry of Health and Welfare of Japan (1999). An internal standards were used in the research work as cleanup standard (cleanup spike) and as syringe standard (syringe spike) shown in Table 2. For dioxins extraction, Soxhlet method was employed for diet sample using acetone-hexane (1 : 1) solution, and alkaline decomposition-solvent extraction method was employed for muscle, abdominal fat, liver and whole egg

Table 2. Internal standards used in the measurement

Cleanup standard	Added amount
[$^{13}C_{12}$] 2, 3, 7, 8-TeCDD [$^{13}C_{12}$] 1, 2, 3, 7, 8-PeCDD [$^{13}C_{12}$] 1, 2, 3, 6, 7, 8-HxCDD [$^{13}C_{12}$] 1, 2, 3, 4, 6, 7, 8-HpCDD	40 pg
[$^{13}C_{12}$] OCDD	80 pg
[$^{13}C_{12}$] 2, 3, 7, 8-TeCDF [$^{13}C_{12}$] 2, 3, 4, 7, 8-PeCDF [$^{13}C_{12}$] 1, 2, 3, 4, 7, 8-HxCDF [$^{13}C_{12}$] 1, 2, 3, 4, 6, 7, 8-HpCDF	40 pg
[$^{13}C_{12}$] OCDF	80 pg
[$^{13}C_{12}$] 3, 3′, 4, 4′-TeCB (#77) [$^{13}C_{12}$] 3, 3′, 4, 4′, 5-PeCB (#126) [$^{13}C_{12}$] 3, 3′, 4, 4′, 5, 5′-HxCB (#169)	400 pg
[$^{13}C_{12}$] 2, 3′, 4, 4′, 5-PeCB (#118) [$^{13}C_{12}$] 2, 3, 3′, 4, 4′-PeCB (#105) [$^{13}C_{12}$] 2, 3, 3′, 4, 4′, 5-HxCB (#156) [$^{13}C_{12}$] 2, 3, 3′, 4, 4′, 5, 5′-HpCB (#189)	10000 pg
Syringe standard	**Added amount**
[$^{13}C_{12}$] 1, 2, 3, 4-TeCDD [$^{13}C_{12}$] 1, 2, 3, 7, 8, 9-HxCDD	40 pg
[$^{13}C_{12}$] 3, 4, 4′, 5-TeCB (#81)	400 pg
[$^{13}C_{12}$] 2, 3, 4, 4′, 5-PeCB (#114)	10000 pg

samples using 1 mol/L potassium hydrate/ethanol solution.

Cleanup standard and solvent (acetone-hexane or potassium hydrate solution in ethanol) were added to each sample to be analyzed. Samples were extracted 3 times with n-hexane, treated with sulfuric acid, washed with water, dehydrated, and condensed. The extracts were poured into the silica gel and 10% silver nitrate silica gel mixed column chromatography, and the bound substances were extracted into hexane. The resulting extract was subjected to alumina column chromatography, with elution into dichloromethane-hexane solution. The obtained extracts were loaded onto silica gel-activated charcoal column chromatography. The columns were washed with dichloromethane-hexane solution and extracted with toluene. These final extracts were fill-upped by using n-decane then subjected to high-resolution capillary column gas chromatography-high-resolution mass spectroscopy (Autospec ULTIMA, Micromass Ltd.) with internal standards (syringe standard in Table 2).

The operating conditions of gas chromatography-high-resolution mass spectroscopy were follows.

Three columns were used ;

1. Fused silica SP-2331 (diameter 0.32 mm × length 60 m, thickness of membrane 0.2 μm) for the separation of tetrachlorodibenzo-p-dioxins (TetraCDDs), tetrachlorodibenzofurans (TetraCDFs), PentaCDDs, PentaCDFs, HexaCDDs and HaxaCDFs

2. Fused silica DB-17 (diameter 0.32 mm × length 60 m, thickness of membrane 0.25 μm) for the separation of HeptaCDDs, OctaCDDs and non-ortho polychlorinated biphenyls (non-ortho PCBs)

3. Fused silica DB-5 (diameter 0.25 mm × length 60 m, thickness of membrane 0.25 μm) for the separation of mono-ortho PCBs

The way of sample injection was split less method.

The temperature at sample inlet was 260℃. And temperature controls of three columns mentioned above were ;

1. 150℃ (1 minute of holding time) → (temperature rising at 15℃/minute) → 200℃ (5 minutes of holding time) → (temperature rising at 2℃/minute) → 250℃ (30 minutes of holding time) for Fused silica SP-2331

2. 150℃ (1 minute of holding time) → (temperature rising at 10℃/minute) → 210℃ (5 minutes of holding time) → (temperature rising at 30℃/minute) → 270℃ (36 minutes of holding time) for Fused silica DB-17

3. 150℃ (1 minute of holding time) → (temperature rising at 15℃/minute) → 200℃ (5 minutes of holding time) → (temperature rising at 2℃/minute) → 250℃ (16 minutes of holding time) for Fused silica DB-5

The temperature at ion source was 260℃. Ionizing method was electron ionization (EI) method. And ionizing voltage and current were 30 eV and 500 μA, respectively. The resolution of the apparatus was 10,000.

The recovery percentages of inner standards (cleanup standards) were 40～120% and were in the range of the analytical method recommended by the Ministry of Health and Welfare of Japan. So we could calculate the contents of the dioxins.

At same time the contents of ethel extract in muscle, abdominal fat and liver were measured. As whole samples of liver at 462 days of age were used, the content of ethel extract was not obtained.

Then the concentrations of 13 isomers of PCDD, 15 isomers of PCDF, and 11 isomers of Co-PCB were measured. The concentration of each component was multiplied by the individual toxic equivalency factor (TEF ; WHO 1997), the values for the isomers of each dioxin component were added together, and a toxic equivalency quantity (TEQ) was derived for each component. The total dioxin concentration of a sample was calculated as the sum of the TEQs for all PCDDs, PCDFs, and Co-PCBs.

Statistical analysis was conducted to examine the difference between obtained values at by t-test.

Results

The birds showed growth typical of laying hens (Fig. 1). Their mean weight (nearly 1600 g) reached a plateau at 224 days, they began to lay at 121 days of age, and 50% of the birds had laid eggs by 144 days of age. The mean daily dietary intake per bird ranged from 75 to 106 g between 140 and 440 days of age (Fig. 2). Because urgent disease control treatment was necessary, the birds were removed from the temparature-controled barn for last 42 days of experiment. During this time, they experienced heat stress, and their live weight, laying rate, and daily intake dropped slightly.

The dioxin concentrations of the diets (Table 3) were in the reported range for domestic feedstuffs (0.0004 to 0.084 pg-TEQ/g ; Ministry of Agriculture, Forestry, and Fisheries of Japan, 2003). Fish meal content in the late growing diet was the least

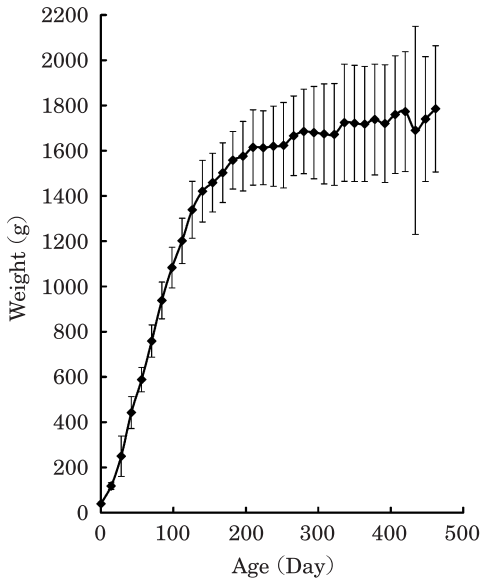

Fig. 1. Body weight of experiment bird
Each point shows mean value±SD.

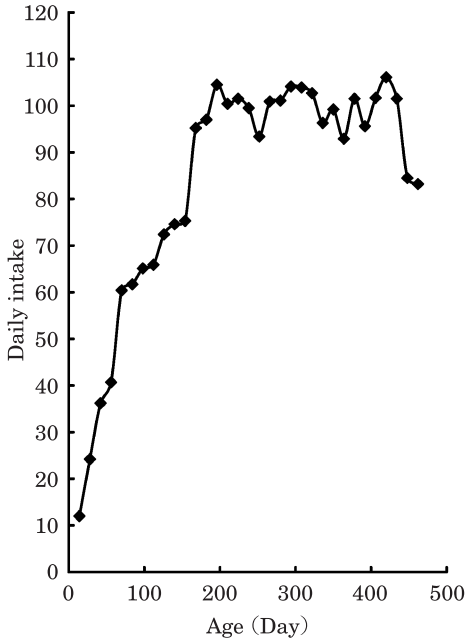

Fig. 2. Feed intake of experiment bird
Daily intake : g/bird/day

Table 3. Concentration of dioxins in diet

Diet	PCDDs	PCDFs	Co-PCBs	Total TEQ
Starting Diet	0.012	0.016	0.051	0.079
Growing Diet (Early)	0.002	0.029	0.037	0.068
Growing Diet (Late)	0.008	0.002	0.014	0.024
Laying Diet	0.014	0.015	0.027	0.056

Concentration : pg-TEQ/g

among the four diets (Table 1), the dioxin concentration in the diet was reduced. In addition, the proportions of the three dioxin components varied among the diets ; the PCDFs fraction in the late growing diet was the lowest among the four diets (Table 3).

The dioxin concentration in muscle increased after 58 days of age (Table 4). The value at 462 days of age (0.21 pg-TEQ/g) was about 5-fold greater than that at 58 days of age (0.045 pg-TEQ/g). These values are in the range reported for domestic chicken meat (0.0007 to 0.265 pg-TEQ/g ; Ministry of Agriculture, Forestry, and Fisheries of Japan, 2003). Although the diets differed at each growing stage and their dioxin compositions and contents differed, the dioxin concentrations in the muscle at 58, 184, and 462 days of age showed rather similar dioxin patterns (Table 4).

Because the newly hatched (1-day-old) chicks lacked abdominal fat, this row in Table 5 is blank (hyphen). The total dioxin concentration in abdominal fat remained nearly constant after 58 days of age. In addition, the relative proportions of the three

Table 4. Concentration of dioxins in muscle

Age (day)	PCDDs	PCDFs	Co-PCBs	Total TEQ
1	0.03	0.034	0.24	0.304
58	0.010	0.010	0.025	0.045
184	0.022±0.011[a] (0.013～0.034)	0.018±0.005[a] (0.013～0.021)	0.034±0.008[a] (0.025～0.039)	0.074±0.022[a] (0.051～0.094)
462	0.072±0.017[b] (0.053～0.086)	0.049±0.010[b] (0.040～0.059)	0.090±0.020[b] (0.079～0.110)	0.211±0.042[b] (0.172～0.255)

Concentration : pg-TEQ/g
Concentration of 184 and 462 days of age are expressed as mean value±SD.
Values in a parenthesis show the range of minimum to maximum value.
Small capitals a and b are allotted in columns. There is significant difference between a and b. ($P<0.01$)

Table 5. Concentration of dioxins in abdominal fat

Age (day)	PCDDs	PCDFs	Co-PCBs	Total TEQ
1	—	—	—	—
58	0.045	0.055	0.91	1.01
184	0.034±0.008 (0.026～0.041)	0.32±0.05 (0.27～0.37)	0.56±0.06 (0.50～0.61)	1.23±0.18 (1.03～1.39)
462	0.30±0.01 (0.29～0.30)	0.29±0.01 (0.28～0.30)	0.54±0.01 (0.53～0.55)	1.12±0.01 (1.11～1.13)

Concentration : pg-TEQ/g
Concentration of 184 and 462 days of age are expressed as mean value±SD.
Values in a parenthesis show the range of minimum to maximum value.

dioxin components were similar at 184 and 462 days of age but differed from that at 58 days of age (Table 5).

The dioxin concentration in the liver did not increase during the period from 58 to 462 days of age. And there was not significant difference 184 and 462 days of age. However, the proportions of the three components in the liver differed at 58, 184, and 462 days of age (Table 6). Dioxin concentrations in the egg at 184 and 462 days of age were in the range (0.0007 to 0.265 pg-TEQ/g) surveyed by Ministry of Agriculture, Forestry, and Fisheries of Japan (2003). Dioxin concentrations and component proportions at the two time points did not differ significantly (Table 7). The ethel extract concentration in muscle, abdominal fat and liver was shown Table 8. The concentration in muscle increased after 58 days of age. The value at 462 days of age (12.5 g/100 g) was up to over 5 times higher than that at 58 days of age (2.2 g/100 g). Meanwhile remarkable increase of concentration in abdominal fat was not observed from 58 days of age (64.8 g/100 g) to 462 days of age (65.3 g/100 g). The concentra-

Table 6. Concentration of dioxins in liver

Age (day)	PCDDs	PCDFs	Co-PCBs	Total TEQ
1	0.30	0.28	0.82	1.40
58	0.069	0.073	0.067	0.209
184	0.025±0.030	0.047±0.010[a]	0.090±0.010	0.162±0.047
	(0.002～0.053)	(0.036～0.053)	(0.081～0.10)	(0.119～0.212)
462	0.089±0.068	0.089±0.033[b]	0.110±0.050	0.288±0.150
	(0.025～0.160)	(0.055～0.120)	(0.061～0.160)	(0.141～0.440)

Concentration : pg-TEQ/g
Concentration of 184 and 462 days of age are expressed as mean value±SD.
Values in a parenthesis show the range of minimum to maximum value.
Small capitals a and b are allotted in column. There is significant difference between a and b. ($P<0.01$)

Table 7. Concentration of dioxins in whole egg

Age (day)	PCDDs	PCDFs	Co-PCBs	Total TEQ
184	0.034±0.007	0.041±0.05	0.054±0.001	0.129±0.012
	(0.026～0.039)	(0.036～0.044)	(0.053～0.054)	(0.116～0.136)
462	0.039±0.007	0.039±0.004	0.057±0.008	0.135±0.018
	(0.035～0.047)	(0.035～0.043)	(0.052～0.066)	(0.122～0.156)

Concentration : pg-TEQ/g
Concentration of 184 and 462 days of age are expressed as mean value±SD.
Values in a parenthesis show the range of minimum to maximum value.

Table 8. Concentration of ethel extract in muscle, abdominal fat and liver

Age (day)	muscle	abdominal fat	liver
1	4.8	—	14.4
58	2.2	64.8	6.8
184	4.8±0.9[a]	75.4±7.0	13.4±0.010
	(3.8～5.6)	(69.2～83.0)	(10.4～16.2)
462	12.5±2.4[b]	65.3±14.1	—
	(10.5～15.1)	(52.4～80.3)	

Concentration : g/100 g
Concentration of 184 and 462 days of age are expressed as mean value±SD.
Values in a parenthesis show the range of minimum to maximum value.
Small capitals a and b are allotted in column.
There is significant difference between a and b. ($P<0.01$)

tion in liver increased slightly from 58 days of age (6.8 g/100 g) to 184 days of age (13.4 g/100 g).

Discussion

During incubation, the dioxins in the egg were transferred to and deposited in the smaller-capacity tissues of the embryo. Because of this concentration, the dioxin concentrations of muscle and liver at 1 day of age were the highest obtained throughout the study. From 58 to 462 days of age, the dioxin concentration in abdominal fat did not increase, whereas that in the muscle did. In the animal body, dioxins primarily are associated with lipids, and with aging, body tissue water is slowly replaced by fat. The dioxin concentration in the muscle rose 5-fold during the experiment due to the increased amount of the ether extract in muscle (Table 4 and 8). Because the dioxin concentration in abdominal fat did not increase from 58 to 462 days of age (Table 5), the increased concentration in muscle was considered to be simply the result of fat deposition.

Dioxins deposited in fatty tissues seem to migrate throughout the animal body. For example, among cattle in the United States, the dioxin content in the back fat was lowest for lactating cows, followed by all other cows, young heifers, and bulls (Fires, 1995). The author concluded that stored nutrients (including dioxin-containing fat) are brought into metabolically active tissues for the production of milk or calf. In our experiment, the dioxin concentration in the abdominal fat seemed to plateau. Further, although ingested dioxins generally are stored in the liver or fatty tissue of animals, our results suggest that the liver is not the dioxin storage organ of fowl.

Like lactation and delivery, ovulation is regarded as a means of excretion of stored dioxins (Rhind, 2002). It seems that the dioxins in the egg were excreted gradually by the laying hen, and that they did not flow out all at once at an early stage of the ovulation. After 224 days of age, the weight of the bird, its daily dietary intake, and laying rate all became stable, thereby maintaining a balance in the dioxin concentration in the hen. From the dioxin concentration of the laying diet (0.056 pg-TEQ/g ; Table 3), daily dietary intake (100 g ; Fig. 2) and rate of intestinal absorption (50% ; WHO, 1997), a hen ingested 4.25 pg-TEQ of dioxins daily. According to the base data of "Phase feeding" of laying hen by Leeson and Summers (1997), the egg weight is 64 g and the laying rate is 83% at 350 days of age. The daily egg production becomes 53 g. Generally egg weight to egg shell ratio is 100 : 9. The daily whole egg's weight can be obtained as 48 g. Mean dioxin concentration in the whole egg is 0.132 pg-TEQ/g (Table 7). A hen daily excreted 10.63 pg-TEQ (4.25 pg-TEQ through feces and 6.38 pg-TEQ through egg), thereby ultimately causing a negative dioxin balance in the laying hen.

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