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Effect of Feeding Milk Replacers on the Tissue Burden of Polychlorinated Dibenzo-para-dioxins, Dibenzofurans, and Dioxin-like Polychlorinated Biphenyls in Suckling Beef Calves

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Abstract

To reduce the intake and accumulation of dioxins and dioxin-like compounds in nursing beef calves, the concentrations of seven polychlorinated dibenzo-para-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), and 12 dioxin-like polychlorinated biphenyls (PCBs) were analyzed and compared in the natural milk of grazing beef cows and bovine milk replacers, and in the blood, testis, and adipose tissue of mother-fed and bottle-fed calves at two months of age. The total toxic equivalent quantities (TEQs) of these compounds were approximately tenfold less ($P < 0.001$) in synthetic milk substitute and starter feed for calves than in natural milk (1.0 and 0.88 vs. 9.28 pg/g-lipid, respectively). The TEQ in the blood of mother-fed calves increased ($P < 0.01$) two months after birth (1.35 vs. 8.44 pg/g-lipid), whereas no difference in TEQ was found in bottle-fed calves between birth and two months of age (0.94 and 0.93 pg/g-lipid, respectively). Consequently, TEQs in the blood, testis, and adipose tissue were less ($P < 0.05$) in bottle-fed calves as compared with mother-fed calves at two months of age (0.93 vs. 8.44, 4.97 vs. 21.7, and 5.17 vs. 18.1 pg/g-lipid, respectively). Regarding individual congeners, the concentrations of dioxins with four or five chlorine substitutions were lower ($P < 0.05$) in synthetic milk substitute and starter feed than in natural milk, whereas the concentrations of hepta- and octa- chlorinated congeners were conversely higher in synthetic milk substitute and starter feed. Similarly, the accumulation of tetra- and penta- chlorinated congeners in adipose tissue of bottle-fed calves was lower ($P < 0.01$) than that in mother-fed calves. In contrast, congener specific accumulation characteristics like PCDDs were not observed in PCDFs and dioxin-like PCBs, and none of these congeners detected in bottle-fed calves were greater than that found in mother-fed calves. These results suggest that the feeding of milk replacers can markedly reduce the intake and accumulation of dioxins and dioxin-like compounds in nursing beef calves as compared with natural feeding, because the milk replacers contain preferably lower concentrations of these compounds than in natural milk.

Discipline: Animal industry
Additional key words: beef cattle, body burden, neonatal calf, organochlorine compound, tissue accumulation

Introduction

Polychlorinated dibenzo-para-dioxins (PCDDs), dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) can be formed as the unintentional byproducts of various chemical reactions and combustion processes. These compounds are ubiquitous and persistent in the environment, and potentially affect development and homeostasis in various species. Of particular interest is that perinatal exposure to these compounds has long-term effects on the development of the central nervous, endocrine, and reproductive systems (Jacobson and Jacobson 1997, Giacomini et al. 2006, Taketoh et al. 2007).

Cattle ingest PCDDs, PCDFs, and dioxin-like PCBs mostly as contaminated roughage during stall feeding or grazing. The contamination of forage plants with these compounds is primarily due to the surface adsorption of atmospheric and soil organochlorine compounds (Uegaki et

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al. 2003). Therefore, grazing animals are likely to ingest a more substantial amount of these compounds than animals fed harvested forages in confinement (Shimoda et al. 2002). Ingested organochlorine compounds accumulate in bovine adipose tissue due to their high lipophilicity (Feil et al. 2000). In suckler cows, these compounds are mobilized from body fat storage and concentrated in the milk (Willett et al. 1990, Huwe and Smith 2005, Hess and Geinoz 2011). Consequently, baby animals ingest more organochlorine compounds than mature animals on a body weight basis. Therefore, studying the transfer of these compounds from dams to suckling calves is apparently important for livestock management and production, because the compounds not only directly interfere with the growth of beef cattle but also indirectly affect human health through the consumption of food. The objectives of this study were to compare the concentrations of PCDDs, PCDFs, and dioxin-like PCBs in milk replacers with those in the natural milk of grazing beef cows, and to characterize the effects of feeding milk replacers on the intake and tissue accumulation of these compounds in nursing beef calves.

Materials and methods

1. Animals and diets

All procedures on animal subjects were reviewed and approved by the Animal Care Committee of the Institute of Livestock and Grassland Science prior to the start of the study. All animals received humane care as prescribed in the Guide for the Care and Use of Experimental Animals, established by the National Agriculture and Food Research Organization (NARO).

Three pairs of male twin Japanese Black (Wagyu) calves produced by the transfer of two embryos to Japanese Black and Holstein crossbred cows were used in this study. Grade 1 sibling blastocysts obtained from superovulated donor cows seven days after insemination were transcervically transferred into the bilateral uterine horns of estrus-synchronized recipient cows seven or eight days after the onset of standing estrus. These cows were allowed to graze in perennial ryegrass and clover pastures at the Nasu branch of the Institute (Nasushiobara, Tochigi, Japan), and were given 1 kg of concentrate (CP=12%, TDN=70% of DM) daily until three weeks before anticipated calving. Cows were subsequently kept in pens individually and fed 1 kg of concentrate and 5 kg (DM) of Orchardgrass and Italian ryegrass mixed hay twice daily. The amount of concentrate fed was increased to 1.5 kg at each feeding after parturition. At birth, the twin calves weighed 26 and 23, 25 and 26 and 28 and 29 kg, respectively, and were reared with their surrogate dams for the first seven days. Thereafter, one calf from each set of twins that weighed 26, 25 and 28 kg, respectively, was continuously reared with its own surrogate dam. During the first month, each dam and calf pair was kept in the same pen as before the calf was born, and then all pairs were kept together in a grass paddock. The other twins that weighed 23, 26 and 29 kg, respectively, were subsequently fed with commercially available synthetic milk substitute (CP=25%, TDN=110% of DM, containing vitamins and minerals as per the NRC nutrient requirements of dairy cattle (2001), milk protein, oligosaccharide, lactoferrin, and probiotic bacteria as supplements) and starter feed (CP=20%, TDN=75% of DM, containing yeast as a supplement) as per the manufacturer's instructions at Nasu farm of the MIC Division of Morinaga Rakunouhanbai Inc. (Nasu, Tochigi, Japan). Briefly, milk substitute was increased weekly until one month of age and thereafter given as 300 g/2.4 L twice a day. Starter feed was increased at the rate of 0.1 kg/week in the first month and 0.2 kg/week during the second month, and given as 1.2 kg/day at two months of age.

2. Sample collection

Maternal blood was collected five or eight days before parturition. Colostrum and calf blood were collected at birth, and before suckling was allowed. Milk, calf blood, testes, and adipose tissue were collected when calves were two months (61 to 65 d) of age, following a six-hour fast. Milk substitute powder and starter feed pellets were also collected at the same time. The body weights of calves fed natural milk and those fed milk replacer at two months of age were 100, 99 and 102 kg and 54, 57 and 58 kg, respectively.

Both colostrum and mature milk were gathered equally from four teats into sterile 50-mL polypropylene conical tubes (Falcon 2098, Corning Japan, Tokyo) after discarding the initial 50 mL of colostrum or initial 100 mL of milk from each teat. Calf blood was collected from the jugular vein into heparinized 10-mL vacuum tubes (Venoject II, Terumo, Tokyo). The testes and adipose tissue were harvested at routine castration under sedation and local anesthesia. All collected samples were immediately cooled and kept at 4˚C for one day, and then stored frozen at -70˚C until being analyzed at the Institute of Environmental Ecology (Shizuoka).

3. Analyses of PCDD/Fs and dioxin-like PCBs

Extraction and measurements of PCDD/Fs and dioxin-like PCBs in the blood and milk (including colostrum) were performed as prescribed in the provisional manual of dioxin analysis in blood (Ministry of Health, Labour and Welfare 2000a) and the provisional manual of dioxin analysis in human breast milk (Ministry of Health, Labour and Welfare 2000b), respectively. Analyses of these compounds in testis and adipose tissue were performed according to the analytical methods outlined in the dioxins survey manual for wildlife of Japan (Ministry of the Environment 2003), and in synthetic milk substitute and starter feed according to the provisional guidelines for the analytical method of dioxin-related compounds (Ministry of Agriculture, Forestry and Fisheries 2004). Briefly, 50 g of liquid samples and 10 g of tissue samples were homogenized after being thawed. Blood samples were mixed with saturated ammonium sulfate solution and ethanol, milk samples were mixed with saturated sodium oxalate solution, diethyl ether, and ethanol, and then lipophilic substances were extracted three times with n-hexane. Tissue and 100 g of feed samples were extracted by the Soxhlet method using a 75:25 mixture of diethyl ether and n-hexane for seven hours, and with toluene for 16 hours. To measure the lipid content, the extract was weighed after the evaporation of solvent. The analyte was further purified using multi-layered silica gel, activated charcoal, and silica gel columns. PCDD/Fs and dioxin-like PCBs in each sample were identified and quantified using high-resolution gas chromatography (HP 6890 series GC system, Agilent Technologies, Santa Clara, CA, USA) with a fused silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 mm; BPX5, SGE International, Melbourne, Australia) and high-resolution mass spectrometry (Auto-Spec-Ultima™ NT®, Micromass UK, Manchester, U.K.). Seven PCDD congeners and 10 PCDFs with a 2,3,7,8-chlorine substitution, as well as four non-ortho and eight mono-ortho PCBs were quantified. The details are shown elsewhere (Hirako et al. 2005). The recovery and quantities of congeners were determined by spiking samples with ^{13}C-labeled internal standards (Wellington Laboratories, Guelph, Canada). The minimum quantification limits in the blood and testis were 1, 2, 4, and 10 pg/g-lipid for PCDD/F congeners with four to five, six to seven, or eight chlorine atoms, and dioxin-like PCBs, respectively. Those in milk (including colostrum), adipose tissue, synthetic milk powder, and starter feed were 0.05, 0.02, 0.005, and 0.06 pg/g-lipid for tetra- and penta-CDD/Fs, 0.1, 0.04, 0.01, and 0.06 pg/g-lipid for hexa- and hepta-CDD/Fs, 0.2, 0.08, 0.1, and 0.6 pg/g-lipid for octa-CDD/F, and 0.5, 0.2, 0.2, and 0.6 pg/g-lipid for dioxin-like PCBs, respectively. The toxic equivalent quantities (TEQs) of individual congeners were calculated in line with the human and mammalian toxic equivalency factors reevaluated by the World Health Organization in 2005 (Van den Berg et al. 2006). The TEQs for PCDD/Fs and dioxin-like PCBs were calculated by excluding any congeners below the minimum quantification limits.

4. Statistics

Values are expressed as means ± SEM and were subjected to GLM-ANOVA (StatView, SAS Institute, Cary, NC, USA). Differences in the concentrations of PCDD/Fs and dioxin-like PCBs between the samples and growth stages of nursing calves were analyzed using a Student's t-test for paired data or non-paired data. The concentrations in synthetic milk substitute and starter feed were compared with the 95% confidence limits of the means in milk samples. For all statistical comparisons, differences were considered significant at $P < 0.05$.

Results

1. Total TEQ values

As shown in Fig. 1, the total TEQ in milk collected at two months postpartum was less ($P < 0.05$) than that in colostrum before the first suckling (9.28 ± 0.74 vs. 12.5 ± 0.1 pg/g-lipid, respectively). However, the total TEQs in synthetic milk substitute and starter feed (1.0 and 0.88 pg/g-lipid, respectively) were much less ($P < 0.001$) than those in natural milk.

The total TEQs of dioxin and dioxin-like compounds in the blood, testis, and adipose tissue of calves reared with milk replacers at two months of age were 0.93 ± 0.003, 4.97 ± 3.02, and 5.17 ± 0.32 pg/g, respectively, on a lipid weight basis, which were three to nine times less ($P < 0.01$ for the blood and $P < 0.05$ for tissues) than those (8.44 ± 0.17, 21.7 ± 4.1, and 18.1 ± 2.6 pg/g-lipid, respectively) of calves reared with their surrogate dams (Figure 2). Blood TEQ levels in bottle-fed calves at two months of age were almost the same as those (0.94 ± 0.18 pg/g-lipid) at birth, whereas the TEQ levels in mother-fed calves increased ($P < 0.01$) at two months of age as compared with those at birth (1.35 ± 0.46 pg/g-lipid).

2. TEQs of compounds

Table 1 lists the concentrations of PCDDs, PCDFs, and dioxin-like PCBs in dam and calf blood, bovine milk and milk replacers, and calf testis and adipose tissue as TEQ on a lipid weight basis. The TEQ levels of all compounds in milk replacers were less ($P < 0.01$) than those in natural bovine milk. The blood and tissue concentrations of these compounds on a lipid weight basis reflected concentrations in the type of diet consumed. Accordingly, the TEQ levels of all compounds in the blood and tissues were less ($P < 0.05$) in bottle-fed calves than in mother-fed calves at two months of age. In the blood of bottle-fed calves, only the TEQ of PCDDs increased at two months, whereas the TEQs of all compounds were elevated ($P < 0.01$) in mother-fed calves at two months of age as compared with those at birth.

As shown in Table 1, the lipid content in adipose tissue was greater in mother-fed calves than in bottle-fed calves, although the lipid content of calf blood and testis was similar between calves fed natural milk and those fed milk replacers. Therefore, the difference in the TEQ in adipose tissue was much larger ($P < 0.001$) between these two groups on a total weight basis. These results suggest

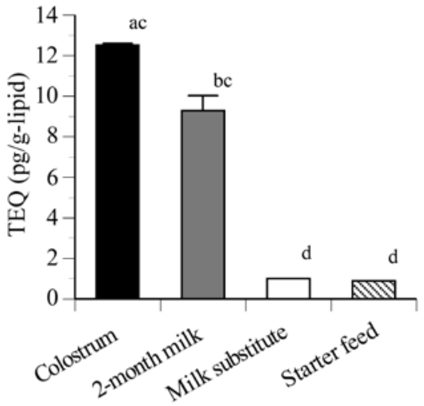

Fig. 1. Total toxic equivalent quantities (TEQs) of polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) on a lipid weight basis in natural bovine milk and milk replacers. Values with different superscripts differ significantly (a, b; *P* < 0.05, c, d; *P* < 0.001).

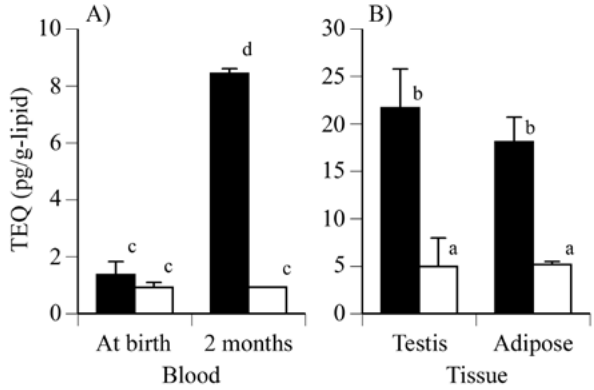

Fig. 2. Total toxic equivalent quantities (TEQs) of polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) on a lipid weight basis in the blood (A) at birth and at two months of age, and tissues (B) of mother-fed (solid) and bottle-fed calves (open) at two months of age. Values with different superscripts differ significantly in each subfigure (a, b; *P* < 0.05, c, d; *P* < 0.01).

Table 1. Toxic equivalent quantities (TEQs) of polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs), and lipid content in maternal and calf blood, milk and milk replacers, and calf tissues at birth and at two months of age

Stage	Sample	Milk fed	PCDDs (pg-TEQ/g lipid)	PCDFs (pg-TEQ/g lipid)	PCBs (pg-TEQ/g lipid)	Lipid (mg/g)
	Maternal blood †		0.58 ± 0.16	2.10 ± 0.23	1.92 ± 0.23	3.1 ± 0.2
At birth	Colostrum		2.93 ± 0.17	5.30 ± 0.06^{a}	4.30 ± 0.25^{a}	67.0 ± 14.8^{a}
	Calf blood ‡		0.008 ± 0.004^{c}	0.85 ± 0.11^{c}	0.29 ± 0.18^{c}	2.6 ± 0.2^{c}
At two months	Milk		2.27 ± 0.15	3.33 ± 0.32^{b}	3.69 ± 0.32^{b}	28.7 ± 2.0^{b}
	Calf blood	Natural	$2.83 \pm 0.19^{d,***}$	$3.33 \pm 0.27^{d,***}$	$2.27 \pm 0.09^{d,***}$	4.1 ± 0.3^{d}
		Substitute	0.31 ± 0.01^{d}	0.50 ± 0.0	0.12 ± 0.01	4.4 ± 0.2^{d}
	Testis	Natural	$7.10 \pm 2.47^{**}$	$9.67 \pm 1.78^{*}$	$5.00 \pm 1.70^{*}$	12.7 ± 0.3
		Substitute	2.35 ± 2.32	2.67 ± 0.82	0.067 ± 0.001	12.3 ± 1.2
	Adipose tissue	Natural	$3.87 \pm 0.43^{**}$	$5.73 \pm 0.39^{***}$	$8.43 \pm 1.84^{*}$	$320 \pm 0^{**}$
		Substitute	1.33 ± 0.12	1.80 ± 0.15	2.07 ± 0.13	109 ± 18
	Synthetic milk substitute §		0.40	0.33	0.27	250
	Starter feed §		0.30	0.22	0.35	37

Values are expressed as mean ± SEM (N = 3 except for maternal blood, calf blood at birth, synthetic milk substitute and starter feed).

† Data at two stages are merged in three cows (N = 6) because of no difference between stages. ‡ Data of three pairs of twin calves are merged (N = 6). § TEQ values are shown as averages of duplicate analyses, and differ significantly compared with natural milk samples (*P* < 0.01). Different letters indicate significant differences between stages in blood and milk samples (a, b; *P* < 0.05, c, d; *P* < 0.01). Asterisks indicate values differ significantly from those in calves fed milk replacers (*; *P* < 0.05, **; *P* < 0.01, ***; *P* < 0.001).

that PCDDs, PCDFs, and dioxin-like PCBs are not only distributed in tissues depending on their intake but also readily accumulate with ingested lipid in the adipose tissue of suckling calves.

3. Concentrations of individual congeners

Congeners are expressed hereafter according to the number of chlorine atoms in the molecule: T_4 as tetra-, P_5 as penta-, H_6 as hexa-, H_7 as hepta- or O_8 as octa-CDD, CDF, or CB (Tables 2 and 3). All 29 congeners analyzed were detected in synthetic milk substitute (Table 2). Except for 1,2,3,7,8,9-H_6CDF and O_8CDF, the other 27 congeners were found in dam's milk and adipose tissue of mother-fed calves (Tables 2 and 3). In addition to these two congeners, 2,3,7,8-T_4CDF, 1,2,3,7,8-P_5CDF, and 1,2,3,4,7,8,9-H_7CDF were also not detected in calves reared with milk replacers, but were detected in mother-fed calves (Table 3). As shown in Table 3, the number of congeners detected in the blood

Table 2. Concentrations of polychlorinated dibenzo-para-dioxin (PCDD), polychlorinated dibenzofuran (PCDF), and dioxin-like polychlorinated biphenyl (PCB) individual congeners in natural milk and milk replacers

	Congener	Colostrum before the first suckling	Milk at two months postpartum	Synthetic milk substitute	Starter feed
PCDD	2,3,7,8-T_4CDD	0.32 ± 0.01^a	0.32 ± 0.02^a	0.06^b	0.08^b
	1,2,3,7,8-P_5CDD	1.73 ± 0.17^a	1.53 ± 0.09^a	0.20^b	0.13^b
	1,2,3,4,7,8-H_6CDD	1.97 ± 0.09^a	0.76 ± 0.05^b	0.20^c	0.09^c
	1,2,3,6,7,8-H_6CDD	4.40 ± 0.17^a	2.53 ± 0.46^b	0.70^b	0.31^b
	1,2,3,7,8,9-H_6CDD	1.63 ± 0.19^a	0.80 ± 0.10^b	0.27^b	0.21^b
	1,2,3,4,6,7,8-H_7CDD	7.00 ± 0.59^a	1.50 ± 0.06^b	2.3^{bc}	2.8^c
	O_8CDD	4.13 ± 0.64^a	0.80 ± 0.04^b	8.9^c	47^d
PCDF	2,3,7,8-T_4CDF	0.090 ± 0.012^a	0.065 ± 0.005^a	0.14^{ab}	0.28^b
	1,2,3,7,8-P_5CDF	0.12 ± 0.02^a	0.077 ± 0.007^a	0.06^a	0.12^a
	2,3,4,7,8-P_5CDF	6.20 ± 0.10^a	4.87 ± 0.39^a	0.41^b	0.24^b
	1,2,3,4,7,8-H_6CDF	6.47 ± 0.23^a	2.47 ± 0.30^b	0.33^c	0.18^c
	1,2,3,6,7,8-H_6CDF	4.47 ± 0.15^a	2.30 ± 0.29^b	0.28^c	0.21^c
	1,2,3,7,8,9-H_6CDF	N.D.	N.D.	0.02	N.D.
	2,3,4,6,7,8-H_6CDF	10.7 ± 0.3^a	4.37 ± 0.64^b	0.40^b	0.23^b
	1,2,3,4,6,7,8-H_7CDF	3.67 ± 0.32^a	0.73 ± 0.07^b	0.56^b	0.61^b
	1,2,3,4,7,8,9-H_7CDF	0.56 ± 0.05^a	0.13 ± 0.01^b	0.05^b	0.08^b
	O_8CDF	N.D.	N.D.	0.20	1.2
Non-ortho PCB	3,3',4,4'-T_4CB	1.33 ± 0.12^a	1.50 ± 0.15^a	3.8^b	12^b
	3,4,4',5-T_4CB	2.77 ± 0.18^a	3.13 ± 0.26^a	0.30^b	0.70^b
	3,3',4,4',5-P_5CB	37.3 ± 1.9^a	32.7 ± 2.6^a	2.0^b	2.6^b
	3,3',4,4',5,5'-H_6CB	24.3 ± 4.3^a	13.5 ± 3.3^b	0.90^{ab}	1.3^{ab}
Mono-ortho PCB	2,3,3',4,4'-P_5CB	223 ± 13^a	187 ± 3^a	68^b	120^b
	2,3,4,4',5-P_5CB	52.3 ± 5.2^a	47.7 ± 6.9^{ab}	6.3^b	6.8^b
	2,3',4,4',5-P_5CB	$1{,}430 \pm 130^a$	$1{,}370 \pm 120^a$	270^b	370^b
	2',3,4,4',5-P_5CB	39.0 ± 1.2^a	32.0 ± 2.5^b	3.0^c	4.3^c
	2,3,3',4,4',5-H_6CB	187 ± 23^a	142 ± 27^b	32^b	38^b
	2,3,3',4,4',5'-H_6CB	66.3 ± 10.2^a	53.7 ± 11.0^b	8.2^{ab}	9.7^{ab}
	2,3',4,4',5,5'-H_6CB	110 ± 15^a	77.7 ± 10.7^b	11^c	19^{abc}
	2,3,3',4,4',5,5'-H_7CB	43.0 ± 8.4^a	25.0 ± 6.4^b	3.3^{ab}	3.0^{ab}

Values are expressed as pg/g-lipid (Mean ± SEM of three samples for natural milk and averages of duplicate analyses for milk replacers). Different letters indicate significant differences in the same row ($P < 0.05$).

and testis of mother-fed calves (18 and 20) was almost twice as many as those detected in calves fed milk replacers (10 and 10, respectively).

Regarding individual congener concentrations in natural milk and milk replacers, highly toxic PCDD congeners with four to five chlorine atoms were lower ($P < 0.05$) in synthetic milk substitute and starter feed than in colostrum and mature milk, whereas highly chlorinated congeners were conversely greater ($P < 0.05$) in synthetic milk substitute and starter feed (Table 2). T_4CDF was greater

Table 3. Concentrations of polychlorinated dibenzo-para-dioxin (PCDD), polychlorinated dibenzofuran (PCDF), and dioxin-like polychlorinated biphenyl (PCB) individual congeners in the blood, testis, and adipose tissue of mother-fed and bottle-fed calves

Congener	Blood		Testes		Adipose tissue	
	Natural	Substitute	Natural	Substitute	Natural	Substitute
2,3,7,8-T_4CDD	N.D.	N.D.	N.D.	N.D.	0.63 ± 0.07[c]	0.20 ± 0.00[d]
1,2,3,7,8-P_5CDD	2.0 ± 0.0	N.D.	4.7 ± 1.2	N.D.	2.50 ± 0.29[c]	0.80 ± 0.10[d]
1,2,3,4,7,8-H_6CDD	N.D.	N.D.	4.0 ± 1.0	N.D.	1.33 ± 0.07[c]	0.53 ± 0.12[d]
1,2,3,6,7,8-H_6CDD	6.7 ± 0.3[c]	3.0 ± 0.0[d]	5.3 ± 0.9	N.D.	4.13 ± 0.41[a]	1.93 ± 0.30[b]
1,2,3,7,8,9-H_6CDD	N.D.	N.D.	2.7 ± 0.3	N.D.	1.45 ± 0.30[a]	0.43 ± 0.03[b]
1,2,3,4,6,7,8-H_7CDD	3.3 ± 0.3	N.D.	16.3 ± 1.9[c]	3.7 ± 0.9[d]	2.53 ± 0.24	2.03 ± 0.20
O_8CDD	8.0 ± 1.2	6.0 ± 0.0	19.7 ± 5.5	N.D.	2.17 ± 0.58	2.50 ± 0.21
2,3,7,8-T_4CDF	N.D.	N.D.	N.D.	N.D.	0.107 ± 0.057	N.D.
1,2,3,7,8-P_5CDF	N.D.	N.D.	N.D.	N.D.	0.093 ± 0.053	N.D.
2,3,4,7,8-P_5CDF	4.0 ± 0.6[c]	1.0 ± 0.0[d]	13.3 ± 2.6[a]	3.7 ± 0.7[b]	8.83 ± 0.58[c]	2.70 ± 0.25[d]
1,2,3,4,7,8-H_6CDF	3.0 ± 0.6	N.D.	10.3 ± 1.3[a]	3.0 ± 1.0[b]	4.07 ± 0.13[c]	1.60 ± 0.15[d]
1,2,3,6,7,8-H_6CDF	5.0 ± 0.0	N.D.	5.0 ± 0.6	N.D.	3.37 ± 0.32[c]	0.93 ± 0.09[d]
1,2,3,7,8,9-H_6CDF	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,4,6,7,8-H_6CDF	5.0 ± 0.6	N.D.	12.7 ± 1.8[a]	3.3 ± 0.9[b]	5.60 ± 0.62[c]	1.97 ± 0.17[d]
1,2,3,4,6,7,8-H_7CDF	4.3 ± 1.3	N.D.	4.3 ± 0.3	N.D.	1.13 ± 0.07	0.57 ± 0.07
1,2,3,4,7,8,9-H_7CDF	N.D.	N.D.	N.D.	N.D.	0.18 ± 0.02	N.D.
O_8CDF	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3,3',4,4'-T_4CB	N.D.	N.D.	N.D.	N.D.	1.8 ± 0.3	1.0 ± 0.0
3,4,4',5-T_4CB	N.D.	N.D.	N.D.	N.D.	12.9 ± 5.8	1.0 ± 0.0
3,3',4,4',5-P_5CB	20 ± 0	N.D.	47 ± 17	N.D.	76.3 ± 17.5[a]	17.3 ± 1.2[b]
3,3',4,4',5,5'-H_6CB	N.D.	N.D.	N.D.	N.D.	26.2 ± 6.5	8.0 ± 1.2
2,3,3',4,4'-P_5CB	110 ± 15	67 ± 3	197 ± 57	50 ± 0	420 ± 121	150 ± 15
2,3,4,4',5-P_5CB	30 ± 6	17 ± 3	50 ± 6[c]	10 ± 0[d]	114 ± 28	35.7 ± 3.0
2,3',4,4',5-P_5CB	1,190 ± 260	650 ± 20	1,300 ± 150[c]	310 ± 10[d]	3,130 ± 900	1,130 ± 70
2',3,4,4',5-P_5CB	27 ± 3[c]	10 ± 0[d]	33 ± 9	N.D.	74.3 ± 17.9[a]	24.0 ± 2.1[b]
2,3,3',4,4',5-H_6CB	93 ± 23	60 ± 0	143 ± 30[a]	40 ± 0[b]	313 ± 92	133 ± 9
2,3,3',4,4',5'-H_6CB	43 ± 7[a]	20 ± 0[b]	54 ± 9[c]	10 ± 0[d]	120 ± 36	42.0 ± 3.0
2,3',4,4',5,5'-H_6CB	87 ± 19	50 ± 0	70 ± 11[a]	17 ± 3[b]	176 ± 60	74.0 ± 4.7
2,3,3',4,4',5,5'-H_7CB	20 ± 6	N.D.	30 ± 6	N.D.	50.3 ± 16.6	18.0 ± 1.7

Values are expressed as pg/g-lipid (Mean ± SEM; N=3). Different superscripts indicate significant differences in the same category (a, b; P <0.05, c, d; P <0.01).

($P < 0.05$) in starter feed, but one P_5CDF and two H_6CDFs were less ($P < 0.05$) in milk replacers as compared with natural milk. No differences were found in the other four PCDF congeners detected in all samples. Concentrations of 3,3',4,4'-T_4CB were greater ($P < 0.05$), but in the other dioxin-like PCB congeners, two non-ortho and three mono-ortho congeners were less ($P < 0.05$) in milk replacers than in natural milk.

With respect to the blood and tissue concentrations of individual congeners in mother-fed and bottle-fed calves, among the three PCDD congeners detected in the blood or testis of bottle-fed calves, the concentrations of two congeners (except for O_8CDD in the blood) were lower ($P < 0.01$) as compared with mother-fed calves (Table 3). Furthermore, T_4, P_5, and H_6CDDs in adipose tissue were less ($P < 0.05$) in bottle-fed calves than in mother-fed calves, although no differences were seen in H_7CDD and O_8CDD. Although only 2,3,4,7,8-P_5CDF was detected in the blood of bottle-fed calves, the concentrations of PCDF congeners detected in bottle-fed calves (except for 1,2,3,4,6,7,8-H_7CDF in adipose tissue) were lower ($P < 0.05$) in all samples as compared with mother-fed calves. Similarly, the values for dioxin-like PCB congeners—2 of 7 detected in the blood of bottle-fed calves, 5 of 6 in the testis, and 2 of 12 in adipose tissue—were less ($P < 0.05$) in bottle-fed calves than in mother-fed calves, whereas no values for dioxin-like PCB congeners were greater in bottle-fed calves than in mother-fed calves.

Discussion

We previously investigated the maternal-fetal and neonatal transfer of PCDDs, PCDFs, and dioxin-like PCBs in cattle, and demonstrated that these compounds are transferred from maternal to fetal circulation through the placenta (Hirako et al. 2005). Additionally, we demonstrated that neonatal calves absorb more lipophilic organochlorine compounds than do prenatal fetuses, that the congener concentrations in milk were correlated with maternal blood levels, and that the congener concentrations in suckling calf blood were dependent on their concentrations in milk (Hirako 2008c). We further demonstrated that highly chlorinated PCDD and PCDF congeners are difficult to transfer from the systemic circulation to mature milk as compared with colostrum, whereas most dioxin-like PCBs are readily transferred in both colostrum and mature milk (Hirako 2008b). We also investigated the tissue distribution of these compounds in suckling beef calves and demonstrated that highly chlorinated PCDD congeners persist in the systemic circulation and tend to accumulate in the testis more than in adipose tissue, whereas dioxin-like PCBs readily accumulate in adipose tissue (Hirako 2008a).

The concentrations of individual PCDD, PCDF, and dioxin-like PCB congeners in colostrum collected before the first suckling and in mature milk collected two months after freshening in this study were consistent with those in a previous study conducted at the same institute (Hirako 2008b, c), although the total TEQ values and congener concentrations obtained in this study were greater than those in the previous study. Similarly, the distribution patterns of individual congeners in the blood, testis, and adipose tissue of mother-fed calves were consistent with those in another study conducted at the same institute (Hirako 2008a).

To the best of our knowledge, this is the first report to show the accumulation of PCDDs, PCDFs, and dioxin-like PCBs in calves fed milk replacers. In humans, the body burden of these compounds is several times greater in breast-fed infants than in formula-fed infants (Lorber and Phillips 2002) due to the difference in content of these compounds in baby formula and breast milk (Pandelova et al. 2010). In this study, similar to what has been observed in humans, the total TEQs were several times greater in the blood, testis, and adipose tissue of mother-fed calves than those of bottle-fed calves at two months of age, and were greater in natural bovine milk than in synthetic milk substitute and starter feed.

Regarding individual congeners in natural milk and replacers, the concentrations of highly toxic dioxin congeners with four to five chlorine substitutions were significantly lower in synthetic milk substitute and starter feed than in natural milk, whereas the concentrations of highly chlorinated congeners were conversely greater in synthetic milk substitute and starter feed. Although the O_8CDD concentration in starter feed is apparently much greater than that in natural milk on a lipid weight basis, its contribution to the total TEQ is almost negligible at this level, as the toxic equivalency factor for O_8CDD is 0.0003 (Van den Berg et al. 2006). Furthermore, the difference between O_8CDD concentrations in natural milk and in starter feed is less evident on a dry weight basis compared with that on a lipid weight basis (Table 2), because pelleted starter feed contains more than 90% DM, whereas mature milk only contains about 12% DM. The intake of O_8CDD derived from starter feed is accordingly estimated to be less. On the other hand, synthetic milk substitute is supposed to have contributed to O_8CDD intake more than starter feed due to its high lipid content and feeding amount, although the O_8CDD concentration was significantly lower in milk substitute than in starter feed on a lipid weight basis. However, O_8CDD was not detected in the testis of calves fed milk replacers, and its concentrations in adipose tissue were almost at the same levels in both mother-fed and bottle-fed calves. In a previous study, Hirako (2008a) reported that the tissue distribution patterns of highly chlorinated PCDD and PCDF congeners differed from four to six chlorinated congeners and dioxin-like PCBs on a lipid weight basis, presumably due to their low affinity for lipoproteins (Patterson et al. 1989). This characteristic might be the reason why highly chlorinated congeners were concentrated in milk replacers but not in the tissue.

In this study, the body weights of calves fed milk replacers were lower than those fed by their surrogate dams at two months of age, although milk replacers were given as per the manufacturer's instructions. The body weight of male Japanese Black calves ranges from 65.9 to 90.5 kg (78.2 kg on average) at two months of age, according to the Japanese Feeding Standard for Beef Cattle (2008) edited by the National Agriculture and Food Research Organization. Mother-fed and bottle-fed calves in this study were over and under this range, respectively. Japanese Black and Holstein crossbred cows yield more than twice the amount of milk than purebred Japanese Black cows (Aoki et al. 1996), because Japanese Black and Holstein breeds are used for beef and dairy, respectively. Therefore, an abundant milk supply from crossbred cows is thought to have promoted the daily weight gain of mother-fed calves to a greater extent than the recommended amount of milk replacer. Additionally, bottle-fed calves were transported by truck, and their rearing environment and feed were drastically changed at one week of age. These stresses might delay the calves' early growth. However, the lipid content of the blood and testis in bottle-fed calves was nearly the same as that in mother-fed calves. The difference in the body weight of these two groups may be caused mostly by the difference in lipid accumulation in adipose tissue, because three times more lipid was accumulated in the adipose tissue of mother-fed calves than in bottle-fed calves. Even if calves were given more milk replacer in order to gain as much weight as the mother-fed calves, the total body burden of dioxins and dioxin-like compounds would most likely be less in bottle-fed calves than in mother-fed calves, because TEQ on a lipid weight basis was lower in milk replacers than in natural milk. In conclusion, it has been clearly demonstrated that the feeding of milk replacers containing lower concentrations of dioxins and dioxin-like compounds can reduce the intake and accumulation of these compounds in nursing beef calves as compared with natural milk feeding.

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