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Presence and Determination of Manure-borne Estrogens from Dairy and Beef Cattle Feeding Operations in Northeast China

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Abstract Four estrogens in cattle feces collected from 24 dairy and beef feeding operations located in the northeast of China were investigated. The average concentration of 17α-estradiol, 17β-estradiol and estrone in dairy feces was 194.6, 104.4, and 262 μg/kg, respectively. While as to beef waste the mean content of 17α-estradiol, 17β-estradiol and estrone was 104.5, 67.7 and 216.4 μg/kg, respectively. Estriol was below the detection limit in all samples. The 17β-estradiol equivalents of all samples ranged from 45.8 to 926.1 μg/kg and dairy together with beef probably generated 16 times more estrogens than the human population in the study area.

Keywords Dairy feces · Beef feces · Natural estrogens · 17β-Estradiol equivalents (EEQ)

Endocrine disrupting chemicals (EDCs) have caused significant concern worldwide due to their serious adverse effects on aquatic organisms even at the ng/L level. Among these EDCs, natural steroid estrogens have the greatest estrogenic potential (Khanal et al. 2006; Kolok and Sellin 2008). Concentrated animal feeding operations (CAFOs) are recognized as one of the main sources of natural estrogens which are excreted in the urine and fecal wastes of livestock (Johnson et al. 2006). Researches have indicated that leaching and migration of manure-borne estrogens from manure piles or agricultural fields fertilized with animal waste expanded the contamination range, posing a serious threat to surrounding groundwater and surface waters (Lee et al. 2007; Arnon et al. 2008). It was also found that the reproductive biology of wild fathead minnows exposed to cattle feedlot effluent was significantly altered (Orlando et al. 2004). Thus, an understanding of the excretion and environmental exposure levels of estrogens from farm manure is very important. However, information relating to natural estrogens in Chinese animal feces has been poorly documented.

The study area is the most important agricultural region and has the highest concentrated animal production base in China. As the first survey on the excretion of estrogens in Chinese animal feces, an accurate method was applied which included extraction and two purification procedures followed by quantitative determination by gas chromatography and tandem mass spectrometry. The purpose of this study was to investigate the residual levels of four natural estrogens in China cattle feces, and to estimate the potential risk of the manure-borne estrogens to the local farmlands.

Materials and Methods

A total of 24 cattle feces were collected from northeast China, concentrations of 17α-estradiol, 17β-estradiol, estriol and estrone were determined by gas chromatograph and tandem mass spectrometry. Random sampling was carried out in December 2007, 24 cattle farms including 17 dairy cattle and 7 beef cattle farms, distributed across the major cattle counties of three provinces (Fig. 1). Fresh solid droppings (<24 h, excluding liquid droppings, cleaning water or bedding materials) were taken directly

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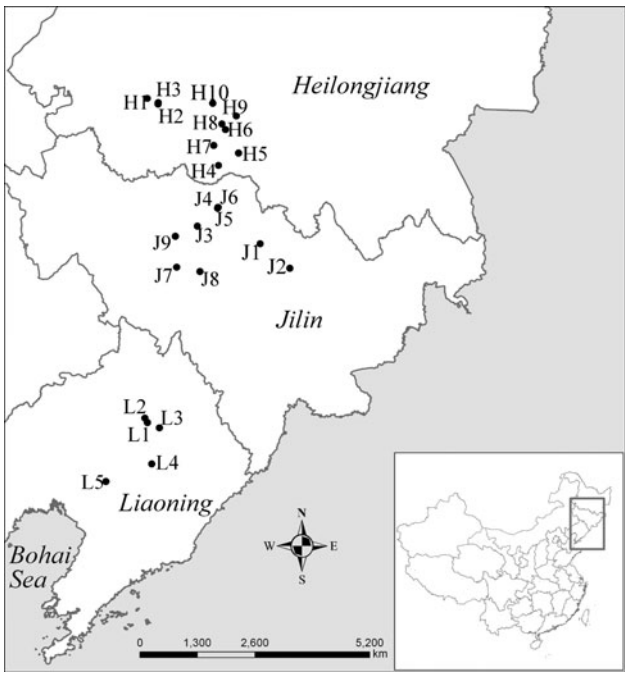

Fig. 1 Map of 24 cattle fecal sampling sites in the northeast provinces, China

from the floor of the barn. The samples were stored in a freezer below −20°C prior to freeze-drying.

Acetone, methanol, hexane, ethyl acetate, and dichloromethane of HPLC grade were purchased from J.T. Baker Company (USA). The derivatization reagent BSTFA:TMCS (99:1) was supplied by Supelco (USA). Standard compounds including 17α-estradiol (17α-E2), 17β-estradiol (17β-E2), estrone (E1), estriol (E3), internal standard androstane, and surrogate compound 17β-estradiol-d2 (17β-E2-d2) of the highest purity commercially available were purchased from Sigma–Aldrich Company (USA).

1 g freeze-dried fecal sample mixed with the surrogate standard was extracted with 20 mL acetone three times, 20 min for each extraction. The slurry was centrifuged at 10,000 rpm for 10 min. The supernatants were concentrated to about 5 mL by rotary evaporation and then filtered through a 0.45 μm nylon filter membrane.

The filtrate was diluted with 500 mL ultrapure water and the pH was adjusted to 2–3 with 1 M hydrochloric acid. The aqueous samples were loaded onto the HLB cartridge (60 mg/3 cc) at a flow rate of 8–10 mL/min, which was previously conditioned with 5 mL ethyl acetate, 5 mL methanol and 10 mL ultra-pure water in sequence. The cartridge was dried thoroughly under vacuum for 30 min after sample loading and was eluted with 8 mL of ethyl acetate.

A cleanup procedure was performed using a silica gel column (Ternes et al. 2002). The former eluate was concentrated to about 1 mL with a gentle stream of nitrogen at 40°C and was added to the silica gel column. The target compounds were finally eluted with 8 mL acetone. The eluate was dried by a gentle nitrogen stream and was reconstituted in 100 μL of the derivative reagent BSTFA:TMCS (99:1). After a reaction time of 1 h at 65°C, the derivative was dried using nitrogen. The residue was dissolved in 1 mL hexane containing 200 ng/mL of the internal standard androstane, finally, the sample was transferred into GC vials for analysis.

The detection of target chemicals were achieved using a Varian GC 3,800 coupled with a mass spectrometer 4,000, equipped with a VF-5 ms gas chromatography column (30 m × 0.25 mm × 0.25 μm film thickness). Helium was used as the carrier gas with a column flow rate of 1 mL/min. The injector and interface temperatures were both set at 280°C. The initial temperature was 80°C without retention time, and rose to 240°C at the rate of 20°C/min with 4 min retention, then increased to 280°C at the rate of 4°C/min. The injection volume was 1 μL in splitless mode. The energy of the ionizing electron was 70 eV. Mass spectra and retention time of analytes from m/z 50 to 550 were obtained in full scan mode, while the quantification was achieved in selected ion monitoring mode. The retention time, quantitative and qualitative ions of the estrogen derivatives are listed in Table 1.

Quality control samples including blanks and spiked samples were analyzed alongside each batch of samples analyzed. None of the target compounds were detected in blank samples. The spiked sample was prepared by adding 1,000 ng of each estrogen and 500 ng of surrogate standard dissolved in acetone to 1 g manure and was placed overnight to ensure that the acetone evaporated completely and the analytes could bind sufficiently to the manure matrix before extraction. Mean recovery of the spiked chemicals, 17α-E2, E1, 17β-E2 and E3 with the three parallels was 92.3%, 98.2%, 102% and 117%, respectively. The method detection limits (MDL) of target chemicals were estimated with a signal/noise ratio (S/N) of 3, and the MDL was 1.06, 5.00, 1.88 and 1.67 μg/kg for 17α-E2, E1, 17β-E2 and E3, respectively. The correlation coefficients of the working

Table 1 Retention time and characteristic ions of the estrogen derivatives

Compounds	Retention time/min	Qualitative ions	Quantitative ion
Androstane	8.870	245/260	245
17α-E2	15.425	416/285/326	416
E1	15.654	342/257/218	342
17β-E2	16.052	416/285/326	416
17β-E2-d2	16.052	418/287	418
E3	19.042	504/386/296	504

curves with seven concentration levels (100–2,000 ng/mL) were all with $r^2 \geq 0.99$.

Results and Discussion

Concentrations of the four natural estrogens in dairy feces are listed in Table 2. Of the target compounds, three of the four natural estrogens were found in 17 dairy fecal samples. E3 was not detected in any of the dairy fecal samples in this survey. In contrast, E1 was measured in all the dairy fecal samples and was the most abundant of the four estrogens. Although the concentrations of 17α-E2 and 17β-E2 in some samples were below detection limits, the highest concentrations were as high as 1,113 and 485 μg/kg, respectively, with a detected frequency of 70% for both estrogens. The mean concentrations of 17α-E2, 17β-E2 and E1 were 194.6, 104.4 and 262.2 μg/kg, E1 accounted for the largest percentage of total hormones in the dairy fecal samples, followed by 17α-E2 and 17β-E2.

In addition to dairy feces, the concentrations of natural estrogens in beef cattle feces were also determined and characterized (Table 3). 17α-E2, 17β-E2 and E1 were all detected in the samples, and the concentrations ranged from bdl to 260 μg/kg, bdl to 242.5 μg/kg and 103.1 to 507.5 μg/kg, with mean values of 104.5, 67.7 and 216.4 μg/kg, respectively. E3 was hardly detected in any of the fecal samples, these results are highly consistent with previous observations in other

Table 2 Natural estrogen concentration in dairy feces (μg/kg, dry weight)

Location[a]	17α-E2	17β-E2	E1	E3	Total estrogens
H1	67.9	66	103.5	bdl	237.4
H2	1,113	109.6	279.9	bdl	1502.5
H3	78	50	103	bdl	231
H4	bdl	bdl	282	bdl	282
H5	bdl	bdl	385.7	bdl	385.7
H6	185.8	158.4	103.1	bdl	447.2
H7	213	116.7	307.9	bdl	637.6
H8	84	51.4	103.8	bdl	239.2
H9	111.1	bdl	101.2	bdl	212.3
H10	353.4	328.4	699.4	bdl	1381.2
J1	bdl	bdl	103	bdl	103
J8	bdl	bdl	370.6	bdl	370.6
J9	283.8	246	164.3	bdl	694.1
L1	131.7	112	122.8	bdl	366.5
L2	84.8	50.4	104.4	bdl	239.5
L3	bdl	bdl	258.8	bdl	258.8
L5	602	485.1	864.7	bdl	1951.8
Min.	bdl	bdl	101.2	bdl	103
Max.	1,113	485.1	864.7	bdl	1951.8
Mean ± SD	194.6 ± 284	104.4 ± 135	262.2 ± 222	bdl	561.2 ± 533

[a] H1–H10 samples collected from Heilongjiang Porvince; J1–J9 samples from Jinlin Province and L1–L5 samples from Liaoning Province

bdl Below the detection limit

Table 3 Natural estrogen concentrations in beef manure (μg/kg, dry weight)

Location[a]	17α-E2	17β-E2	E1	E3	Total estrogens
J2	52	50.4	103.1	bdl	205.5
J3	bdl	bdl	368.8	bdl	368.8
J4	85.6	bdl	161.4	bdl	247
J5	260	242.5	507.5	bdl	1,010
J6	80	60.7	123.8	bdl	264.5
J7	253.8	120	146.5	bdl	520.2
L4	bdl	bdl	103.5	bdl	103.5
Min.	bdl	bdl	103.1	bdl	103.1
Max	260	242.5	507.5	bdl	1,010
Mean ± SD	104.5 ± 91	67.66 ± 76	216.4 ± 146	bdl	388.5 ± 281

[a] J1–J9 samples collected from Jinlin Province; and L1–L5 samples from Liaoning Province

bdl Below the detection limit

studies (Zheng et al. 2008). Moreover, E3 was shown to be less measurable than the other steroid estrogens in liquid effluent from CAFOs (Hutchins et al. 2007).

The mean concentrations of 17α-E2, 17β-E2 and E1 in dairy feces were 86.3%, 54.2% and 21.2% higher than those in beef feces, respectively, it is clear that female dairy cows may excrete more estrogens than beef cattle. The investigation concerning estrogen content in beef cattle was very limited, Lange et al. (2002) concluded that bulls excreted less estrogen than cycling and pregnant cattle. Thus, the hormone load to the environment from beef feces is less compared with dairy feces when applied at the same agronomic rates. However, no significant differences in estrogen concentrations ($p > 0.05$) between beef and dairy cow feces were found in this study, therefore more attention should be paid not only to the dairy sector but also to beef feeding operations which are relatively less studied than dairy production at present in term of estrogen excretion.

The relatively large variation in the concentrations of the three estrogens suggests that, besides the differences in species, age and sex, the status of the reproductive stage of the cattle (pregnant or not and the stage of pregnancy) may also lead to significant variation in manure-borne estrogen concentrations. Hanselman et al. (2003) reported that the fecal estrogen excretion rate in dairy cattle not pregnant or pregnant for less than 80 days was 300–600 μg/d 1,000 kg live animal mass, which increased to 1,500–11,400 μg/d 1,000 kg live animal mass when the animal was pregnant for more than 80 days. Moreover, the application of growth promoters may be an influencing factor. High-potency growth-promoting compounds, for example, 17β-E2 are often administered to beef cattle to stimulate growth either alone or with other pharmaceuticals in most cattle feedlots in the USA (Kolok and Sellin 2008). Even though owners guarantee that hormone materials are not administered, this information is suspect at least in some farms where hormone growth promoters might be applied now, however, the actual situation is unclear.

In order to assess the totally potential environment risk of estrogenic for the cattle fecal samples, the estrogenic potential of dairy and beef cattle feces was expressed as the 17β-E2 equivalents (EEQ). The mean relative potency of 17β-E2, E1, 17α-E2 and E3 is adopted from Borgert et al. (2003).

$$\mathrm{EEQ} = [17\mathrm{b}\text{ - }\mathrm{E2}] + 0.444[\mathrm{E1}] + 0.094[17\mathrm{a}\text{ - }\mathrm{E2}] + 0.171[\mathrm{E3}]$$

The EEQ concentrations of all fecal samples ranged from 45.8 to 926 μg/kg, with a mean concentration of 220.1 μg/kg. For dairy cattle feces, the EEQ concentration ranged from 45.8 to 926.1 μg/kg, and for beef cattle feces the range was 46–492.5 μg/kg. In contrast, the EEQ concentration for dairy cattle feces in another study was much higher than in the present study, and the highest concentration of 3,048 μg/kg was reported by Lorenzen et al. (2004). This disagreement can be explained by the different monitoring methods used in the two studies, because the bioassays (recombinant yeast assays) they applied could overestimate the hormone activity of the samples due to cross contamination (Hanselman et al. 2003; Lee et al. 2007). Moreover, other estrogenic materials may have contributed to the total hormone activity, such as phytoestrogens and their degradation products which would be included when using bioassays but would not be determined by instrumental detection such as GC/MS or LC/MS/MS (Lorenzen et al. 2004; Lee et al. 2007). In contrast, Lorenzen et al. (2004) observed that EEQ values for estrogen receptor gene transaction activities in 9 beef cattle fecal pats were generally below 20 μg/kg, which are relatively lower than those observed in this study, this may imply the possibility of the application of estrogenic growth promoter compounds in the beef farms in the current study.

Table 4 Comparison of the excretion of estrogens from residents and cattle in the northeast district of China in 2007 (kg, dry weight)

	Population (million)	17α-Estradiol	Estrone	17β-Estradiol	EEQ[d]
Dairy	2.4[a]	1,369	1,844	734.4	1683.2
Beef	12.7[a]	2,961	6,131.5	1917.1	4920.9
Human	108.5[a]	–[b]	269[c]	403[c]	403

[a] Data from national bureau of statistics of China

[b] Data not available

[c] According to the prediction by Johnson et al. (2006)

[d] EEQ is 17β-E2 equivalents

The estrogen load due to cattle production in the northeast district was calculated and compared with that due to humans (Table 4).

As illustrated in Table 4, estrogens due to cattle feces were much higher than those generated by humans, indicating the potential magnitude of cattle wastes as a source of estrogens, at least in this region, due to the predominance of dairy and beef cattle farming. In terms of EEQ concentrations, dairy and beef cattle together probably generated estrogens at a rate 16 times greater than the human population in the northeastern areas, which is far above the rate of four times the estrogens excreted by farm animals compared with the human population in the UK (Johnson et al. 2006). Human feces will partly enter the municipal waste water treatment system, E1, 17α-E2 and 17β-E2 have been detected in sewage effluents and municipal sewage sludge, however, the levels of these estrogens were far lower than those in cattle feces (Ternes et al. 2002; Chang et al. 2009). Zheng et al. (2008) also revealed that

land application of solid dairy waste caused a greater hormone contamination risk compared with the use of effluent from dairy farms, because the solid waste contained significantly higher levels of estrogens than wastewater in lagoons. Therefore, the environmental risk posed by the application of cattle waste as soil fertilizer may be significantly more than that caused by land-use of municipal sewage sludge and effluent.

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References

Arnon S, Dahan O, Elhanany S, Cohen K, Pankratov I, Gross A, Ronen Z, Baram S, Shore LS (2008) Transport of testosterone and estrogen from dairy-farm waste lagoons to groundwater. Environ Sci Technol 42:5521–5526

Borgert CJ, Lakind JS, Witorsch RJ (2003) A critical review of method for comparing estrogenic activity of endogenous and exogenous chemicals in human milk and infant formula. Environ Health Perspect 111:1020–1036

Chang H, Wan Y, Hu JY (2009) Determination and source appointment of five classes of steroid hormones in urban rivers. Environ Sci Technol 43:7691–7698

Hanselman TA, Graetz DA, Wilkie AC (2003) Manure-borne estrogens as potential environmental contaminants: A review. Environ Sci Technol 24:5471–5478

Hutchins SR, White MV, Hudson FM, Fine DD (2007) Analysis of lagoon samples from different concentrated animal feeding operations for estrogens and estrogen conjugates. Environ Sci Technol 41:738–744

Johnson AC, Williams RJ, Matthiessen P (2006) The potential steroid hormone contribution of farms animals to freshwaters the united kingdom as a case study. Sci Total Environ 362:166–178

Khanal SK, Xie B, Thompson ML, Sung S, Ong SK, Van LJ (2006) Fate transport and biodegradation of natural estrogens in the environment and engineered systems. Environ Sci Technol 40:6537–6546

Kolok AS, Sellin MK (2008) The environmental impact of growth-promoting compounds employed by the united states beef cattle industry: history current knowledge and future directions. Rev Environ Contam T 195:1–30

Lange IG, Daxenberger A, Schiffer B, Witters H, Ibarreta D, Meyer HHD (2002) Sex Hormones originating from different livestock production systems: fate and potential disrupting activity in the environment. Anal Chim Acta 473:27–37

Lee LS, Carmosini N, Sasman SA, Dion HM, Sepuleveda MS (2007) Agricultural contributions of antimicrobials and hormones on soils and water quality. Adv Agron 93:2–67

Lorenzen A, Hendel JG, Conn KL, Bittman S, Kwabiah AB, Lazarovitz G, Masse D, Mcallister TA, Top E (2004) Survey of hormone activities in municipal biosolids and animal manures. Environ Toxicol 19:216–225

Orlando EF, Kolok AS, Binzcik GA, Gates JL, Horton MK, Lambright CS Jr, EG Soto AM, Jr LJG (2004) Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species the fathead minnow. Environ Health Perspect 112:353–358

Ternes TA, Andersen H, Gilberg D, Bonerz M (2002) Determination of estrogens in sludge and sediments by liquid extraction and GC/MS/MS. Anal Chem 74:3498–3504

Zheng W, Yates SR, Bradford SA (2008) Analysis of steroid hormones in a typical dairy waste disposal system. Environ Sci Technol 42:530–535