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17 β -Estradiol and testosterone in drainage and runoff from poultry litter applications to tilled and no-till crop land under irrigation

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ABSTRACT

Thirteen metric tons of poultry litter are produced annually by poultry producers in the U.S. Poultry litter contains the sex hormones estradiol and testosterone, endocrine disruptors that have been detected in surface waters. The objective of this study was to evaluate the potential impact of poultry litter applications on estradiol and testosterone concentrations in subsurface drainage and surface runoff in irrigated crop land under no-till and conventional-till management. We conducted an irrigation study in fall of 2001 and spring of 2002. Four treatments, no-till plus poultry litter, conventional-till plus poultry litter, no-till plus conventional fertilizer, and conventional-till plus conventional fertilizer, were evaluated. Flow-weighted concentration and load ha^{-1} of the two hormones were measured in drainage and runoff. Soil concentrations of estradiol and testosterone were measured. Based on comparisons to the conventional fertilizer (and control) treatments, poultry litter did not add to the flow-weighted concentration or load ha^{-1} of either estradiol or testosterone in subsurface drainage or surface runoff. Significant differences were, however, observed between tillage treatments: flow-weighted concentrations of estradiol were greater for no-till than conventional-till plots of the June irrigation; and runoff loads of both estradiol and testosterone were less from no-till than conventional-till plots for the November irrigation. Although the differences between no-till and conventional-tillage appeared to affect the hydrologic transport of both hormones, the differences appeared to have inconsequential environmental impact.

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1. Introduction

As animal feeding operations have increased in size and production, quantities of livestock manure have subsequently increased and might pose environmental problems. Livestock manures contain natural steroidal estrogen and androgen hormones, particularly 17 β -estradiol, the most bioactive of natural estrogens, and testosterone (Bushee et al., 1998; Finlay-Moore et al., 2000; Nichols et al., 1997, 1998; Raman et al., 2001; Shore et al., 1993, 1995) both of which are classified as endocrine disrupting compounds (EDC) (Lintelmann et al., 2003). A concern is that livestock manure could contribute to the load of EDCs in surface and groundwater resources. In 2002 over 8.7 billion chickens were

produced in the U.S (Georgia Agricultural Statistics Service, 2004), and since one chicken can produce 1.5 kg of litter, 13 million metric tons of poultry litter were also amassed (Perkins et al., 1964). Most poultry litter is applied to croplands and pastures as a source of N, P, and K (Moore et al., 1995).

Concentrations of estradiol have been observed to range between 14 and 904 $\mu\text{g kg}^{-1}$ dry weight of litter (Hanselman et al., 2003; Shore et al., 1993). Lange et al. (2002) estimated the annual total estrogen (the sum of estradiol, estrone, and estriol) load from poultry litter to be 2.7 Mg. Estradiol has been detected in surface waters across the U.S. and Europe (Adler et al., 2001; Kolpin et al., 2002), and has the potential to affect ecological and public health.

The bioactivity of estrogens (estradiol and estrone) in poultry litter was observed in non-pregnant heifers which, after consuming chicken manure silage, showed premature udder growth and lactation (Shore et al., 1988). Tyler and Routledge (1998) demonstrated the adverse effects of estrogens from sewage treatment plants on wild fish; estradiol concentrations between 10 and 100 ng l^{-1} can affect the development of trout. Environmental

Abbreviations: NT, no-till; CT, conventional-till; PL, poultry litter; CF, conventional fertilizer.

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estrogens have been linked to decreased sperm counts, testicular, prostate, and breast cancer, and male reproductive disorders (Epstein, 1997; Harrison et al., 1997; Sharpe and Skakkebaek, 1993; Toppari et al., 1996).

Several studies have reported estrogen concentrations in surface water affected by applications of poultry litter (Finlay-Moore et al., 2000; Nichols et al., 1997, 1998; Shore et al., 1995). Few studies have reported the effects of poultry litter on subsurface or groundwater resources. Peterson et al. (2000) measured concentrations of estradiol between 6 and 66 ng l⁻¹ in water from five springs in a mantled karst aquifer in Arkansas, and linked its presence to animal waste. Shore et al. (1995) and Wicks et al. (2004) detected estradiol in spring water at a concentration ranging between 5 and 80 ng l⁻¹ and inferred that the estradiol infiltrated the soil profile from a source of manure.

The naturally occurring male sex hormone, testosterone, has been detected in surface waters of the U.S. (median concentration 116 ng l⁻¹; Kolpin et al., 2002). Although testosterone is classified as an EDC (Lintelmann et al., 2003), no reports of its adverse effects in the environment have been published (Hakk et al., 2005). Androgenic compounds from pulp and paper mills have, however, been reported to affect the sexual development of fish (Cody and Bortone, 1997; Larsson et al., 2000). Testosterone, thus, has potential to have an adverse effect on aquatic ecology. The two principle sources of testosterone in the environment are sewage treatment plants and animal agriculture (Kirk et al., 2002; Lintelmann et al., 2003).

Testosterone's aqueous solubility is greater than estradiol's and it is bound less to soil and soil organic matter (Casey et al., 2004; Lee et al., 2003). Therefore, it possesses a greater potential to move through the soil profile into groundwater where it has been detected (Shore et al., 1997; Shore and Shemesh, 2003). Although microcosm studies have indicated that testosterone is biodegradable in agricultural soils (Casey et al., 2004; Lorenzen et al., 2005), and degradation pathways of bacteria have been elucidated (Horinouchi et al., 2005), it is still detected in surface and subsurface water (Finlay-Moore et al., 2000; Jenkins et al., 2006; Shore et al., 2004).

Conservation tillage has been widely adopted in regions of the U.S. because it reduces soil erosion and increases water retention (CTIC, 2005). Reducing tillage and increasing accumulation of crop residues at the soil surface often results in increased infiltration rates and greater frequency of macropores compared to conventional-tillage (Radcliffe et al., 1988). More rapid leaching of solutes under no-till than conventionally tilled soils has been reported (Andreini and Steenhuis, 1990; Isensee et al., 1990). Combining conservation tillage and poultry litter applications may, therefore, lead to a greater potential for groundwater contamination with sex hormones. The objective of this study was to evaluate the potential for poultry litter applications at recommended agronomic rates to affect estradiol and testosterone concentrations in subsurface drainage and surface runoff from cropped land under no-till and conventional-till management.

2. Materials and methods

2.1. Experimental site, instrumentation, and experimental design

The experimental site was at the USDA-ARS J. Phil Campbell, Sr., Natural Resource Conservation Center, Watkinsville, GA. The soil at the site is a Cecil sandy loam (fine, kaolinitic, thermic, typic kanhapludults) with a 20-cm thick brown sandy loam Ap-horizon underlain by a 5- to 10-cm thick BA horizon of red sandy clay loam to clay loam texture (Bruce et al., 1983). Soil organic carbon ranged between 0.822 and 1.012% for conventional-tilled plots, and between 0.734 and 1.262% for no-till plots. Average daily

temperature in winter ranges between 6 and 8 °C, and in summer it ranges between 23 and 27 °C. Mean annual precipitation is 1240 mm with greatest precipitation in March and least in October. Before this study, poultry litter had been applied periodically to the plots designated for poultry litter applications.

The site has twelve 10 by 30 m plots. Each one is drained by five 30-m length flexible, slotted 10-cm diameter PVC tiles, installed on a 1% grade and spaced 2.5 m apart and approximately 1 m deep at the outlet. To isolate the plots from surface lateral water flow plots have sheets of polyethylene plastic (4 mil thick) around them that were installed vertically from bottom of the drain line to the soil surface.

Drain lines terminate into tipping buckets housed in a chamber at the end of each plot. Each bucket measured 30.5 cm wide by 35.6 cm long and the tipping pair was separated by a 0.65 cm sampling slot. A portion of the effluent was captured as the sampling slot crossed the drain line stream and was then directed into a 300 ml steel cylinder reservoir. Each tip was directly proportioned to a known volume, and the sampler was calibrated to sub-sample at predetermined volume intervals. When the sampler was activated, a peristaltic pump emptied the 300 ml reservoir in the collection barrel into a 1000 ml container in an ISCO model 3700 FR sequential waste water sampler. Numbers of bucket tips were recorded by way of an encapsulated reed switch which interfaced with the sampler and a Campbell Scientific CR10X data logger. This combination allowed for sequential sampling of the effluent throughout a drainage event.

Sample temperature was maintained at 4 °C by the ISCO unit to prevent degradation of sample. The drainage volume was calculated hourly from the number of tips.

A surface runoff collector (14 gauge galvanized steel) spans the plot width (10 m) at the base of each plot. Each collector channels the surface runoff from a single plot to a HS flume, where water height was measured and recorded continuously during an event to provide runoff volume. Each HS flume discharged onto a Coshocton wheel, where the runoff was sub-sampled for analysis of sediment load and solute concentration. As was the case of drainage samples, runoff samples were collected sequentially, and stored under refrigeration (4 °C) by ISCO model 3700 FR waste water samplers. Moisture Point TDR probes (Environmental Sensors, Inc., Victoria, British Columbia, Canada) had been installed in each plot and volumetric soil water content was measured at depth increments from 0 to 30 and 90 to 120 cm.

The design was a randomized complete block with a factorial combination of tillage (conventional- vs. no-tillage) and primary fertility source (poultry litter vs. conventional fertilizer). Conventional fertilizer (CF) plots were considered as controls for measuring background levels of hormones. Conventional-tillage (CT) consisted of chisel plowing and disking for seedbed preparation and incorporation of broiler litter and fertilizer. No-tillage (NT) consisted of using a planter equipped with coulters and double disc openers for seeding operations only. Corn (*Zea mays* L) was the main summer row-crop and rye (*Secale cereal* L) was a winter cover crop. The CF (N, P, K and lime) treatment followed current University of Georgia recommendations. In November 2001 128 kg ha⁻¹ N and in May 2002, 168 kg ha⁻¹ N was applied as NH₄SO₄. For both experiments, 44.8 kg ha⁻¹ of P and K were applied. The rate of PL application was determined to match the N application of the mineral fertilizer (Table 1) based on the potential mineralization of N in the litter (Ritz and Merka, 2004). Irrigation occurred on November 14–15, 2001 29 days after litter application and planting of rye, and on June 4–5, 2002, 14 days after litter application and planting of corn (Table 2). Irrigation was accomplished with a set of eight laterals at 10-m spacing, each with ten risers and sprinkler heads spaced at 9 m. Sprinkler heads were

Table 1

Rates of broiler litter, and load of estradiol and testosterone applied to plots receiving poultry litter (treatments: no-till with poultry litter [NTPL] and conventional-till with poultry litter [CTPL]).

Date	Treatment	Litter (kg ha ⁻¹)	Estradiol (mg ha ⁻¹)	Testosterone (mg ha ⁻¹)
16 Oct '01	NTPL	7,397	19.7	2.5
	CTPL	7,397	19.7	2.5
21 May '02	NTPL	11,096	11.0	4.4
	CTPL	11,096	11.0	4.4

arranged in a triangular pattern to insure uniform distribution of water. Amount of spatial distribution of irrigation was determined using seventy rain gauges set on a 7.5 by 13.5-m grid. Rain gauges were read hourly during the irrigation event. The water was from an on-site pond; hormones in the pond water were not detected.

2.2. Broiler litter

Fresh broiler litter (composite of feces, wood chips as bedding, and feathers) was trucked to the experimental site within a day after removal of chickens from the broiler house. Before spreading the litter, five grab samples were collected in sterile whirl-pack containers, placed on ice and taken to the laboratory. The samples were frozen at -80°C until analyzed for estradiol and testosterone. Additional grab samples of litter were also taken for moisture and nutrient analysis which were performed by the Soil Testing Laboratory at the University of Georgia.

2.3. Soil sampling

Before and after each irrigation soil samples (5 random cores from each plot) were collected with an auger (1.8-cm diameter) to a depth of 30 cm, and subdivided into 10 cm increments. The cores were composited by depth, air-dried, and passed through a 2-mm sieve and stored at -80°C until analyzed for the hormones (Finlay-Moore et al., 2000).

2.4. Analysis of estradiol and testosterone

Estradiol and testosterone concentrations in soil, litter and unfiltered drainage and runoff samples were measured with a commercial competitive enzyme-linked immunosorbent assay (EIA) (Caymen Chemical Company, Ann Arbor, MI). Within two hours of collecting drainage and runoff samples they were stored at -80°C until their analysis. Hormones were extracted from 1-g subsample of air-dried and sieved (2-mm) composite soil sample and 1-g composite litter sample with 5 ml of ethylacetate in 15-ml glass centrifuge tubes. Tubes were secured on a reciprocal shaker and shaken at high speed for 60 min, then centrifuged at $480 \times g$ at 10°C for 30 min. The solvent phase was removed and evaporated under a stream of ultrapure N_2 . Residue was then dissolved in the manufacturer's enzyme immunoassay buffer and hormone content

Table 2

Irrigation rates, duration, and total mean quantities of water. Note: a rain event occurred the night of 4 June 2002.

Date	Duration (h)	Rate (mm h ⁻¹)	Quantity mm (l)	Total mm (l)
14 November '01	7	9.83	68.83 (20,537)	123.73 (37,228)
15 November '01	5	9.83	54.90 (16,691)	
4 June '02	7.25	7.57	54.90 (16,691)	134.50 (40,888)
(rain at night)	ND ^a	ND	13.1 (3,982)	
5 June '02	8	8.31	66.5 (20,216)	

^a ND = no data.

measured as described above. The assay procedure for runoff, soil and litter samples has been described in detail by Finlay-Moore et al. (2000). We performed the assay according to the manufacturer's protocol. Microtiter plates were washed with an automatic strip washer (AM60 Multi-Reagent Washer, Dynex Technologies, Chantilly, VA) to remove unbound reagents. Color intensity of reactions between free hormones and tracer was measured on a spectrophotometer (μ Quant, Bio-Tek Instruments, Winooski, VT) with intensity being inversely proportional to free estradiol or testosterone. Limit of quantification for estradiol and testosterone were 8 and 6 pg ml^{-1} , respectively.

2.5. Data analysis

The total load in runoff and drainage from each plot was the sum of the products of estradiol and testosterone concentrations and their respective volumes from initiation of runoff and drainage to their respective cessation. Flow-weighted concentrations of estradiol and testosterone were calculated by dividing the total load of each hormone by the total volume of drainage or runoff for each experimental plot. The load of hormone in drainage and runoff coming off a plot was normalized to hectares. Analysis of variance was conducted on the natural log transformed hormone concentration data and untransformed hydrologic and soil moisture data using the Mixed Procedure of SAS (SAS, 2004; Littell et al., 1996) with fertilizer and tillage being fixed effects for runoff and drainage data, and for soil data depth was an additional fixed effect. The model contained the effects of tillage, fertilizer, and their interaction for runoff and drainage data. For soil data, the model contained the effects of sampling depth, tillage, fertilization and their interactions. Where significant differences ($P=0.05$) were assigned to the transformed means, data are presented in the original units.

3. Results

3.1. Soil moisture, irrigation rates, and volumes of drainage and runoff

No initial soil moisture data were available for the November 2001 irrigation. Soil moisture before initiating the June 2002 irrigation indicated no differences between treatment plots at soil depths between 0 and 60 cm (data not shown). Mean soil percent moisture content of 20.3 and 22.8 for soil depth increments of 0–15 and 15–30 cm, respectively, were, however, significantly less than the percent soil moisture of 39.6% for the 30–60 cm depth increment. Irrigation rates (Table 2) for the two events were similar; each occurred over a two-day period. The rate for each day of the November 2001 irrigation was the same, although the second day's duration was 2 h less than the first day and, thus, less in quantity. Minor variations occurred in the rates of water application for each day of the June 2002 irrigation which also varied in duration and quantity. The main difference in irrigation protocol between November 2001 and June 2002 was an unexpected rain event on the night between June 4 and 5, 2002 that added 13.1 mm (3982 l) of water. The mean quantity of drainage for the November 2001 irrigation indicated no differences between treatments and, although differences were observed in volumes of runoff between CT and NT treatments (with a mean difference between them of 1425 l), no differences were observed for the sum of drainage and runoff volumes (i.e., total volumes) between tillage treatments (Table 3). Between 28 and 35% of the applied irrigation left the plots in runoff and drainage. The rest went to satisfy evapotranspiration needs, as soil water storage, and possibly as percolation below the drainage lines. In contrast to the November 2001 irrigation, differences in drainage (mean difference of 13,611 l), runoff (mean

Table 3

Drainage, runoff, and their sum as total water coming off the treatment plots: conventional-till with conventional fertilizer (CT-CF), conventional-till with poultry litter (CT-PL), no-till with conventional fertilizer (NT-CF) and no-till with poultry litter (NT-PL).

Date	Treatment	Drainage (l)	Runoff (l)	Total (l)
14–15 Nov '01	CT-CF	11,456 ^a	1,632 ^a	13,088 ^a
	CT-PL	10,697 ^a	1,384 ^a	12,081 ^a
	NT-CF	11,271 ^a	74 ^b	11,335 ^a
	NT-PL	10,098 ^a	93 ^b	10,417 ^a
4–5 June '02	CT-CF	15,678 ^a	9,330 ^a	25,008 ^a
	CT-PL	14,456 ^a	12,197 ^a	26,653 ^{ab}
	NT-CF	29,067 ^b	2,724 ^b	31,791 ^b
	NT-PL	28,289 ^b	2,835 ^b	31,124 ^b

Means within a date and under headings of Drainage, Runoff, and Total that are followed by different letters are significantly different at $P < 0.05$.

difference of 7984 l), and total volumes (mean difference of 5627 l) for the June 2002 irrigation were observed between tillage treatments (Table 3). Drainage volume from the NT plots was greater than the CT plots; runoff volume from the CT plots was greater than the NT plots; and the total volume from the NT plots was greater than the CT plots. Between 76 and 78% of the applied irrigation exited the NT plots as runoff and drainage compared to between 61 and 65% for the CT plots.

3.2. Estradiol and testosterone in litter, soil, drainage, and runoff

Differences were observed in concentrations of estradiol and testosterone in litters applied for each experiment (Table 1). Testosterone concentrations of the two batches of litter were less than their respective concentrations of estradiol.

Differences in soil concentrations of estradiol and testosterone were not observed between treatments (data not shown). Mean concentrations of estradiol of 243.5, 144.2, and 80.4 ng kg⁻¹ soil, and testosterone of 68.1, 27.1, and 17.3 ng kg⁻¹ soil for depth increments of 0–10, 10–20, and 20–30 cm, respectively, were significantly different for each depth sampled. Mean bulk soil (0–30 cm) concentrations of testosterone of 41.5 ng kg⁻¹ for plots amended with poultry litter was greater than the mean testosterone concentration of 24.3 ng kg⁻¹ for conventionally fertilized plots.

Both estradiol and testosterone were detected in subsurface drainage and overland runoff from these field scale plots (Tables 4 and 5). Results of the November 2001 irrigation indicated no differences in flow-weighted estradiol concentrations and load ha⁻¹ in the drainage between the four treatments (Table 4). No differences in flow-weighted concentrations of estradiol in runoff were observed between treatments (Table 4). A greater load from runoff was observed for CT compared to NT (Table 4) and reflected

Table 4

Mean flow-weighted concentrations, and mean load ha⁻¹ of estradiol from drainage, runoff, and sum of the two for total load ha⁻¹ of estradiol from the four triplicate treatment plots: conventional-till with conventional fertilizer (CT-CF), conventional-till with poultry litter (CT-PL), no-till with conventional fertilizer (NT-CF) and no-till with poultry litter (NT-PL).

Date	Treatment	Drainage		Runoff		Total
		Flow-weighted (ng l ⁻¹)	Load ha ⁻¹ (mg ha ⁻¹)	Flow-weighted (ng l ⁻¹)	Load ha ⁻¹ (mg ha ⁻¹)	Load ha ⁻¹ (mg ha ⁻¹)
14–15 Nov '01	CT-CF	27.6 ^a	10.4 ^a	22.7 ^a	1.3 ^a	11.7 ^a
	CT-PL	78.5 ^a	27.7 ^a	28.5 ^a	1.3 ^a	29.0 ^a
	NT-CF	18.1 ^a	6.5 ^a	34.4 ^a	0.08 ^b	6.6 ^a
	NT-PL	12.8 ^a	4.2 ^a	19.2 ^a	0.06 ^b	4.3 ^a
4–5 June '02	CT-CF	18.0 ^a	9.4 ^a	35.0 ^a	10.3 ^a	19.7 ^a
	CT-PL	15.2 ^a	7.3 ^a	19.3 ^a	7.7 ^a	15.0 ^a
	NT-CF	8.0 ^a	7.5 ^a	158.0 ^b	7.4 ^a	14.9 ^a
	NT-PL	9.4 ^a	8.6 ^a	389.1 ^b	33.6 ^a	42.2 ^a

Means within a date and category of unit that are followed by different letters are significantly different at ($P \leq 0.05$).

differences in volume of runoff between the two tillage practices (Table 3). Differences in the total load ha⁻¹ (drainage plus runoff) of estradiol between tillage treatments were, however, not observed (Table 4).

For the June 2002 irrigation, no differences in flow-weighted concentrations or load ha⁻¹ of estradiol in drainage between treatments were observed (Table 4). The flow-weighted concentration of estradiol in runoff from the NT-PL treatment was greater than the CT-PL treatment; no differences between NT-PL and control treatment NT-CF, however, indicated that the flow-weighted concentrations of estradiol were not greater than background. No differences in total load ha⁻¹ of estradiol between treatments were observed.

Although the total load ha⁻¹ of estradiol from the CT-PL treatment was greater than the input load from the November 2001 litter application, it was, however, not significantly different from the total loads ha⁻¹ of the other treatments all of which were less than the input load. On the other hand, total loads ha⁻¹ of estradiol for all treatments of the June 2002 irrigation experiment were greater than the input from its respective litter application (Table 4).

No differences were observed between flow-weighted concentrations and load ha⁻¹ of testosterone in drainage, flow-weighted concentrations of testosterone in runoff, and total load ha⁻¹ of testosterone from either the November 2001 or June 2002 irrigation experiments (Table 5). Differences in load ha⁻¹ of testosterone in runoff were, however, observed between tillage treatments for November 2001 irrigation (Table 5). The total loads ha⁻¹ of testosterone for both irrigations and all treatments were greater than the input from the litter applications (Table 5).

4. Discussion

4.1. Irrigation, drainage, and runoff

Under conditions of drought, irrigation was necessitated to produce measurable subsurface drainage and overland runoff from the experimental field plots. Hydrologic differences observed between tillage practices appeared to reflect previous observations and conceptual models that conservation tillage is more effective in increasing infiltration and reducing runoff than conventional-tillage (Bruce et al., 1995; Endale et al., 2002; Langdale et al., 1979).

4.2. Estradiol and testosterone in litter, soil, drainage, and runoff

Broiler litter for the November 2001 and June 2002 experiments were obtained from two different commercial broiler producers. Variations in concentrations of estradiol and testosterone in broiler litter have been reported (Hemmings and Hartel, 2006; Jenkins

Table 5

Mean flow-weighted concentrations, and mean load ha⁻¹ of testosterone from drainage, runoff, and sum of the two for total load ha⁻¹ of testosterone from the four replicated treatment plots: conventional-till with conventional fertilizer (CT-CF), conventional-till with poultry litter (CT-PL), no-till with conventional fertilizer (NT-CF) and no-till with poultry litter (NT-PL).

Date	Treatment	Drainage		Runoff		Total
		Flow-weighted (ng l ⁻¹)	Load ha ⁻¹ (mg ha ⁻¹)	Flow-weighted (ng l ⁻¹)	Load ha ⁻¹ (mg ha ⁻¹)	Load ha ⁻¹ (mg ha ⁻¹)
14–15 Nov '01	CT-CF	7.4 ^a	2.8 ^a	8.7 ^a	0.47 ^a	3.3 ^a
	CT-PL	8.9 ^a	3.1 ^a	7.9 ^a	0.40 ^a	3.5 ^a
	NT-CF	9.9 ^a	3.6 ^a	7.3 ^a	0.02 ^b	3.6 ^a
	NT-PL	9.1 ^a	3.0 ^a	6.4 ^a	0.01 ^b	3.0 ^a
4–5 June '02	CT-CF	6.0 ^a	3.1 ^a	8.7 ^a	2.7 ^a	5.8 ^a
	CT-PL	6.0 ^a	2.8 ^a	6.8 ^a	2.8 ^a	5.6 ^a
	NT-CF	12.3 ^a	11.6 ^a	7.1 ^a	1.3 ^a	12.9 ^a
	NT-PL	7.3 ^a	6.8 ^a	19.3 ^a	1.9 ^a	8.7 ^a

Means within a date and category of unit that are followed by different letters are significantly different at ($P \leq 0.05$).

et al., 2006). The natural occurring loads of these two hormones applied with their respective batches of litter were greater than the loads that Jenkins et al. (2006) applied to four cropped catchments, but less than the loads Finlay-Moore et al. (2000) and Nichols et al. (1998) applied to their respective experimental sites. The range of concentrations and loads of estradiol and testosterone reported to date indicate differences in hormone concentrations between litters from different operations (Finlay-Moore et al., 2000; Hemmings and Hartel, 2006; Jenkins et al., 2006; Nichols et al., 1998, 1997). Variations in flock management or differences in flock physiology may account for these differences. Since Hemmings and Hartel (2006) demonstrated minimal degradation of these hormones within litter under controlled temperature and moisture condition, hormone mineralization within litter appeared not to account for hormone variations between litters.

The lack of differences in soil concentrations of estradiol between fertilization treatments appeared to indicate that the load of estradiol applied to the field plots did not add to background soil estradiol concentrations. The soil concentrations of estradiol were within the range of background soil concentrations that Jenkins et al. (2006) observed in soils from cropped catchments, and comparable to the soil concentrations of estradiol from hayed and grazed fields of the southern Piedmont that Finlay-Moore et al. (2000) observed. The mean total bulk soil concentration of testosterone associated with the PL treatment was greater than the CF treatment. This difference, however, does not indicate that litter applications increased background testosterone concentrations since soil testosterone concentrations after litter applications were not greater than before litter applications.

The differences in soil estradiol and testosterone concentrations observed between soil depths were indicative of a stratification likely attributable to their hydrophobicity and sorption to soil organic matter (Casey et al., 2004; Lee et al., 2003). In a transport study of estradiol and testosterone through intact soil columns taken from the same field plots of this study, Sangsupan et al. (2007) demonstrated that only a small fraction of either hormone leached through the soil profile and most remained in the upper 5 cm.

The flow-weighted concentration of estradiol in runoff from the NT plots for the fall 2001 irrigation was within the range that could affect the development of trout (Tyler and Routledge, 1998). These flow-weighted concentrations of estradiol appeared to reflect background levels from a soil reservoir of estradiol and not directly from the poultry litter applications. With the exception of the NT-PL treatment for the spring 2002 irrigation, the flow-weighted concentrations of estradiol and testosterone in runoff that we observed from both irrigations were below or within the range of background concentrations that Finlay-Moore et al. (2000) and

Nichols et al. (1997) measured in runoff. Their data provide an example of the persistence of estradiol in the soil environment, and may indicate hormones within manure or litter might aggregate and resist abiotic, UV and microbial degradation (Lange et al., 2002). Estradiol and testosterone outside of the manure or litter matrix and in soil and soil water can be mineralized as Colucci et al. (2001) and Lorenzen et al. (2005) have reported. This study and others (Finlay-Moore et al., 2000; Jenkins et al., 2006; Nichols et al., 1997) have, however, indicated that fractions of both estradiol and testosterone appeared to persist and resist mineralization in soil.

If 10–100 ng l⁻¹ of estradiol can have an adverse effect on the physiology of wild trout (Tyler and Routledge, 1998), then the flow-weighted concentrations of estradiol in the drainage and runoff of both litter treated and control plots of the November 2001 irrigation, as well as the runoff flow-weighted concentrations of estradiol of the June 2002 irrigation could, if not further diluted, have a negative environmental impact. Nevertheless, the application of poultry litter appeared to add inconsequential amounts of these sex hormones to the background levels when applied at rates recommended for the nitrogen nutrition of the row-crop to be planted.

5. Conclusion

Under conditions of drought and conventional and no-till crop management, applications of broiler litter at rates recommended for crop nutrient requirements followed by irrigation appeared not to contribute to the loads of estradiol and testosterone coming off the edge of the replicated field plots as either runoff or subsurface drainage. As Jenkins et al. (2006) observed for cropped catchments in the Southern Piedmont, soils that have or have not received regular applications of broiler litter appeared to be a reservoir of background concentrations of estradiol and testosterone. Sources of background levels of these hormones could be random inputs from wildlife and avian activity. Variations in rates of mineralization may also play a role in the variability of the background levels observed. Although the differences between no-till and conventional-tillage appeared to affect the hydrologic transport of both hormones the differences have inconsequential environmental impact.

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References

- Adler, P., Steger-Hartmann, T., Kalbfus, W., 2001. Distribution of natural and synthetic estrogenic steroid hormones in water samples from southern and middle Germany. *Acta Hydrochim. Hydrobiol.* 29, 227–241.
- Andreini, M.S., Steenhuis, T.S., 1990. Preferential paths of flow under conventional and conservation tillage. *Geoderma* 46, 85–102.
- Bruce, R.R., Dane, J.H., Quisenberry, V.L., Powell, N.L., Thomas, A.W., 1983. Physical Characterization of Soil in the Southern Region: Cecil. In: Southern Coop. Series Bull. No. 267. University of Georgia, Athens, GA.
- Bruce, R.R., Langdale, G.W., West, L.T., Miller, W.P., 1995. Surface soil degradation and soil productivity restoration and maintenance. *Soil Sci. Soc. Am. J.* 59, 654–660.
- Bushee, E.L., Edwards, D.R., Moore, P.A., 1998. Quality of runoff from plots treated with municipal sludge and horse bedding. *Trans. ASAE* 41, 1035–1041.
- Casey, F.X.M., Larsen, G.L., Hakk, H., Simunek, J., Larsen, G.L., 2004. Fate and transport of testosterone in agricultural soils. *Environ. Sci. Technol.* 38, 790–798.
- Cody, R.P., Bortone, S.A., 1997. Masculinization of mosquito fish as an indicator of exposure to Kraft mill effluent. *Bull. Environ. Contam. Toxicol.* 58, 429–436.
- Colucci, M.S., Bork, H., Topp, E., 2001. Persistence of estrogenic hormones in agricultural soils: 17 β -estradiol and estrone. *J. Environ. Qual.* 30, 2070–2076.
- CTIC, 2005. National Crop Residue Management Survey. Conservation Technology Information Center, West Lafayette, IN.
- Endale, D.M., Radcliffe, D.E., Steiner, J.L., Cabrera, M.L., 2002. Drainage characteristics of a Southern Piedmont soil following six years of conventionally tilled or no-till cropping systems. *Trans. ASAE* 45, 1423–1432.
- Epstein, S., 1997. Don't eat extra estrogen. *Ecologist* 27, 89.
- Finlay-Moore, O., Hartel, P.G., Cabrera, M.L., 2000. 17 β -estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. *J. Environ. Qual.* 29, 1604–1611.
- Georgia Agricultural Statistics Service, 2004. Georgia Poultry Facts. Georgia Agricultural Statistics Service, Athens, GA.
- Hakk, H., Millner, P., Larsen, G., 2005. Decrease in water-soluble 17 β -estradiol and testosterone in composted poultry manure with time. *J. Environ. Qual.* 34, 943.
- Hanselman, T.A., Graetz, D.A., Wilkie, A.C., 2003. Manure-borne estrogens as potential environmental contaminants: a review. *Environ. Sci. Technol.* 37, 5471–5478.
- Harrison, P.T.C., Holmes, P., Humfrey, C.D.N., 1997. Reproductive health in humans and wildlife: are adverse trends associated with environmental chemical exposure? *Sci. Total Environ.* 205, 97–106.
- Hemmings, S.N.J., Hartel, P.G., 2006. Mineralization of hormones in breeder and broiler litters at different water potentials and temperatures. *J. Environ. Qual.* 35, 701–706.
- Horinouchi, M., Hayashi, T., Koshino, H., Kurita, T., Kudo, T., 2005. Identification of 9,17-dioxo-1,2,3,4,10,19-hexanorandrostane-5-oic acid, 4-hydroxy-2-oxohexanoic acid, and 2-hydroxyhexa-2,4-dienoic acid and related enzymes involved in testosterone degradation in *Comamonas testosterone* TA441. *Appl. Environ. Microbiol.* 71, 5275–5281.
- Isensee, A.R., Nash, R.G., Helling, C.S., 1990. Effect of conventional vs. no-tillage on pesticide leaching to shallow groundwater. *J. Environ. Qual.* 19, 434–440.
- Jenkins, M.B., Endale, D.M., Schomberg, H.H., Sharpe, R.R., 2006. Fecal bacteria and sex hormones in soil and runoff from cropped watersheds amended with poultry litter. *Sci. Total Environ.* 358, 164–177.
- Kirk, L.A., Tyler, C.R., Lye, C.M., Sumpter, J.P., 2002. Changes in estrogenic and androgenic activities at different stages of treatment in wastewater treatment works. *Environ. Toxicol. Chem.* 21, 972–979.
- Kolpin, D.A., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams 1999–2000: a national reconnaissance. *Environ. Sci. Technol.* 36, 1202–1211.
- Lange, I.G., Daxenberger, A., Schiffer, B., Witters, H., Ibarreta, D., Meyer, H.H.D., 2002. Sex hormones originating from different livestock production systems: fate and potential disrupting activity in the environment. *Anal. Chim. Acta* 473, 27–37.
- Langdale, G.W., Barnett, A.P., Leonard, R.A., Flemming, W.G., 1979. Reduction of soil erosion by the no-till system in the Southern Piedmont. *Trans. ASAE* 22, 82–86.
- Larsson, D.G.J., Hällman, H., Förlin, L., 2000. More male fish embryos near a pulp mill. *Environ. Toxicol. Chem.* 19 (2911), 2917.
- Lee, L.S., Strock, T.J., Sarmah, A.K., Rao, P.S.C., 2003. Sorption and dissipation of testosterone, estrogens and their primary transformation products in soils and sediment. *Environ. Sci. Technol.* 37, 4098–4105.
- Lintelmann, J., Katayama, A., Kurihara, N., Shore, L., Wenzel, A., 2003. Endocrine disruptors in the environment. *Pure Appl. Chem.* 75, 631–681.
- Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, W.R., 1996. SAS Systems for Mixed Models. SAS Institute, Cary, NC.
- Lorenzen, A., Chapman, R., Hendel, J.G., Topp, E., 2005. Persistence and pathways of testosterone dissipation in agricultural soil. *J. Environ. Qual.* 34, 854–860.
- Moore Jr., P.A., Daniel, T.C., Sharpley, A.N., Wood, C.W., 1995. Poultry manure management: environmentally sound options. *J. Soil. Water Conserv.* 50, 321–327.
- Nichols, D.J., Daniel, T.C., Edwards, D.R., Moore Jr., P.A., Pote, D.H., 1998. Use of grass filter strips to reduce 17 β -estradiol in runoff from fescue-applied poultry litter. *J. Soil Water Conserv.* 53, 74–77.
- Nichols, D.J., Daniel, T.C., Moore Jr., P.A., Edwards, D.R., Pote, D.H., 1997. Runoff of estrogen hormone 17 β -estradiol from poultry litter applied to pasture. *J. Environ. Qual.* 26, 1002–1006.
- Perkins, H.F., Parker, M.B., Walker, M.L., 1964. Chicken Manure- its Production, Composition and use as a Fertilizer. In: Georgia Agric. Exp. Stn. Bulletin 123. University of Georgia, Athens.
- Peterson, E.W., Davis, R.K., Orndorff, H.A., 2000. 17 β -Estradiol as an indicator of animal waste contamination in mantled karst aquifers. *J. Environ. Qual.* 29, 826–834.
- Radcliffe, D.E., Tollner, E.W., Hargrove, W.L., Clark, R.L., Golabi, M.H., 1988. Effect of tillage practices on infiltration and soil strength of a Typic Hapludult soil after ten years. *Soil Sci. Soc. Am. J.* 52, 798–804.
- Raman, D.R., Layton, A.C., Moody, L.B., Easter, J.P., Sayler, G.S., Burns, R.T., Mullen, M.D., 2001. Degradation of estrogens in dairy waste solids: Effects of acidification and temperature. *Trans. ASAE* 44, 1881–1888.
- Ritz, C.W., Merka, W.C., 2004. Maximizing poultry Manure Use Through Nutrient Management Planning. Bulletin 1245. Georgia Cooperative Extension Service, College of Agriculture and Environmental Sciences, University of Georgia, Athens, GA. Verified August 16, 2006. Available at: <http://pubs.caes.uga.edu/caespubs/pubcd/B1245.htm> (accessed 13.06.08).
- Sangsupan, H.A., Radcliffe, D.E., Hartel, P.G., Jenkins, M.B., Vencill, W.L., Cabrera, M.L., 2007. Sorption and transport of 17 β -estradiol and testosterone in intact soil columns. *J. Environ. Qual.* 35, 2261–2272.
- SAS, 2004. SAS/STAT 9.1 User's Guide. SAS Institute, Cary, NC.
- Sharpe, R.M., Skakkebaek, N.E., 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract. *Lancet* 341, 1392–1395.
- Shore, L.S., Correll, D.L., Chakraborty, P.K., 1995. Relationship of fertilization with chicken manure and concentrations of estrogens in small streams. In: Steele, K. (Ed.), *Animal waste and the land-water interface*. Lewis Publishers, New York, pp. 155–162.
- Shore, L.S., Hall, D.W., Shemesh, M., 1997. Estrogen and testosterone in ground water in the Chesapeake Bay Watershed. In: *Dahlia Greidinger International Symposium on fertilization and the environment*, Technion, Haifa, Israel, p. 250–255.
- Shore, L.S., Harel-Markowitz, E., Gurevich, M., Shemesh, M., 1993. Factors affecting the concentration of testosterone in poultry litter. *J. Environ. Sci. Health A* 28, 1737–1749.
- Shore, L.S., Reichmann, O., Shemesh, M., Wenzel, A., Litaor, M.I., 2004. Washout of accumulated testosterone in a watershed. *Sci. Total Environ.* 332, 193–202.
- Shore, L.S., Shemesh, M., 2003. Naturally produced steroid hormones and their release into the environment. *Pure Appl. Chem.* 75, 1859–1871.
- Shore, L.S., Shemish, M., Cohen, R., 1988. The role of oestradiol and oestrone in chicken manure silage in hyper-oestrogenism in cattle. *Aust. Vet. J.* 65, 68.
- Toppari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette Jr., L.J., Jegou, B., Jensen, T.K., Jounnet, P., Keiding, K., Leffers, H., McLachlan, J.A., Meyer, O., Muller, J., Rajpert De Meyts, E., Scheike, T., Sharpe, R., Sumpter, J., Skakkebaek, N.E., 1996. Male reproductive health and environmental xenoestrogens. *Environ. Health Perspect.* 104 (Suppl. 4), 741–803.
- Tyler, C.R., Routledge, E.J., 1998. Oestrogenic effects in fish in English rivers with evidence of their causation. *Pure Appl. Chem.* 70, 1795–1804.
- Wicks, C., Kelley, C., Peterson, E., 2004. Estrogen in a karstic aquifer. *Ground Water* 42, 384–389.