Hormones and Antibiotics in Surface Runoff

M.L. Cabrera[1], M. Bera[1], V.A. Calvert[1], S. Doydora[1], M. Durant[1], D. Endale[4], B. Fairchild[2], D.H. Franklin[1], P.G. Hartel[1], S. Hassan[1], C.H. Huang[3], D.E. Kissel[1], S. Pavlostathis[3], D.E. Radcliffe[1], J.A. Rema[1], P. Sun[3], A. Thompson[1], and W.K. Vencill[1]

[1]Department of Crop & Soil Sciences, [2]Department of Poultry Science, University of Georgia
[3]Civil and Environmental Engineering, Georgia Institute of Technology
[4]USDA – Agricultural Research Service

Hormones in Broiler Litter

The poultry industry in the United States produces about 8 billion broilers (*Gallus gallus domesticus*) each year, with the generation of approximately 11 Mg of broiler litter, a mixture of bird excreta, bedding material, feathers, and wasted feed. Broilers excrete large amounts of hormones in their manure, especially 17-β estradiol (the female hormone) and testosterone (the male hormone). Testosterone is usually present in concentrations two to three times greater than estradiol (Table 1). When broiler houses are cleaned, producers remove either the caked broiler litter only (cake cleanout) or all of the broiler litter (full cleanout). Caked cleanout has greater concentrations of estradiol and testosterone than full cleanout (Table 1).

Table 1. 17β-Estradiol and testosterone concentration in full (130 samples) and cake (51 samples) cleanouts of broiler litter. (Cabrera et al., unpublished results)

Litter Type	17β-Estradiol	Testosterone
	------- ng g^{-1} dry litter -------	
Cake cleanout	57 a†	165 a
Full cleanout	37 b	85 b

†Within each column, means followed by the same letter are not significantly different according to Fisher's LSD at p=0.05.

Because a significant proportion of the broiler litter removed from broiler houses is surface applied to grasslands as fertilizer, there is a risk of contaminating surface runoff with hormones, which in turn can lead to contamination of streams, rivers, and lakes. The presence of these hormones in surface waters can have negative impacts on wildlife and human populations that drink the contaminated water (Colborn et al., 1993). This is especially true for estradiol, which can have effects at concentrations as low as 10 ng L^{-1}. In contrast, testosterone can have steroidal effects at much greater concentrations (1000 ng L^{-1}).

Broiler litter is commonly stored in stack houses for one to two months before producers apply it to grasslands. Therefore, it is important to determine if hormone degradation occurs during the storage period. With that objective in mind, we sampled a stack house immediately after broiler litter was stacked and 4 to 8 weeks later. Stacking broiler litter reduced the concentrations of estradiol and testosterone (Table 2).

Table 2. Initial and final (4 and 8 weeks) hormone concentrations in stacked broiler litter (Cabrera et al., unpublished results.

Variable	Initial	4 Weeks	8 Weeks	p>t	% Change
Water content (g g^{-1} dry)	0.64	0.60	0.60		
Estradiol (ng g^{-1} dry)	103a	49b	52b	0.0001	-50
Testosterone (ng g^{-1} dry))	202a	125b	128b	0.0001	-37

It is also important to evaluate the decomposition of hormones in broiler litter when they are applied to the soil surface. For that purpose, we conducted laboratory studies to determine the effects of temperature and soil water potential on the decomposition of radiolabeled estradiol and testosterone mixed with broiler litter and incubated on the surface of four soils for 168 days. Data of cumulative C mineralization (Cmin) for each replication was used to fit a one-pool exponential model of the form:

$$Cmin = C_0(1-e^{-kt}),$$

where C_0 is the C mineralization potential expressed as a percentage of the ^{14}C applied, k is the first-order rate constant, and t is time in days. In general, potentially mineralizable C in estradiol increased with increasing temperature and increasing water potential although the range was relatively small (7.6 to 13.8 %; Table 3).

Table 3. Effect of temperature, and soil water potential, on ^{14}C-labeled estradiol in broiler litter applied on the surface of a Cecil sandy loam and incubated for 168 d. Means followed by different letters are significantly different ($p \leq 0.05$). Upper-case letters compare values within the same column (separated by soil type); lower-case letters compare values within the same row. (Adapted from Durant et al., 2012).

Temperature	Soil Water Potential (MPa)		
(°C)	-0.03	-0.75	-1.5
	--Mineralizable estradiol % of applied ^{14}C -		
10	9.4 Ab	7.6 Aa	8.0 Aa
20	10.6 Bb	8.3 Ba	8.7 Aa
30	13.8 Cc	8.5 Ba	9.3 Bb

Similar to estradiol, mineralizable testosterone C generally increased with increasing water content, however, dissimilar to estradiol, mineralization of testosterone decreased with increasing temperature (Table 4).

Table 4 Effect of temperature, and soil water potential, on ^{14}C-labeled testosterone in broiler litter applied on the surface of a Cecil sandy loam and incubated for 168 d. Upper-case letters compare values within the same column (separated by soil type); lower-case letters compare values within the same row. (Adapted from Durant et al., 2012).

Temperature (°C)	Soil Water Potential (MPa) -0.03	-0.075	-1.5
	--% cumulative testosterone mineralized--		
10	44.4 Bab	43.8 Aa	47.7 Bb
20	45.5 Ba	45.1 Aa	44.5 ABa
30	40.7 Aa	40.3 Aa	42.3 Aa

After observing the relative large amounts of undecomposed hormones remaining in the soil after 168 days of incubation, a second study was conducted to evaluate the effect of time on incorporation of ^{14}C into soil fractions. For both hormones, the results showed a trend for the percentage in the water- and acetone-extractable fractions to decrease while the percentage found associated with the soil organic matter fractions increased (Tables 5 and 6). These results suggest a process of humification of the hormones.

Table 5. Distribution of the remaining ^{14}C-estradiol in the soil into water- and acetone-soluble fractions, and soil organic matter fractions. Means followed by different letters are significantly different ($p \leq 0.05$). Upper-case letters compare values within the same column; lower-case letters compare values within the same row. Percentages may not add up to 100% due to rounding. (Adapted from Durant et al., 2012).

Days	Water- and Acetone-Soluble Fractions			Soil Organic Matter Fractions			
	Water	Acetone	Total	Fulvic Acid	Humin	Humic Acid	Total
	--------------%						
0	9.8 ABa	59.1 DEb	68.9	11.5 Aa	4.7 Aa	14.9 Ba	31.1
1	11.4 ABa	62.9 Eb	74.3	11.6 Aa	4.6 Aa	9.6 Aa	25.8
3	14.2 BCb	57.7 Dc	71.9	13.2 Ab	5.3 Aa	9.7 Aab	28.2
7	19.5 Db	43.7 Cc	63.2	12.6 Aa	11.4 Ba	12.8 ABa	36.8
14	16.1 CDa	24.6 Bb	40.7	15.2 ABa	22.0 Cb	22.2 Cb	59.4

28	13.6 BCa	7.8 Aa	21.4	15.9 ABa	28.4 Db	34.3 Db	78.6
56	6.6 Aa	5.0 Aa	11.6	18.2 Bb	30.2 Dc	40.0 Ec	88.4

Table 6. Distribution of the remaining ^{14}C-testosterone in the soil into water- and acetone-soluble fractions, and soil organic matter fractions. Means followed by different letters are significantly different ($p \leq 0.05$). Upper-case letters compare values within the same column; lower-case letters compare values within the same row. Percentages may not add up to 100% due to rounding. (Adapted from Durant et al., 2012).

Days	Water- and Acetone-Soluble Fractions			Soil Organic Matter Fractions			
	Water	Acetone	Total	Fulvic Acid	Humin	Humic Acid	Total
	---%---						
0	13.2 ABa	63.1 Eb	76.3	8.9 Aa	11.1 Aa	3.6 Aa	23.6
1	15.0 ABCa	56.8 Db	71.8	11.4 Aa	13.1 Aa	3.7 Aa	28.2
3	18.3 BCc	48.7 Cd	67.0	13.4 Abc	12.8 Ab	6.8 ABa	33.0
7	13.6 ABb	60.2 Dc	73.8	10.1 Aab	9.6 Aab	6.5 ABa	26.2
14	19.3 Cb	47.6 Cc	66.9	11.2 Aa	12.6 Aa	9.3 Ba	33.1
28	17.9 BCab	24.5 Bc	42.4	21.0 Bbc	20.4 Babc	15.3 Ca	56.7
56	12.2 Aa	13.7 Aa	25.9	27.5 Cb	28.8 Cb	17.8 Cab	74.1

Broiler litter remaining on the soil surface may contaminate surface runoff with significant amounts of hormones. To investigate worse-case scenarions, three rainfall simulations were conducted, one in September 2008, one in February 2009, and one in August 2009 to determine the effect broiler litter addition (4,667 kg ha^{-1}) and time of rainfall simulation (0, 1, 2, or 4 weeks after application) on hormone concentrations in surface runoff. Runoff from litter-treated plots had greater concentrations of estradiol and testosterone than control plots (Table 7), but there was no effect of time of runoff (0, 1, 2, or 4 weeks after litter application) on hormone concentrations in runoff.

Table 7. Concentrations of estradiol and testosterone in runoff from control and litter-treated plots in three rainfall simulation studies (Cabrera et al., unpublished results).

Treatment	September 2008		February 2009		August 2009	
	Estradiol	Testost.	Estradiol	Testost.	Estradiol	Testost.
	------------------------------- ng L^{-1} -------------------------------					
Control	25	48	13	19	11	65
Litter	68	206	83	380	27	123
P>t	0.0007	0.001	0.0001	0.0001	0.0008	0.21

Summary for Hormones

Broiler litter contains the sex hormones 17-β estradiol and testosterone, which may undergo partial but not complete decomposition if the broiler litter is stacked for 4 to 8 weeks. Once broiler litter is applied to soil, up to 14% of estradiol and 48% of testosterone may undergo decomposition. Of the undecomposed hormones remaining in the soil, approximately 75 to 88% appears to be slowly incorporated into soil organic matter fractions (humification) whereas 10 to 15% remains water soluble. As a result, surface runoff from grasslands receiving broiler litter (5.6 Mg ha^{-1}) may have elevated concentrations of estradiol and testosterone. Of these, elevated concentrations of estradiol are of the most concern as this hormone can have effects at concentrations as low as 10 ng L^{-1}.

Antibiotics in Broiler Litter

Monensin and salinomycin are among the most commonly used antibiotics in the US broiler industry. These antibiotics can pass through the digestive track of the broilers and appear in the manure. Thus, when broiler litter is applied on the surface of pastures, there is a risk of surface water contamination with antibiotics. The presence of these compounds in surface waters may cause adverse effects on native biota and potentially increase bacterial resistance to these drugs. (Boxall et al., 2003).

To determine if monensin and salinomycin are decomposed during litter storage we conducted a 112-d study in which broiler litter spiked with these hormones was stored in wooden bins (1.2 m x 1.2 m x 1.2 m) in a stack house (building with a roof but without walls). We found that stacking the litter for 112 days did not affect antibiotic concentrations, even though the stacks underwent heating caused by biological activity.

We also carried out a rainfall simulation study to determine if antibiotics in the litter would appear in surface runoff from grassed plots that received surface-applied broiler litter. We found average runoff concentrations of 1.4 μg monensin L^{-1} and 2.5 μg salinomycin L^{-1}. We also found that broiler litter treated with aluminum sulfate (at 200 g kg^{-1}) had runoff concentrations of these antibiotics that were about half of those measured with untreated broiler litter.

Summary for Antibiotics

Broiler litter may contain antibiotics that are used to control or prevent diseases during broiler production. When broiler litter is applied to the surface of grasslands, surface runoff can carry these antibiotics to surface waters, where they may have an impact on native biota and increase pathogen resistance to these drugs. In contrast with what was observed for hormones, the antibiotics monensin and salinomycin are not likely to be decomposed during normal stacking of broiler litter.

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