

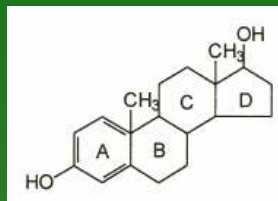
Thermophilic Composting of Poultry Layer Manure: Significantly Reduces Endocrine Compounds and Pathogens while Stabilizing Organic Matter

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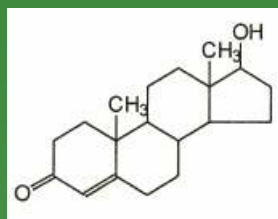
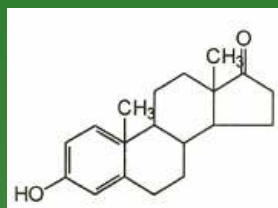
Beltsville Agricultural Research Center and
Red River Valley Agricultural Research Center





Objectives:

- To evaluate the water extractability of the potent endogenous hormones, 17β-estradiol and testosterone, during the course of aerobic, thermophilic composting in windrows
- To evaluate the extent of decomposition of hormones, 17β-estradiol and testosterone, during the course of aerobic, thermophilic composting in an adiabatic composter, and anaerobic digestion at RT.



Windrow Composting



Poultry Litter from a commercial egg-laying operation in Pennsylvania was composted at the ARS Beltsville compost facility to produce soil amendment.



Composting Process

- » Inputs (by volume) included:
 - » Poultry layer manure: 3.3 parts
 - » Hay, 2 parts
 - » Straw: 2 parts
 - » Leaves: 4 parts
 - » Starter Compost (10% by volume) of existing thermophilic compost: 2 parts
 - » Christiana clay (one pile): 1 part

- » C:N initially: 30:1
- » Moisture initially: 60%
- » Pile size: 1.7 m (w) x 0.16 m (h) x 53 m (l)
- » Compost duration: 12 weeks
- » Windrows turned weekly for weeks 2-6; bi-weekly for weeks 8-12.
- Pile Monitoring: Temperature, CO₂, O₂



Sampling

- Piles (rep 1): 3 composites (~200 g each) collected on Days 0, 6, and 13; 2 composites on Days 27, 55, 69, 83, 111, and 139.
- Piles (rep 2) (constructed 6 da after the first): 3 composites collected on Days 0 and 7; 2 composites sampled at Days 21, 49, 63, 77, 105, and 133.
 - Composite sample: 3 subsamples equidistant from a site within the windrow; then mixed to homogenize.
- Samples frozen at -20C, shipped O/N in coolers, frozen blue-ice gel to Fargo, ND ARS lab. Then immediately frozen at -30C and stored until analyzed.
 - analyses performed within 2wks of receipt.
- Data from replicate windrows were combined for statistical analyses.

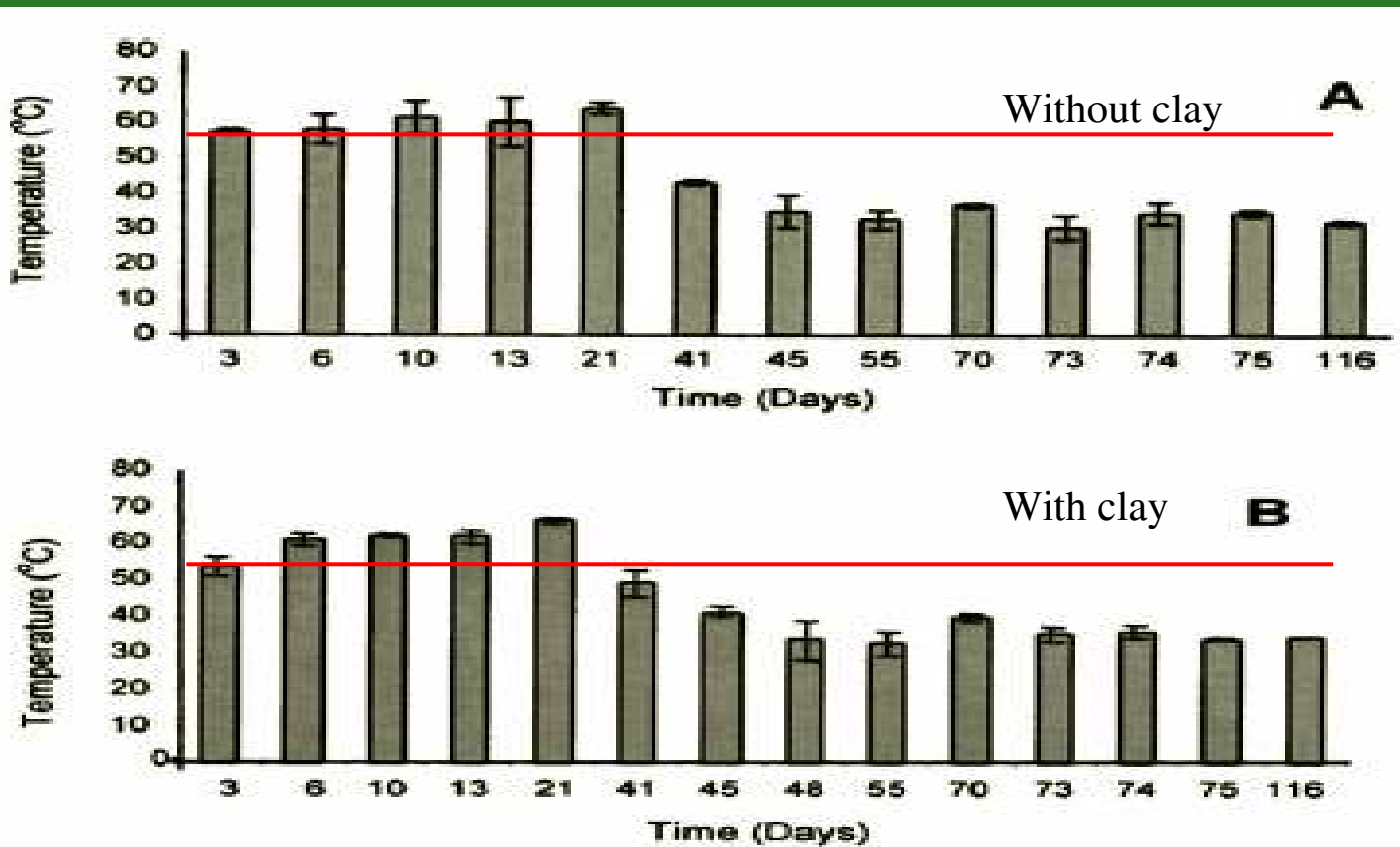
Sample Processing

- Blending (5 min): ~ 200 g + dry ice
- Dry ice evaporation: -20C
- 1.0-1.5 g sample (wet wt), dried at 50C, moisture content determinations
- 2-3 sample determination for first two sample dates
- Extraction: 200 mg + 50 mL ddH₂O; 2 hr, 25C, reciprocating shaker water bath
- Centrifugation: 1124g
- Aqueous supernatant retained for analyses

Immunoassays of Hormones

- Estradiol and testosterone EIA kits (Cayman Chemical, Ann Arbor, MI)
- 3 or 4 replicates with blanks (straw, hay, leaves, clay, compost) & stds; nonspecific and maximum binding determined.
- Testosterone EIA kit cross-reactivities:
 - 21% for 5-dihydro testosterone, 12.4% for 11-keto testosterone, 10% for 5-dihydro testosterone, 3.6% for androstenedione, 1.2% for 11-hydroxy testosterone, and less than 1% for all other steroids.
- 17-estradiol EIA kit cross-reactivities:
 - 17% for 17-estradiol-3-glucuronide; 4% for estrone, 0.57% for estriol, 0.1% for testosterone & 5-dihydrotestosterone

Mean Temperatures of Windrows (2 core temps and one end temp ea time) for 116 da



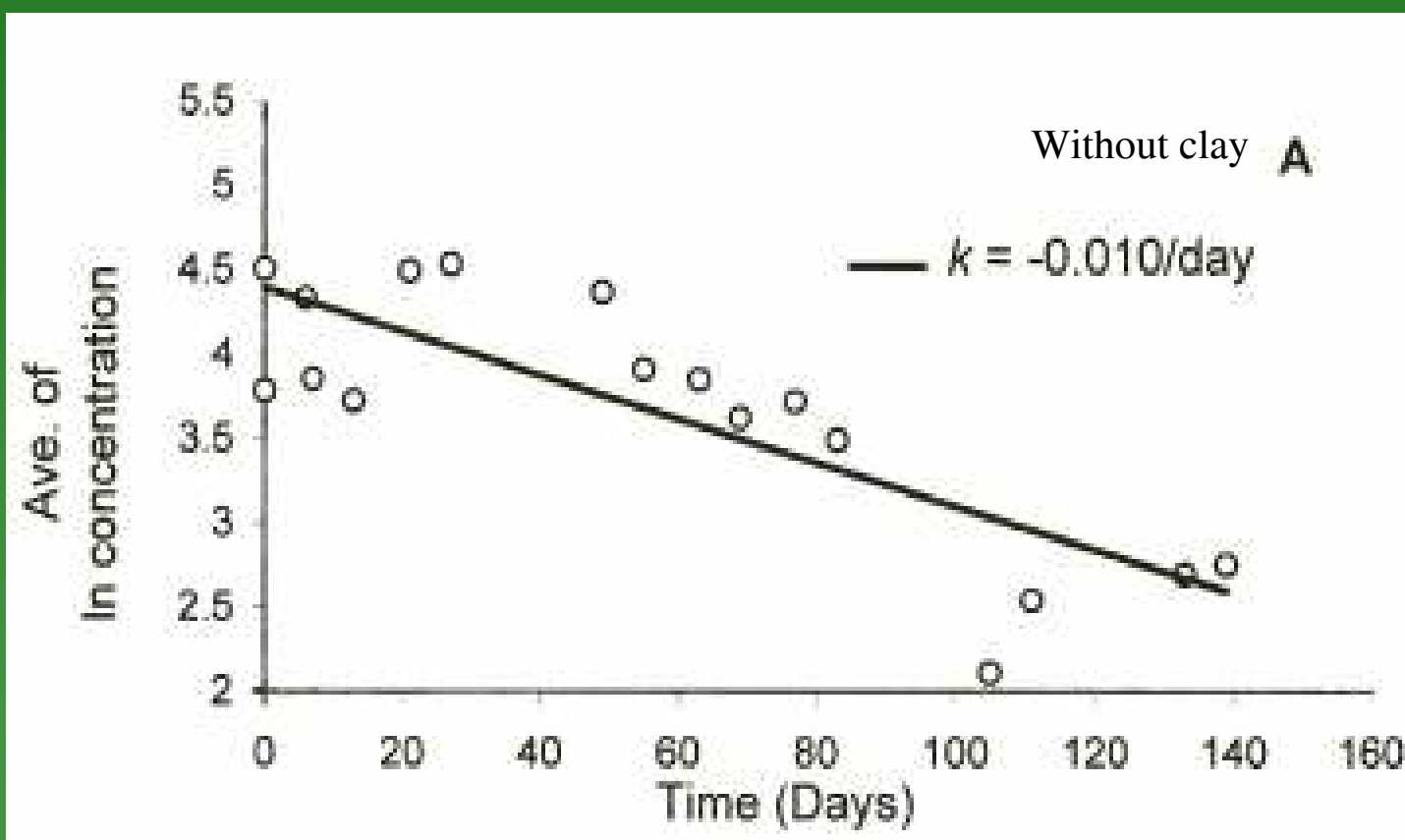
Percent recovery of spiked [^{14}C]17-estradiol or [^{14}C]testosterone (0.33 g) into an aliquot of Day 0 layer manure compost (414-558 mg). Recoveries after successive water extractions (50 mL 3; $n=3$) or successive water, methanol, and acetone extractions (50 mL 5; $n=5$).†

Extraction solvent	[^{14}C]17 β -estradiol-spiked compost	[^{14}C]testosterone-spiked compost
	%	
Water #1	51.9 \pm 1.9‡	61.8 \pm 2.0‡
Water #2	15.7 \pm 0.7	14.9 \pm 0.3
Water #3	7.9 \pm 0.6	6.2 \pm 0.8
Cumulative	75.5 \pm 0.9	82.9 \pm 2.2
Water	43.4 \pm 3.9	54.5 \pm 1.7
Methanol	54.6 \pm 3.1	55.6 \pm 2.8
Acetone	1.5 \pm 0.4	1.0 \pm 0.7
Cumulative	99.5 \pm 6.5	111.1 \pm 2.5

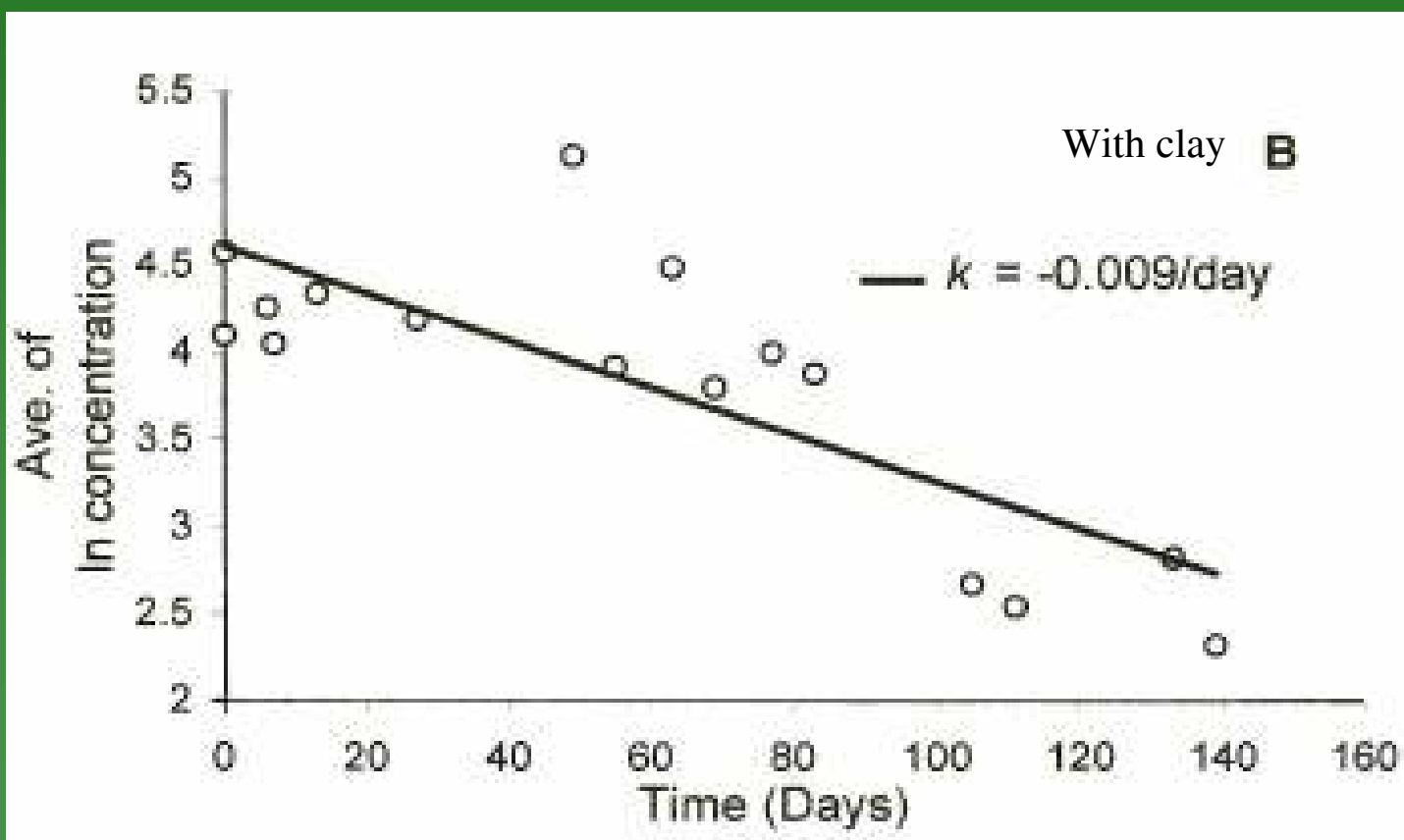
† Values are means \pm SD.

‡ Replicate, 24-h aqueous extraction recoveries ($n = 3$): 17 β -estradiol (48.2 \pm 4.8%) and testosterone (59.6 \pm 4.2%). Replicate, low hormone concentration aqueous extraction recoveries ($n = 3$): 17 β -estradiol (49.1 \pm 4.9%) and testosterone (59.8 \pm 11.1%).

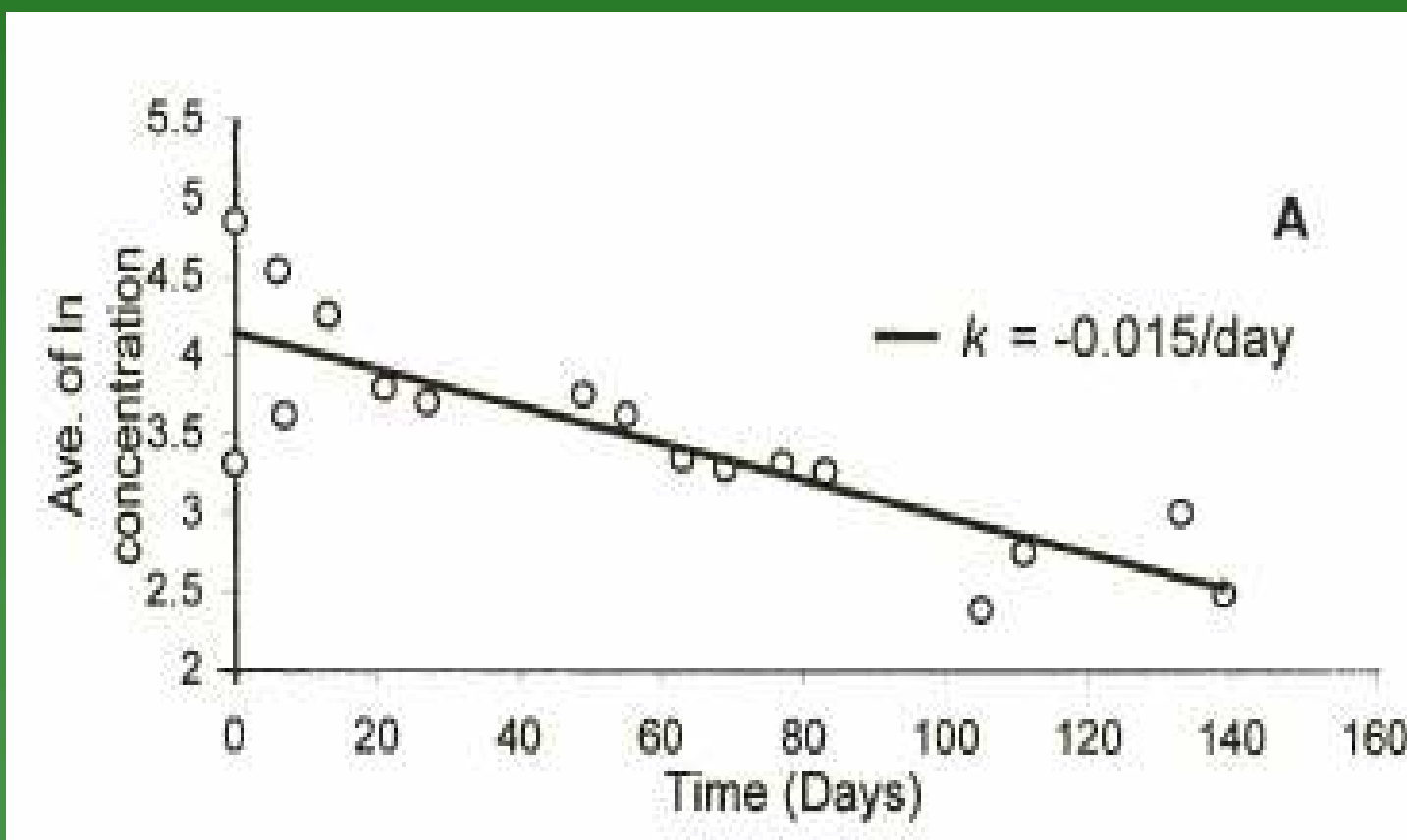
Mean natural logarithm (ln) of the concentration of water-extractable 17-estradiol and degradation rate constant, no Clay



Mean natural logarithm (ln) of the concentration of water-extractable 17-estradiol and degradation rate constant; Clay



Mean natural logarithm (ln) of the concentration of water-extractable testosterone and degradation rate constant, no Clay



Summary of Phase 1: field studies

- Aerobic thermophilic composting reduced the amount of WExtr 17 β -estradiol and testosterone in poultry manure compost.
- Initial, Mean concentrations WExtr 17 β -estradiol and testosterone in compost = 83 and 115 ng/g compost dry wt., respectively
- At da 139 these hormone concentrations were 13 and 11 ng/g, respectively.
 - an **84 and 90% reduction**, respectively
 - water-soluble levels of these hormones were reduced substantially during composting, but not completely eliminated

Adiabatic Composter: Lab Studies

- Used mixed feedstocks with poultry manure
- Ability to trap and analyze gaseous by-products
- Radioisotopically-labelled compounds can be spiked and decomposition products quantitated.

Adiabatic Composting vs Anaerobic Digestion

- Lab Bench-scale studies in progress

- **Moisture content is critical: should not drop below ~40%**
 - **Low moisture limits microbial metabolism, particularly in high temperature composting**
 - **WExtr testosterone degradation in 24 da similar for compost and anaerobic**
 - **Longer degradation times (>24 da) will be needed**
 - **Additional studies with other manures in progress**

Field vs. Lab Scale Compost Results

- 84 – 90% reduction in hormone concentrations in water extracts in field windrows, 0 – 139 da
 - Moisture content ranged from 60% initially to 50% final
- Small decreases in hormone concentration in water extracts of manure in adiabatic compost and anaerobic digestion, 24 da
 - Moisture content critical
- Bench-scale composting and digestion good for tracer studies capture of decomposition products & CO₂; full field scale studies represent 'real world' situations without radio-tracers.

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