Raising the steaks: A pilot study on the effects of cattle-associated, hormonally-active compounds in Texas watersheds

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Abstract. Globally, there is demand for increased meat production. Texas, a leader in cattle production in the United States, has met this demand by utilizing confined animal feeding operations (CAFOs) containing hundreds to thousands of cattle. To increase production efficiency, cattle receive growth-promoting hormone treatments to enhance growth and increase cattle mass. These hormonally-active compounds (HACs) have been found in cattle waste, feedlot runoff, and surface waters. The ultimate goal of this project was to identify watershed characteristics that promote the transport of cattle-associated HACs to surface waters. Therefore, the objectives of this pilot study were to: 1) identify and define a study area for evaluating HACs in Texas watersheds and 2) begin preliminary assessments of HAC activity in surface water downstream of cattle feedlots. A suitable study area was identified using satellite imagery, elevation data and the ArcGIS hydrology tool pack. Sample sites were selected within this area based on geographical features and position to CAFOs. Caged fish studies, followed by analysis of estrogen-responsive gene expression, were utilized to assess the presence and activity of HACs. Though no statistically significant alterations in estrogen-responsive gene expression metrics were observed, females from three of the four sites downstream of CAFOs experienced 2.9 to 3.7-fold and 1.9 to 5.3-fold decreases in the expression of estrogen receptor alpha and vitellogenin, respectively. This could have larger implications as previous research by Miller et al. 2007 forecasted that a 25% reduction in vitellogenin plasma concentration could result in up to a 70% decrease in population size.

Introduction

- Cattle-based confined animal feeding operations (CAFOs, Figure 1) are routinely used as a means of producing beef in high volumes. Texas alone accounts for approximately 25% of the cattle on feedlots in the United States.[1]
- To increase CAFO efficiency, synthetic hormones (trenbolone acetate, melengestrol acetate, estradiol, etc.) are administered to enhance livestock growth and increase mass.[2-4] These compounds, have been found in cattle manure, feedlot runoff and surface waters.[5-7] This is alarming given the immense amount of waste produced by CAFOs, as well as the fact that these compounds have been identified as hormonally-active, with the ability to disrupt normal reproductive endocrine function.[8]
- Given the potential environmental impact, **the goal of this project was to assess the biological significance of these cattle-associated, hormonally-active compounds in Texas watersheds**. To achieve this goal there were two objectives.

Figure 1. Confined animal feeding operation.[9]

Objective 1: *To identify and define a study area for evaluating hormonally-active compounds (HACs) in Texas watersheds*

Methods

- Erath and Comanche counties were selected as the boundary for our study area given their geographical location in northern Texas (Figure 2) and known prevalence of cattle feedlots.
- Satellite imagery of the counties (Google Earth) was scanned, and upon the presence of certain factors (i.e., barren patch, feeding troth, retention basin) indicating the presence of a feedlot, the location was pinned (Figure 3).
- CAFO locations were spatially referenced to Erath and Comanche county boundary data (TNRIS) using ArcMap software.[10]
- Given the location of the CAFOs within our study area, several sites of possible field deployment were visited (Figure 4), and basic water quality measurements were taken (Table 1).
- Utilizing ArcMap, 10 meter elevation data (USDA) was hydrologically corrected in order to calculate water flow direction and accumulation in the counties of interest.[11] This information could then be used to identify watersheds and the bodies of water located within them.

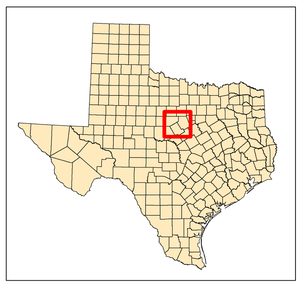

Figure 2. Location of Erath and Comanche county in northern Texas.

Figure 3. Satellite imagery depicting an example of a located cattle feedlot.

Table 1. Table indicating the name of the body of water, spatial relationship to CAFOS, soil type, slope, water temperature and pH at each of the five sampling sites of interest.

Site #	Water source	CAFO nearby	Soil type	Slope	Water temp (°C)	pH
1	Sabana River	No	Sand	High	27.8	8.02
2	Mercer Creek	Yes	Clay	High	31.4	8.33
3	Green Creek	Yes	Clay	High	30.3	7.93
4	Armstrong Creek	Yes	Sand	High	27.3	7.77
5	South Fork N. Bosque River	Yes	Sand	High	29.1	8.25

Results

- Figure 5 indicates the location of CAFOs, sample sites, watersheds, rivers, streams and large bodies of water in our counties of interest.

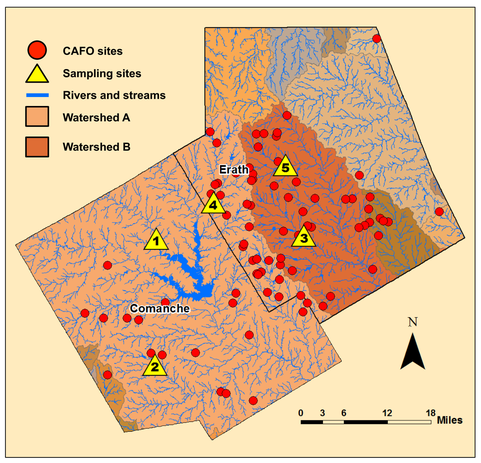

Figure 5. Map depicting the location of CAFOs and sampling sites, hydrologic features and defined watersheds in the counties of interest.

- Five sites were identified for potential deployment of fish in a pilot study:
  - Site 1: Control
  - Sites 2-5: Downstream of CAFO
- Sites 1, 2 and 4 were contained within watershed A, while sites 3 and 5 were contained within watershed B
- Of the 81 total CAFOs identified:
  - 41 were located in watershed A
  - 29 were located in watershed B
- CAFOs averaged about 9.2 miles from a sample site in watershed A, while CAFOs averaged 5.3 miles from a sample site in watershed B.

Figure 4. Images of potential field deployment sites.

Conclusions

- Findings suggest that sampling sites 3 and 5 will detect greater levels of HAC activity given the closer proximity of CAFOs to sampling sites in the watershed, despite fewer total feedlots.

Objective 2: *To begin preliminary assessments of HAC activity in surface waters downstream of cattle feedlots*

Methods

- In June of 2016, 100 fathead minnows were deployed in the field utilizing the cage design depicted in Figure 6.

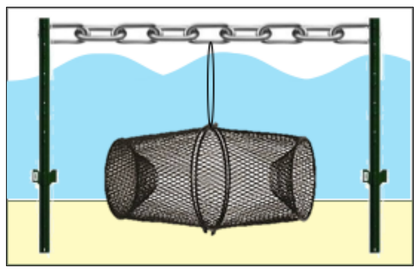

Figure 6. General setup of cages deployed in the field. Cages were hung from chain staked into the ground and rested against the sediment in approximately 3 feet of water.

- 10 males and 10 females were deployed together at each of our five sampling sites identified in objective 1.
- After a weeklong exposure, fish were collected, sacrificed, weighed and dissected in the field. Liver, gonad and gill tissue were taken for future gene expression analysis.
- Two genes of interest were examined using qPCR methods and are listed in Table 2.

Table 2. A list of the target genes of interest and their respective biological significance.

Target gene	Biological significance
Vitellogenin (*vtg*)	Egg yolk precursor protein; biomarker of estrogenic activity
Estrogen receptor alpha (*ERα*)	Mediates estrogen signaling activity

Results

- For males, no significant differences in *vtg* or *ERα* RNA levels were detected in liver tissues taken from sites 1 to 5.
- Among females, 1.9 to 5.3-fold decreases in *vtg* expression were identified in liver tissue, though no significant differences were detected (Figure 7).
- Among females, 1.5 to 3.7-fold decreases in *ERα* expression were identified in liver tissue, though no significant differences were detected (Figure 8).
- Decreases in *vtg* or *ERα* RNA expression levels indicate potential anti-estrogenic activity from cattle-associated HACs.

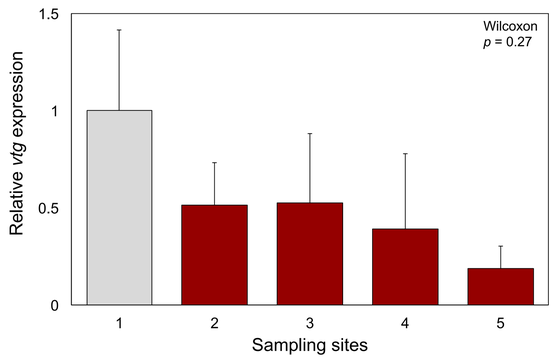

Figure 7. The average relative expression of vitellogenin (*vtg*) in fathead minnows from sampling sites 1-5. Red indicates sites downstream of CAFOs. Error bars represent standard error. n = 5.

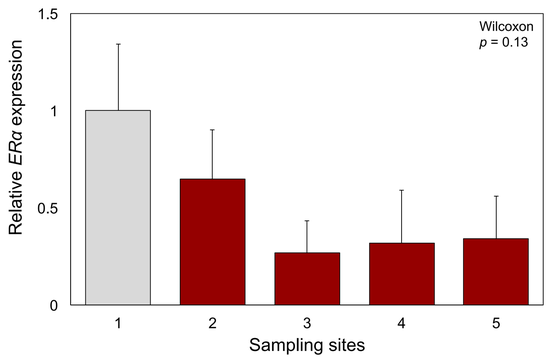

Figure 8. The average relative expression of estrogen receptor alpha (*ERα*) in fathead minnows from sampling sites 1-5. Red indicates sites downstream of CAFOs. Error bars represent standard error. n = 5.

Conclusions & Future Directions

- Though no significant differences were identified, downregulation of estrogen-related genes in female fish suggests the presence of anti-estrogenic compounds in watersheds downstream from CAFOs, as fold changes greater than 2.0 are often considered biologically significant.
- The inability to detect significant differences is likely due to large variation observed at each sample site. This variation could be driven by a variety of factors including sample size, sexual maturity of the fish, etc.
- Future studies should aim to evaluate external characteristics as a means of reducing variation.

References. [1] USDA National Agriculture Statistics Service. 2015. Texas cattle on feed. Issue No.: PR-109-15. [2] Preston, R.L. 1999. *Adv Drug Deliver Rev* 38:123-138. [3] Montgomery, T.H. et al. 2001. *J Anim Sci* 79:296-306. [4] Johnson, B.J. et al. 2014. *Animal Frontiers* 3:8-13. [5] Lange, I.G. et al. 2002. *Anal Chim Acta* 473:27-37. [6] Kolok, A.S. et al. 2007. *Sci Total Environ* 388:104-115. [7] Bartelt-Hunt, S.L. et al. 2012. *Environ Sci Technol* 46:1352-1360. [8] Ankley, G.T. et al. 2003. *Environ Toxicol Chem* 22:1350-1360. [9] occoquanbayperformance.com [10] Erath and Comanche Boundaries. 2016. [shapefile]. TNRIS. Retrieved from: https://tnris.org [11] National Elevation Dataset 10 meter. 2017. [DEM]. USDA. Retrieved from: https://datagateway.nrcs.usda.gov

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