**Assessing the removal of hormones and their potency as endocrine disrupting chemicals in dairy wastes through an anaerobic digestion system**

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Manure generated by dairy farms contains excreted natural estrogens and their conjugates, as well as other hormones that have been used to increase milk production. Natural estrogens are known potent endocrine disruptor compounds. Therefore, when manure is applied to fertilize croplands, these hormones may be spread in the environment and can potentially reach surface water by run-off, especially when a heavy rain event occurs immediately after fertilization. Livestock farms implement manure storage and treatment systems to decrease nutrient loading to the environment. Hence, it is important to evaluate the extent at which estrogens are removed during storage or treatment of manure prior to land application. However, data regarding degradation of steroid hormones in digested dairy manure are very limited. The aim of this study is to evaluate the fate and change in potency of animal hormones and their conjugates, in dairy wastes through an anaerobic digestion system.

In this study, the concentrations of estrogens [estrone (E1), 17β–estradiol (E2), estriol (E3)] and their conjugated forms (glucuronides and sulfates) were evaluated in an advanced digestion facility (CH4 BiogasTM). This facility includes a pasteurization step, followed by a 22-day anaerobic digestion process. Pasteurization combine with anaerobic digester is a feature not commonly used, and its effect on estrogen removal has not been evaluated. Samples were collected before and after pasteurization, and also after the final anaerobic digestion. These samples were analyzed by liquid chromatography-tandem mass spectrometry (LC/MS/MS), enzyme-linked immunosorbent assay (ELISA) and yeast estrogen screen (YES) assay. Detection of analytes by LC/MS/MS was done using Tso’s method1 slightly modified. Quantification was done by isotope dilution mass spectrometry. Table 1 shows the results obtained so far. None detection of E2 by LC/MS/MS suggests that current LOD is too high in the manure matrix. ELISA determination of E2 concentration was evaluated with a commercial kit according to manufacturer’s instructions (Abraxis, P.A, USA). Figure 1 shows the effect of sample dilution on ELISA concentration for estrogens. These results indicate that ELISA is affected significantly by high matrix effects, and clean up may be necessary. The estrogenic activity of liquid manure samples was determined utilizing protocols described previously by Desbrow2 and Holbrook3. Table 2 shows concentration of E1 and E2 obtained by the different techniques.

Table 1. LC/MS/MS Results for Estrogen analysis (in ng/L)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **[E1]** | **[E2]** | **[E3]** | **[E2-3G]** | **[E2-17S]** | **[E2-3S]** | **[E1-3S]** |
| RawManure | 813 | < LOD | < LOD | < LOD | 2671 | 6435 | 118 |
| AfterPasterization | 345 | < LOD | < LOD | < LOD | 32088 | 2778 | 227 |
| PostDigestor | 2363 | < LOD | < LOD | < LOD | 3024 | 130 | 8 |

E1: Estrone, E2: 17β-estradiol, E3 : Estriol, E2-3G :17β-estadiol-3-glucuronide, E2-17S : 17β-estradiol-17-sulfate E2-3S:17β-estradiol-3-sulfate, E1-3S: Estrone-3-sulfate

Figure 1. Effect of Sample Dilution on ELISA for Estrogens

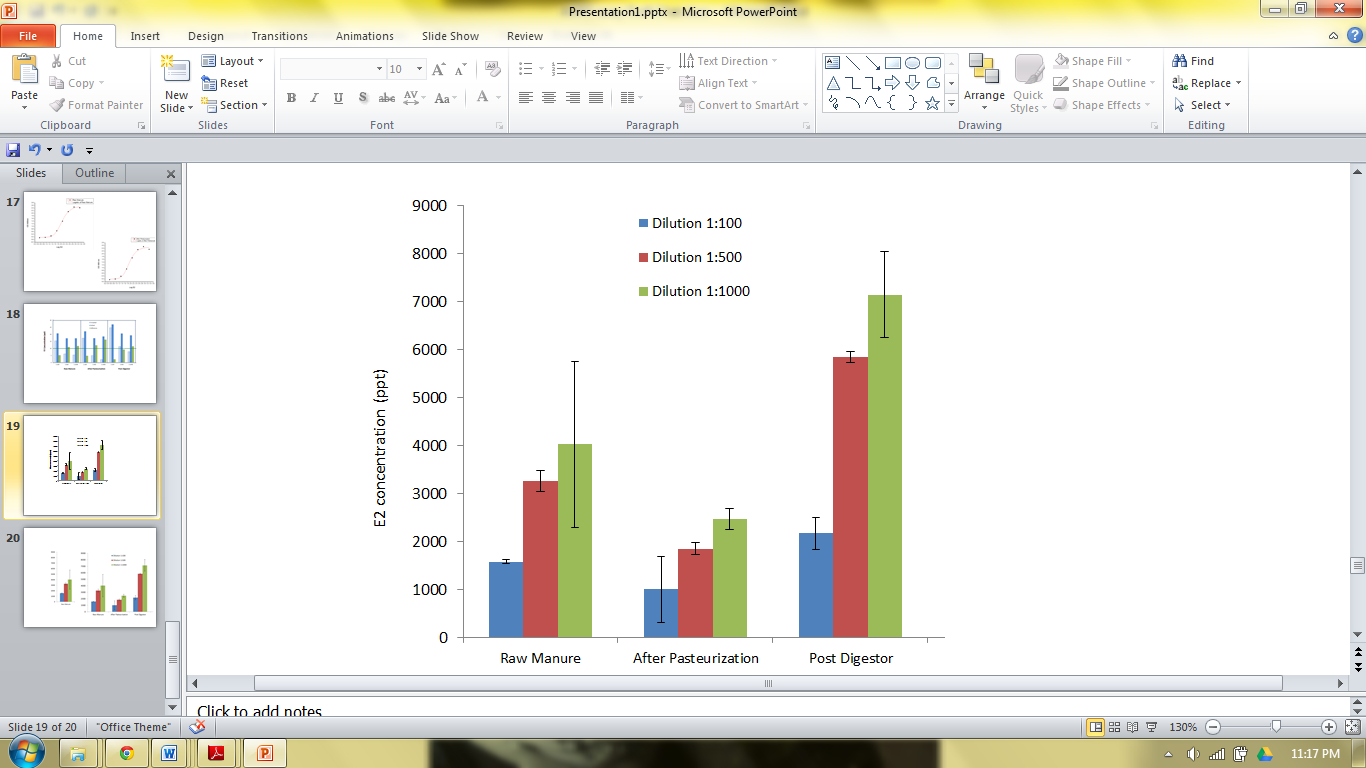


Table 2. Yeast Estrogen Screening (YES) Assay vs. LC/MS/MS vs. ELISA concentration of E2 and E1 (ppt)

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **E2-**  **Equivalent YES** | **E1-**  **LC/MS/MS** | **E2-**  **Equivalent ELISA** |
| Raw Manure | 28,113 | 813 | 1,578 |
| After Pasteurization | 14,975 | 345 | 1,003 |
| Post Digester | 1,645 | 2,364 | 2,167 |

Preliminary results from YES assay showed a much higher E2-equivalent than the E2 concentrations observed in ELISA. This could mean that there are many other hormones present in the manure that have significant estrogenic activity. Increase in E1 concentration after digestion indicates deconjugation of glucuronides and sulfated conjugates. The sample matrix affected the analysis using both ELISA and LC/MS/MS; hence improved sample preparation is necessary.

References:

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