Comparison of the welfare of beef cattle in housed and grazing systems: hormones, health, and behaviour

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Supplement E –Assay protocols

## Cortisol assay

Cortisol assays were conducted by competitive ELISA using the Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit, produced by Salimetrics (USA) and further details can be found on their website. In breif:

1. Reagents were brought up to room temperature
2. 24 ml of assay diluent was measured into a sterile tube.
3. 25 µl of each of six standards (3.0, 1.0, 0.333, 0.111, 0.037, 0.012 µg dl-1) was pipetted into wells. 25 µl of blanks (assay diluent) and samples (no dilution) were added to their wells.
4. The enzyme conjugate was diluted (1:1600) into the assay diluent. 200 µl of this solution was added to each well.
5. The plate was placed on a shaker for 5 mins at 100 rpm, it was then incubated at 20°C for 1 hr.
6. Well liquid content was discarded, and the plate was washed four times using the buffer solution (diluted to 1x). For each wash, 300 µl of wash buffer was added to each well before being discarded. The plate was then blotted onto clean paper towels after each discard.
7. 200 µl of TMB substrate solution was added to each well.
8. The plate was placed on a shaker for 5 mins at 100 rpm before being incubated, in the dark, at 20°C for 25 mins.
9. 50 µl of stop solution was added to each well
10. The plate was placed on a shaker for 3 mins at 100 rpm.
11. The plate was scanned at 450 nm using a plate reader (Infinite 200 Pro, Tecan Life Sciences, Switzerland).
12. The optical densities of standards were used to create a four parameter logistic curve from which sample from which sample concentrations were interpolated (MyAssays Ltd.).

## Serotonin assay

Serotonin ELISAs were conducted using a Serotonin High Sensitivity ELISA kit produced by DLD-Diagnostika GMBH (Germany) (product no. EA 630/96). Additional details and a full protocol can be found via their website, in breif:

1. Reagents were prepped in accordance to manufacturer instructions.
2. 20 µl of samples were pipetted into their respective wells.
3. 25 µl of acylation buffer was added.
4. 10 µl of acylation reagent was added.
5. The plate was incubated at room temperature for 1 hr.
6. 25 µl of deactivator solution was added.
7. The plate was covered and incubated at room temperature for 3 hrs.
8. 50 µl was taken from each well and added to a coated microtiter wells.
9. The plate was covered and incubated at 5°C for 18 hrs.
10. The plate was washed 4x with 300 µl wash buffer.
11. 100 µl of enzyme conjugate was added to all wells.
12. The plate was incubated at room temperature on an orbital shaker at 100rpm, for 60 mins.
13. The plate was washed 4x with 300 µul wash buffer.
14. 100 µl of substrate was added to all wells.
15. The plate was incubated at room temperature on an orbital shaker at 100rpm, for 30 mins.
16. 100 µl of stop solution was added to all wells.
17. The plate was scanned at 450 nm using a plate reader (Infinite 200 Pro, Tecan Life Sciences, Switzerland).
18. The optical densities of standards were used to create a four parameter logistic curve from which sample from which sample concentrations were interpolated (MyAssays Ltd.).