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

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Supplement D – Hair and nasal mucus preparation

Hair samples were taken at the same time as physical checks were conducted. Hair was taken using an electric shearer, in the area around the base of the neck and between shoulder blades and stored at -20ºC prior to further preparation. Approximately 250 mg of hair was placed in a beaker to be washed four times. For the first two washes: 5 ml of water was added to the beaker which was then shaken at 100 rpm for 3 mins, after which the water was strained off. The third and fourth wash followed a similar process but with isopropanol in place of water. After the final wash samples were dried at 30ºC for 3 days. Hair samples were then ground in a ball mill at 50 hz for 2 mins until a powder of approximately 2 mm. A 50 mg sub-sample of the resulting ground hair was then weighed into a 2 ml microcentrifuge tube and 1.5 ml of methanol added. Tubes were vortexed for 10 secs, then sonicated for 30 minutes, and then placed in an incubator-shaker at 100 rpm and 30ºC for 18 h. Samples were then centrifuged at 7,000 x g for 2 mins, after which 750 µl of the supernatant was transferred to a new 2 ml microcentrifuge tube. Tubes were then placed in a block heater at 38ºC for 18hrs to evaporate off the methanol from the supernatant. The sample was then resuspended by adding 150 µl of PBS and vortexing for 30 secs. Samples were then stored -20ºC until analysis.

Nasal mucus was collected using sterile swabs (Sterilin F155CA). Whilst animals were appropriately restrained the swab was inserted into the nostril at a depth of approximately 5 cm (not so far enough to feel resistance) and rotated around the inside of it. Samples were then stored -20ºC until analysis. Microcentrifuge tubes (2 ml) were weighed and a 45 µm filter then added to the tube. Swab tips were cut off and placed in the filter basket, 500µl of methanol was added. Samples were then placed in a shaker at 100 rpm for 18 h at 20°C. Tubes were centrifuged at 10,000 x g for 2 minutes to draw the methanol extract through the filter. The filter was then removed, and tubes placed in a block heater at 30ºC for 18h, to evaporate off the methanol. Tubes were re-weighed and the addition in weight considered to be equal to the mass of material extracted from the swab. The extracted material was re-suspended by adding 150 µl of PBS and vortexing for 30 seconds.