These data are from the experiment described as “Post mortem observations on ruminal histology and gene expression and ruminal and caecal content of beef cattle fattened on barley-based rations” Conducted by N.N. Jonsson 1,2, H. Ferguson 1,a, H.H.C. Koh-Tan 1, C.A. McCartney 3, R.C. Cernat 3, E.M. Strachan 1, W. Thomson 2, T.J. Snelling 3, C.D. Harvey 2, I. Andonovic 4, C. Michie 4, R.J. Wallace 3 (1 Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G61 1QH, UK; 2 Harbro Ltd., Turriff, Aberdeenshire AB53 4PA, UK ; 3 Rowett Institute, University of Aberdeen, Aberdeen AB25 2ZD, UK ; 4 Department of Electrical and Electronic Engineering, Strathclyde University, Glasgow G1 1XW, UK)

Corresponding author: Nicholas N Jonsson.

Email: nicholas.jonsson@glasgow.ac.uk

Materials and Methods

Farms and animals

Ten beef farms situated in Aberdeenshire completed a questionnaire on dietary and farm management in the year prior to the study. Six were selected based on dietary composition and perceived susceptibility to acidosis (three high-risk and three low-risk) based on the questionnaire and on-farm observations. In total, 119 steers and heifers with an average age of 700 days and carcase weight of 371 kg were sampled at slaughter. Breeds included Aberdeen Angus, Blonde d’Aquitaine, Charolais, Limousin, Saler British Blue, Shorthorn and Simmental, and crosses of these breeds. All animals were finished indoors in the Grampian region close to Aberdeen (mean annual temperature 7.5 °C, rainfall 1130 mm) between January and July 2013.

Feed analysis

A representative sample of the total mixed ration (TMR) was assessed for each of the farms, once during the feeding period for each cohort of animals. Samples were taken for measurement of crude protein, crude fibre, starch, and neutral-detergent fibre (NDF) on a dry matter basis using near infrared reflectance (NIR). NIR was conducted using a FossNIRSystems 5000+ machine, using calibrations provided by Trouw Nutrition GB. Particle size of TMR was measured by Trouw Nutrition GB using a Penn State particle separator (PSPS), with a 1.18 mm screen to enable quantification of fine particles. All cattle had *ad libitum* access to feed, and were estimated to consume 11-13.5 kg dry matter feed per day. Cattle at farm BH6 also had *ad libitum* access to barley straw.

Post-mortem sampling

In the abattoir, once the rumen was separated from the hindgut and incised, a sample of its contents was taken and strained through four layers of gauze muslin into two 50 ml plastic centrifuge tubes. Full details on the sampling protocol are provided in the Supplementary Materials section.

Gross assessment of rumen wall

The opened and washed wall of the ventral sac of the rumen was examined and photographed. Papillae were subjectively assessed for size and shape and the complete, open surface of the sac was characterised according to the prevalence of lesions and colour. The scoring system is shown in Table S1.

Histamine measurement in ruminal fluid

Histamine concentration in the rumen fluid was measured using the Abnova Histamine ELISA Kit (Abnova, Catalogue Number KA1888), designed for analysis of histamine in human faeces. Duplicate measurements were used for all standards, controls and samples. Before use on research samples, several dilutions were tested to optimise the assay for ruminal fluid. A dilution of 1:300 provided the most consistent results and was subsequently used on all samples. The absorbance of the solution in the wells was read using a microplate reader set to 450 nm. Standard curves were plotted for ruminal and plasma samples and unknown concentrations were calculated from the regression equation.

Lipopolysaccharides in ruminal and caecal fluid

For measurement of LPS, a modified, scaled-down method of Li et al. (2012) was created. This was carried out immediately upon return from the abattoir, in order to minimise lysis of the Gram-negative bacteria and further release of LPS into the extracellular fluid. LPS was measured in the processed rumen and caecum fluid by the *Limulus* Amebocyte Lysate Assay (Kinetic-QCL, Lonza Group Ltd.) according to the manufacturer’s instructions. Full details are provided in the Supplementary Materials.

Short-chain fatty acids (SCFA) in ruminal fluid

SCFA were measured on centrifuged rumen fluid using gas chromatography (Richardson et al. 1989). SCFA were measured on centrifuged rumen fluid using gas chromatography (Richardson et al. 1989). Samples were shipped to the laboratory on dry ice and analysed for: acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, succinate, formate, lactate. Two standards were tested in duplicate for each run.

Histology

The rumen samples collected at the abattoir and fixed in 10% formalin for 48 h and stored in PBS were cut into 1-3 segments, depending on their size, removing any rough edges and ensuring that all layers of the rumen wall were present in each segment. Fixation of the segments continued with a fresh aliquot of 10% formalin. They were then rinsed in PBS and stored in PBS or 70% ethanol, before paraffin-embedding and micro-sectioning on a microtome. Sections of 3 μm thickness were cut, placed onto slides and baked in an oven at 60°C for 1-2 h, until excess wax had melted and the tissues adhered to the slides. Haematoxylin and Eosin staining (H&E) and Elastin Martius Scarlet Blue staining (EMSB) were then carried out by hand (EMSB stain is a Martius Scarlet Blue (MSB) protocol modified to include a Miller’s Elastin stain).

Immunohistochemistry

The immunohistochemistry (IHC) staining was performed by Veterinary Diagnostic Services (VDS) at the University of Glasgow’s School of Veterinary Medicine. Five slides were stained per sample for all samples; major histocompatibility complex class 2 (MHCll), myeloperoxidase, cluster of differentiation 3 (CD3), a negative rabbit and a negative mouse immunoglobulin control. Full details are presented in the Supplementary Materials file.

Examination, image capture, storage and analysis

All slides were initially scanned under low and high power using an Olympus CX41 microscope. Images of typical and atypical examples of all features of interest were captured using GXCam software, calibrated as recommended by the manufacturer, using a ×4, ×10 and ×40 graticule. The stratum corneum (SC) thickness was determined by taking the mean of 5 measurements in µm across the SC over 2 fields, using ×40 magnification and H&E stain. The same approach was taken for the stratum granulosum (SG) thickness. The vascular diameter (VASCD) was taken as the mean diameter of the single largest vessel in each of two papillae using ×40 magnification and H&E stain. CD3+ and MHCII+ cells were estimated as the count of the total number of CD3+ or MHCII+ cells in a single image taken at ×40 magnification. All histological measurements and counts in this study were undertaken manually by one operator (HJF).

Gene Expression

Gene expression methods are presented in detail in the Supplementary Materials file. RNA extraction from rumen tissue was carried out using the miRNeasy® mini kit (Qiagen) and DNase® kit (Qiagen). TaqMan real-time quantitative PCR (qPCR) was used to quantify gene expression levels from the ruminal cDNA using TaqMan Gene Expression Master Mix assay kit (Life Technologies, UK) and the respective probes as shown in Table S2. The assay was carried out using a 384-well microplate (Thermo Fisher, UK) with 3 technical replicates for each sample to reduce measurement error. The assays were carried out in singleplex or duplex, depending on the assay efficiency pre-determined in a prior optimisation assay. A pooled cDNA sample from all cDNA samples was used to generate a series of 3-fold serial dilution used as standard curve in every assay. Thermal cycling was carried out using the 7900HT Fast Real-Time PCR System (Applied Biosystems).