SUPPLEMENTARY INFORMATION

*animal* journal

*Post mortem o*bservations on rumen wall histology and gene expression and ruminal and caecal content of beef cattle fattened on barley-based rations

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Short title: *Post mortem* ruminal findings in beef cattle

Table S1 Subjective, categorical scoring system for gross pathology in the ventral sac of the rumen of cattle at post mortem examination.

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| Feature | Score | Description |
| Papillae Colour |  |  |
| 0 | A | Black/Brown |
| 1 | B | Grey/Brown |
| 2 | C | Grey/Brown small areas with pink tips |
| 3 | D | Grey/Brown large areas with pink tips |
| 4 | E | Pink |
| 5 | F | Yellow |
| Papillae Shape |  |  |
| 0 | A | Long + Thin |
| 1 | B | Long + Oval |
| 2 | C | Short + Thin |
| 3 | D | Short + Oval |
| 4 | E | Short + Brittle |
| Ventral Sac  |  |  |
| 0 | A | No evidence of any damage |
| 1 | B | Small areas bare of papillae |
| 2 | C | Large areas bare of papillae |
| 3 | D | Small areas of excoriation/scarring |
| 4 | E | Red/Bloody areas |
| 5 | F | Parakeratosis |

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| Table S2List of all the Taqman Gene expression assays that were used in the study on cattle |
| Gene | Assay ID |
| *ACTB\** | Bt03279175\_g1 |
| *CCL11* | Bt03244553\_m1 |
| *GAPDH\** | Bt03210913\_g1 |
| *IFNγ* | Bt03212723\_m1 |
| *IL-10* | Bt03212724\_m1 |
| *IL-1β* | Bt03212745\_m1 |
| *IL-2* | Bt03217368\_m1 |
| *NHE3* | Bt04309751\_m1 |
| *RPLP0\** | Bt03218082\_g1 |
| *TLR2* | Bt03223212\_m1 |
| *TLR4* | Bt03251671\_m1 |
| \*house-keeping genes |

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| Table S3 Results of Shapiro-Wilk normality tests on continuous variables for the full dataset of 119 cattle, for which complete sets of observations were available.  |
| Variable |  W |  *p* |  |
| Age | 0.94756 | 0.0004986 | \*\*\* |
| Cold Weight | 0.90969 | 3.424e-06 | \*\*\* |
| RF Histamine | 0.77318 | 3.004e-11 | \*\*\* |
| RF LPS | 0.80141 | 2.074e-10 | \*\*\* |
| RF Acetate | 0.97855 | 0.09577 | . |
| RF Propionate  | 0.88139 | 1.664e-07 | \*\*\* |
| RF isoButyrate | 0.96141 | 0.004523 | \*\* |
| RF Butyrate | 0.86582 | 3.772e-08 | \*\*\* |
| RF IsoValerate | 0.95326 | 0.0012 | \*\* |
| RF Valerate | 0.92728 | 2.945e-05 | \*\*\* |
| RF Lactate | 0.95703 | 0.002192 | \*\* |
| RF Succinate | 0.079727 | < 2.2e-16 | \*\* |
| RF Prop:Pro | 0.89348 | 5.724e-07 | \*\*\* |
| RF Prop:Ace | 0.89565 | 7.204e-07 | \*\*\* |
| RF Prop:But | 0.87254 | 7.061e-08 | \*\*\* |
| RF Ace:Pro | 0.98896 | 0.5671 | NS |
| RF Total SCFA | 0.98016 | 0.1286 | NS |
| CF LPS | 0.80018 | 1.899e-10 | \*\*\* |
| CF Formate | 0.81117 | 4.218e-10 | \*\*\* |
| CF Acetate | 0.9845 | 0.2795 | NS |
| CF Propionate  | 0.98591 | 0.3546 | NS |
| CF isobutyrate | 0.98044 | 0.1353 | NS |
| CF Butyrate | 0.70239 | 4.443e-13 | \*\*\* |
| CF isovalerate | 0.95884 | 0.002951 | \*\* |
| CF Valerate | 0.98657 | 0.3953 | NS |
| CF Lactate | 0.92658 | 2.689e-05 | \*\*\* |
| CF Succinate | 0.23552 | < 2.2e-16 | \*\*\* |
| CF Ace:Pro | 0.90526 | 2.064e-06 | \*\*\* |
| CF Total SCFA | 0.98951 | 0.6111 | NS |
| *TLR4* RE | 0.84794 | 7.773e-09 | \*\*\* |
| *IL1B* RE | 0.32455 | < 2.2e-16 | \*\*\* |
| *CCL11* RE | 0.35602 | < 2.2e-16 | \*\*\* |
| *NHE3* RE | 0.79918 | 1.769e-10 | \*\*\* |
| *IL2* RE | 0.12574 | < 2.2e-16 | \*\*\* |
| *IFNG* RE | 0.19145 | < 2.2e-16 | \*\*\* |
| SC | 0.75313 | 8.384e-12 | \*\*\* |
| SG | 0.80126 | 2.051e-10 | \*\*\* |
| Vascular diameter | 0.69995 | 3.893e-13 | \*\*\* |
| CD3 | 0.96883 | 0.01634 | \* |
| MHCII | 0.85897 | 2.03e-08 | \*\*\* |
| Key: NS p > 0.1, . p < 0.1; \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001; ace = acetate, but = butyrate, CD3 = cluster of distinction 3, CF = cecal fluid, LPS = lipopolysaccharide,MHCII = major histocompatibility 2, Pro = propionate, Prop = proportion of total, RE = relative expression, RF = rumen fluid, SC = stratum corneum, SCFA = short-chain fatty acids, SG = stratum granulosum. |

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| Table S4 Count of cattle with post mortem papillae characterised by colour, grouped by farm of origin |
|  |  | Farm of origin |
| Description | Score | BH1 | BH6 | BH7 | BL2 | BL3 | BL7 |
| Black/Brown | A | 3 | 5 | 10 | 14 | 1 | 16 |
| Grey/Brown | B | 7 | 4 | 5 | 4 | 2 | 3 |
| Grey/Brown small areas with pink tips | C | 5 | 6 | 2 | 0 | 6 | 0 |
| Grey/Brown large areas with pink tips | D | 4 | 5 | 2 | 1 | 6 | 0 |
| Pink | E | 1 | 0 | 0 | 1 | 5 | 0 |
| Yellow | F | 0 | 0 | 0 | 0 | 0 | 0 |

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| Table S5 Count of cattle with post mortem papillae characterised by shape, grouped by farm of origin |
|  |  | Farm of origin |
| Description | Score | BH1 | BH6 | BH7 | BL2 | BL3 | BL7 |
| Long + Thin | A | 10 | 12 | 9 | 8 | 12 | 17 |
| Long + Oval | B | 9 | 6 | 8 | 11 | 6 | 2 |
| Short + Thin | C | 0 | 2 | 0 | 0 | 0 | 0 |
| Short + Oval | D | 0 | 0 | 2 | 1 | 1 | 0 |
| Short + Brittle | E | 1 | 0 | 0 | 0 | 1 | 0 |

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| Table S6 Count of cattle with post mortem ruminal luminal wall characterised by presence of lesions, grouped by farm of origin |
|  |  | Farm of origin |
| Description | Score | BH1 | BH6 | BH7 | BL2 | BL3 | BL7 |
| No evidence of any damage | A | 15 | 19 | 19 | 18 | 14 | 19 |
| Small areas bare of papillae | B | 2 | 1 | 0 | 0 | 5 | 0 |
| Large areas bare of papillae | C | 2 | 0 | 0 | 0 | 1 | 0 |
| Small areas of excoriation/scarring | D | 1 | 0 | 0 | 0 | 0 | 0 |
| Red/Bloody areas | E | 0 | 0 | 0 | 0 | 0 | 0 |
| Parakeratosis | F | 0 | 0 | 0 | 2 | 0 | 0 |

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| Table S7 Correlation matrix of selected variables from 119 cattle. |
|  | Age | ColdWt | RFSCFA | CFSCFA | RFLact | RFLPS | CFLPS | *IFNG* | *IL1B* | *NHE3* | *TLR4* | *CCL11* | RFHist | SC | SG | VASCD | CD3 |
| ColdWt | 0.29 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| RF SCFA | -0.40 | -0.36 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| CF SCFA | 0.14 | -0.01 | -0.09 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| RF Lact | 0.09 | 0.50 | 0.03 | -0.20 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| RF LPS | 0.10 | 0.00 | -0.04 | 0.05 | -0.04 |  |  |  |  |  |  |  |  |  |  |  |  |
| CF LPS | 0.19 | 0.03 | -0.02 | 0.06 | 0.09 | -0.16 |  |  |  |  |  |  |  |  |  |  |  |
| *IFNG* | -0.01 | -0.27 | 0.08 | -0.05 | -0.14 | -0.24 | 0.15 |  |  |  |  |  |  |  |  |  |  |
| *IL1B* | -0.08 | -0.16 | 0.25 | -0.16 | -0.10 | 0.23 | 0.01 | 0.33 |  |  |  |  |  |  |  |  |  |
| *NHE3* | -0.10 | 0.10 | 0.14 | -0.14 | 0.05 | 0.07 | -0.01 | 0.17 | 0.29 |  |  |  |  |  |  |  |  |
| *TLR4* | 0.08 | 0.21 | -0.11 | -0.16 | 0.18 | 0.03 | -0.11 | 0.36 | 0.28 | 0.44 |  |  |  |  |  |  |  |
| *CCL11* | -0.10 | -0.14 | 0.09 | 0.05 | -0.07 | -0.18 | 0.12 | 0.57 | 0.11 | -0.05 | 0.27 |  |  |  |  |  |  |
| RF Hist | -0.25 | -0.08 | 0.30 | -0.27 | 0.02 | -0.09 | -0.28 | -0.03 | 0.13 | 0.30 | 0.13 | -0.07 |  |  |  |  |  |
| SC | 0.15 | 0.36 | -0.40 | -0.11 | 0.07 | 0.28 | -0.32 | -0.13 | -0.11 | -0.03 | 0.11 | -0.19 | -0.02 |  |  |  |  |
| SG | 0.10 | 0.21 | -0.28 | -0.15 | 0.07 | 0.33 | -0.38 | -0.15 | -0.07 | 0.01 | 0.17 | -0.20 | 0.07 | 0.81 |  |  |  |
| VASCD | 0.12 | 0.17 | -0.26 | -0.16 | -0.05 | 0.17 | -0.21 | -0.13 | 0.11 | 0.21 | 0.24 | -0.24 | 0.21 | 0.53 | 0.63 |  |  |
| CD3 | 0.09 | -0.13 | 0.08 | 0.16 | -0.14 | -0.24 | 0.29 | 0.19 | 0.06 | -0.16 | -0.17 | -0.03 | -0.10 | -0.20 | -0.29 | -0.13 |  |
| MHCII | -0.06 | -0.30 | 0.18 | 0.19 | -0.12 | -0.11 | 0.24 | 0.30 | 0.02 | 0.00 | -0.07 | 0.17 | 0.10 | -0.31 | -0.35 | -0.21 | 0.31 |
| CD3 = cluster of distinction 3, CF = caecal fluid, Cold Wt = cold weight of carcase after slaughter, Lact = lactate concentration, hist = histamine concentration, LPS = lipopolysaccharide, MHCII = major histocompatibility complex 2, Pro = propionate, Prop = proportion of total, RE = relative expression, RF = rumen fluid, SC = stratum corneum, SCFA = short-chain fatty acids, SG = stratum granulosum, VASCD = vascular diameter,  |

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| Table S8 Summary of linear regression models for each of the selected outcome variables against the dietary input variables (percentage dry matter of barley, silage, straw, crude protein and crude fibre, and percentage of fines in TMR). For each outcome variable (rows) the best predictor from among the dietary input variables is underlined and in bold.  |
| Variable | Barley | Silage | Straw | TMR Fines % | Crude Protein | Crude Fibre |
|  | Adj R2 | *P*-value | Adj R2 | *P*-value | Adj R2 | *P*-value | Adj R2 | *P*-value | Adj R2 | *P*-value | Adj R2 | *P*-value |
| RF Histamine | 0.010 | 0.919 | 0.045 | 0.019 | 0.028 | 0.053 | 0.109 | <0.001 | 0.089 | 0.001 | **0.208** | **<0.001** |
| RF LPS | 0.016 | 0.112 | 0.012 | 0.142 | 0.009 | 0.793 | 0.050 | 0.008 | **0.181** | **<0.001** | 0.070 | 0.002 |
| CF LPS | 0.108 | 0.001 | 0.157 | <0.001 | 0.008 | 0.599 | 0.008 | 0.718 | **0.157** | **<0.001** | 0.004 | 0.440 |
| RF Lactate | 0.067 | 0.005 | 0.078 | 0.003 | 0.062 | 0.007 | 0.216 | <0.001 | **0.285** | **<0.001** | 0.084 | 0.001 |
| CF Lactate | 0.161 | <0.001 | **0.248** | **<0.001** | 0.010 | 0.861 | 0.009 | 0.960 | 0.047 | 0.012 | 0.006 | 0.550 |
| RF Total SCFA | 0.268 | <0.001 | 0.077 | 0.003 | 0.303 | <0.001 | **0.389** | **<0.001** | 0.095 | <0.001 | 0.295 | <0.001 |
| CF Total SCFA | 0.022 | 0.081 | 0.016 | 0.113 | 0.010 | 0.771 | 0.007 | 0.645 | **0.047** | **0.012** | 0.009 | 0.929 |
| *TLR4* RE | 0.054 | 0.011 | 0.033 | 0.038 | 0.047 | 0.018 | 0.088 | 0.001 | **0.165** | **<0.001** | 0.037 | 0.020 |
| *IL1B* RE | 0.054 | 0.012 | 0.011 | 0.148 | 0.071 | 0.004 | **0.111** | **<0.001** | <0.001 | 0.310 | 0.065 | 0.003 |
| *CCL11* RE | 0.008 | 0.180 | 0.002 | 0.363 | 0.024 | 0.066 | 0.004 | 0.487 | **0.021** | **0.065** | 0.008 | 0.938 |
| *NHE3* RE | 0.018 | 0.097 | 0.011 | 0.155 | 0.010 | 0.859 | 0.004 | 0.488 | **0.088** | **0.001** | 0.009 | 0.979 |
| *IL2* RE | 0.023 | 0.073 | 0.004 | 0.439 | 0.015 | 0.115 | 0.008 | 0.778 | **0.082** | **0.001** | 0.009 | 0.926 |
| *IFNg* | 0.064 | 0.007 | 0.072 | 0.004 | 0.039 | 0.028 | 0.079 | 0.001 | **0.180** | **<0.001** | 0.009 | 0.150 |
| SC | 0.271 | <0.001 | 0.114 | 0.001 | 0.261 | <0.001 | 0.314 | <0.001 | **0.380** | **<0.001** | 0.208 | <0.001 |
| SG | 0.208 | <0.001 | 0.076 | 0.004 | 0.187 | <0.001 | 0.202 | <0.001 | **0.331** | **<0.001** | 0.166 | <0.001 |
| VASCD | 0.136 | <0.001 | 0.040 | 0.030 | 0.161 | <0.001 | 0.094 | 0.001 | **0.192** | **<0.001** | 0.062 | 0.005 |
| CD3+ | 0.052 | 0.015 | 0.033 | 0.043 | 0.048 | 0.020 | 0.119 | <0.001 | **0.349** | **<0.001** | 0.059 | 0.006 |
| MHCII+ | 0.011 | 0.950 | 0.002 | 0.288 | 0.041 | 0.028 | 0.103 | <0.001 | **0.135** | **<0.001** | 0.050 | 0.010 |
| CD3 = cluster of distinction 3, CF = caecal fluid, LPS = lipopolysaccharide, MHCII = major histocompatibility complex2, Pro = propionate, Prop = proportion of total, RE = relative expression, RF = rumen fluid, SC = stratum corneum, SCFA = short-chain fatty acids, SG = stratum granulosum, TMR = total mixed ration, VASCD = vascular diameter, |



Figure S1 This panel summarises the distribution of the six variables in the diets of 119 cattle that were considered as possible predictors for variation in the dependent variables measured in the study. Farms labelled as BH1,BH6, BH7 were classed *a priori* as high-risk and those labelled BL2, BL3, BL7 were low-risk. It is clear that there is considerable potential for interaction among the variables, which is not amenable to statistical modelling. TMR = total mixed ration



Figure S2 Concentrations of acetate, propionate and butyrate in the rumens of cattle, by farm of origin (above) and risk (below). Farms labelled as BH1,BH6, BH7 were classed *a priori* as high-risk and those labelled BL2, BL3, BL7 were low-risk.



Figure S3 Concentrations of isobutyrate, valerate and isovalerate in the rumens of cattle from each farm (above) and by risk classification of the farm of origin (below). Farms labelled as BH1,BH6, BH7 were classed *a priori* as high-risk and those labelled BL2, BL3, BL7 were low-risk.



Figure S4 Box and whisker plots showing acetate, propionate, and butyrate concentration in caecal fluid of the cattle on six beef finishing units (above) and by risk category of farm of origin (below). Farms labelled as BH1,BH6, BH7 were classed *a priori* as high-risk and those labelled BL2, BL3, BL7 were low-risk.



Figure S5 Box and whisker plots showing isobutyrate, valerate, isovalerate and lactate concentrations in caecal fluid of the cattle on six beef finishing units (above) and by risk category of farm of origin (below). Farms labelled as BH1,BH6, BH7 were classed *a priori* as high-risk and those labelled BL2, BL3, BL7 were low-risk.



Figure S6 Box and whisker plots showing the relative gene expression of *TLR4*, *IL1B*, *CCL11*, *NHE3*, *IL2* and *IFNG* in the ruminal wall of cattle from each of the farms. Farms labelled as BH1,BH6, BH7 were classed *a priori* as high-risk and those labelled BL2, BL3, BL7 were low-risk. Note that *IL1B*, *CCL11*, *IL2* and *IFNG* relative expression values have been natural log transformed (ln) for ease of visualisation.



Figure S7 Box and whisker plots showing the main histological indicators in rumen of cattle, by *a priori* risk classification of farm of origin. Note that stratum corneum and granulosum thickness and vascular diameter are natural log-transformed (ln) for ease of visualisation.CD3 = cluster of distinction 3, hpf = high-power field, MHCII = major histocompatibility complex 2



Figure S8 Box and whisker plots showing ruminal and caecal concentrations of lactate and lipopolysaccharide (LPS), and concentration of histamine in the rumen of cattle against the strongest potential predictor input variable for each.



Figure S9 Box and whisker plots showing total short-chain fatty acids (SCFA) concentrations and the ratios of acetate to propionate in ruminal and caecal fluid of cattle against the strongest potential predictor input variable for each.



Figure S10 Box and whisker plots showing total relative expression of six genes (*TLR4*, *IL1B*, *CCL11*, *NHE3*, *IL2* and *IFNG)* in ruminal wall against the strongest potential predictor input variable for each. Note that *IL1B*, *CCL11*, *IL2* and *IFNG* have been natural log-transformed (ln) for ease of visualisation.



Figure S11 Box and whisker plots showing histological variables from ruminal wall tissue against the strongest potential predictor input variable for each (crude protein in all cases). CD3 = cluster of distinction 3, hpf = high-power field, MHCII = major histocompatibility complex 2.