Supplementary Material

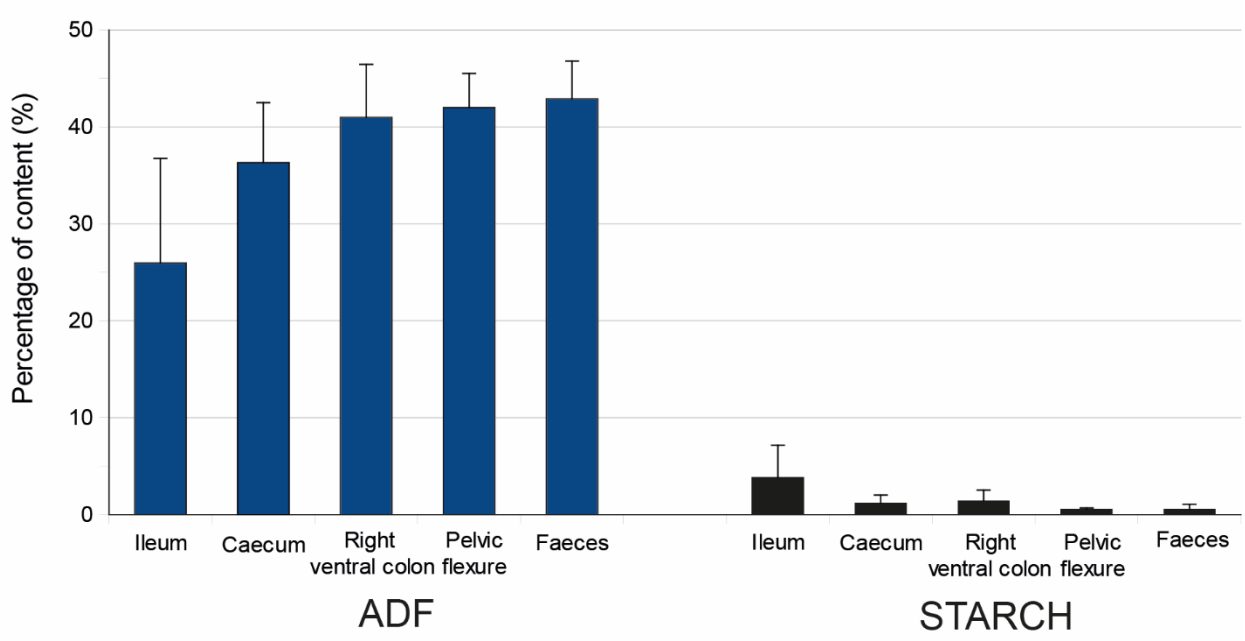

Figure S1: The average percentage of ADF (acid detergent fiber) and starch within the gut content from five areas of the equine gastrointestinal system from three horses (1, 2 and 3 in Table S1). Error bars show standard deviation of the values within each group.

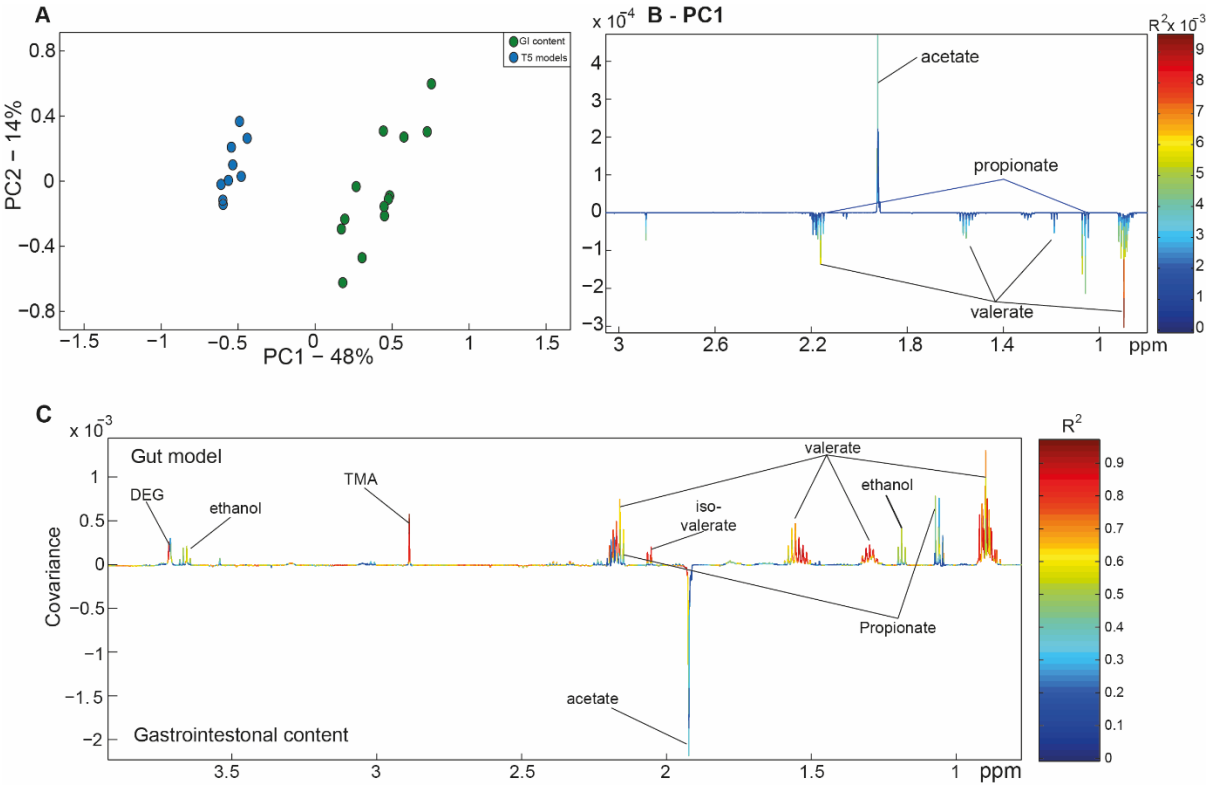

Figure S2: Comparison of metabolic profiles of *ex* vivo gut content and the gut model at steady state (T5). Multivariate models built with the ^{1}H NMR spectra from samples of GI content and samples from all vessels of the three separate gut models in the concordance study (inoculated with feces from different horses). A PCA model (R^2 = 0.64) was built and A) scores plot and B) the loading plot for PC1 (representing 48 % of the total variance in the dataset) was visualised. C) The correlation coefficient plot from the OPLS-DA model built with these spectra (Q^2Y = 0.82). PC, principle component; DEG, diethylene glycol; TMA, trimethylamine.

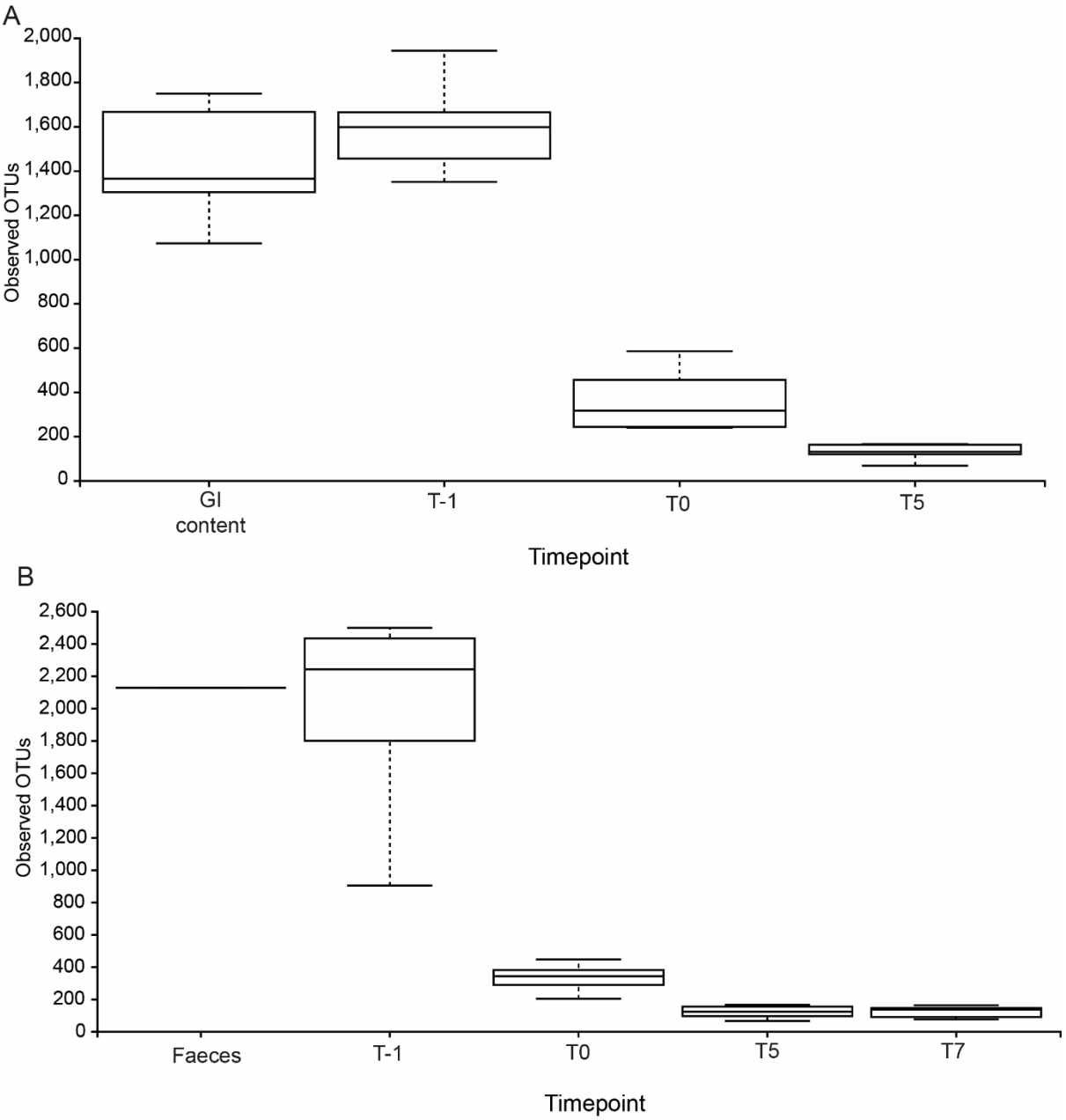

Figure S3: Alpha rarefaction boxplots showing bacterial diversity of the gastrointestinal contents/feces and the time points of the gut models of: A) the concordance study and B) the repeatability study.

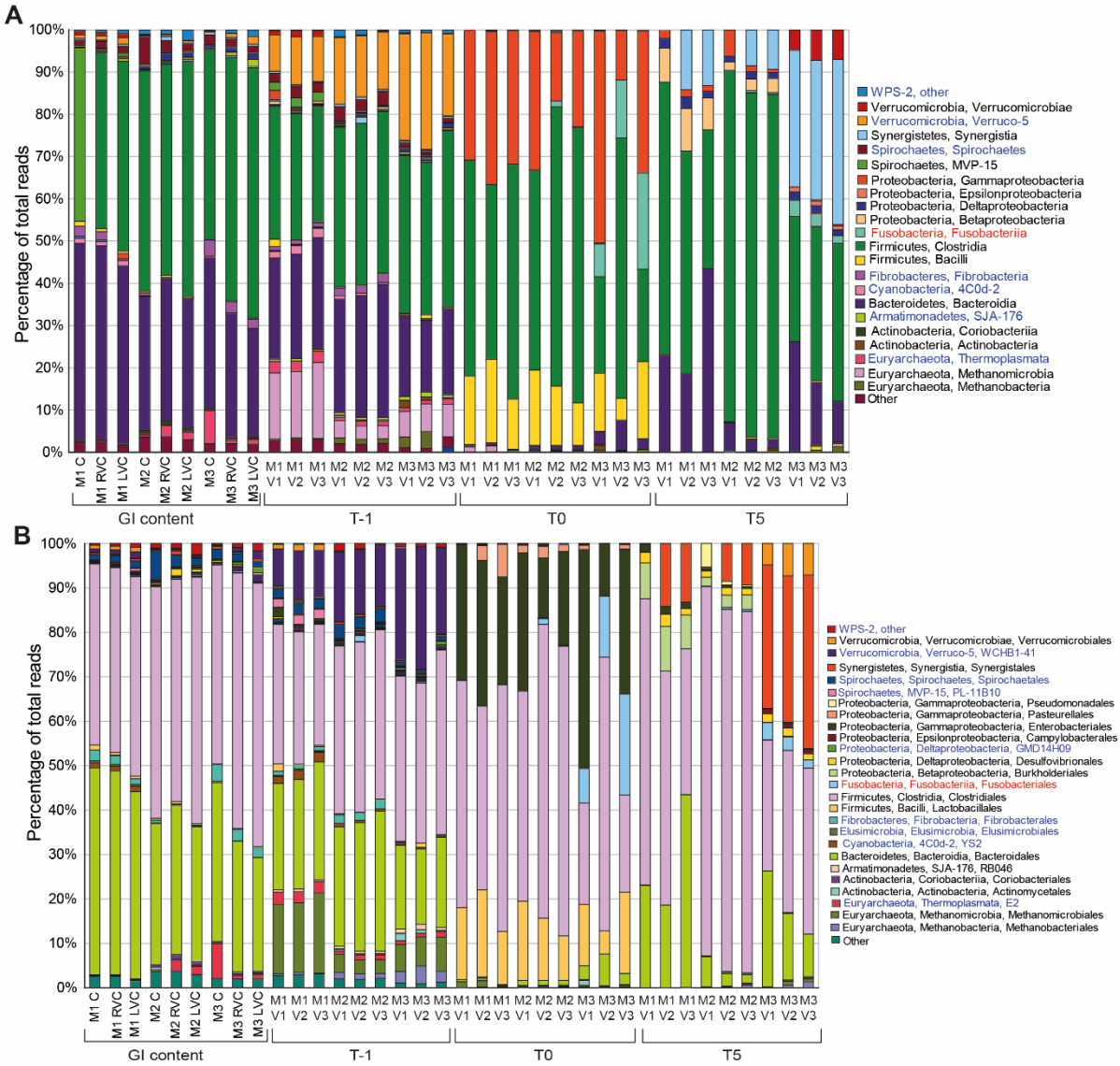

Figure S4: Relative abundance of bacterial A) classes and B) orders identified in samples from the GI content and all vessels the three gut models of the concordance study (inoculated with feces from three different horses). The key indicates whether bacterial phyla are: identified in both gastrointestinal and gut model samples at T5 (black), only in gastrointestinal samples (blue) or only in gut model samples (red). C, cecum; RVC, right ventral colon; LVC, left ventral colon; V, vessel; T, turnover.

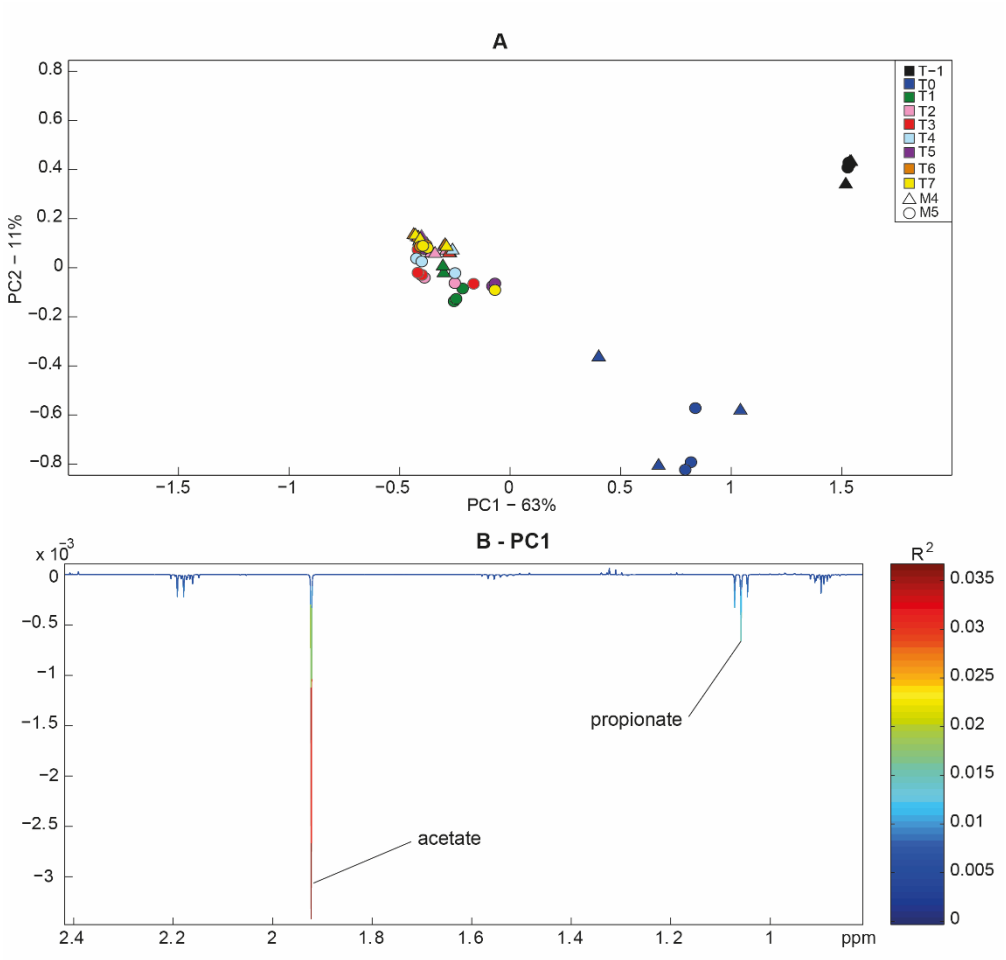

Figure S5: Demonstration of the repeatability of the metabolic profiles of the gut models from the repeatability study. PCA model constructed with the ^{1}H NMR spectra gained from the all vessels of the two gut models inoculated with the feces from the same horse (R^2 = 0.75). A) The PCA scores plot for this model and B) the loading plot for PC1 (representing 63 % of the total variance in the dataset). T, turnover; PC, principle component; M, model.

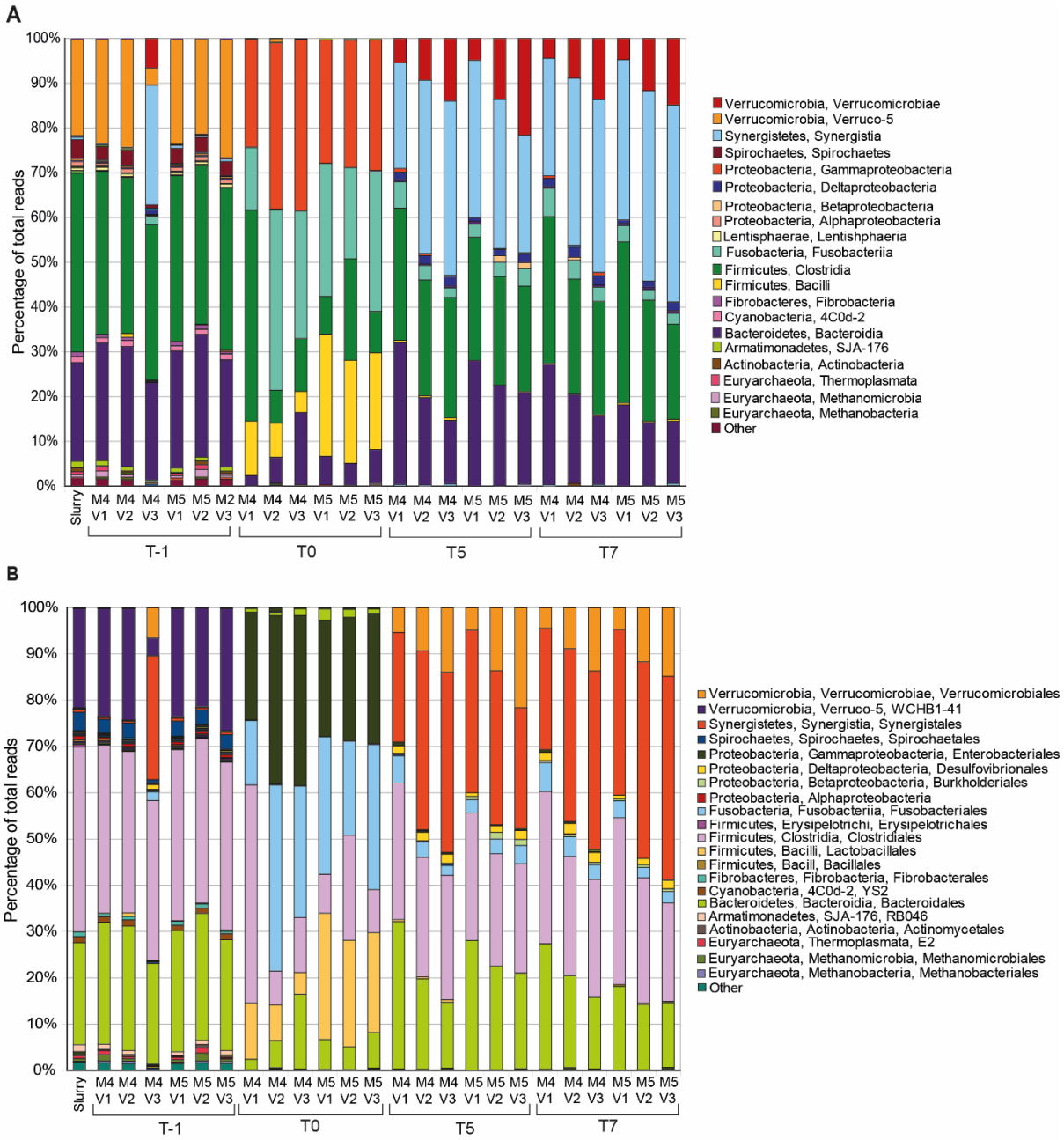

Figure S6: Relative abundance of bacterial A) classes and B) orders identified in samples from all vessels the two gut models of the repeatability study (inoculated with feces from the same horse).C, cecum; RVC, right ventral colon; LVC, left ventral colon; V, vessel; T, turnover; M model.

Table S1: Information on horses sampled for gastrointestinal contents *post*-mortem and faeces *pre*-mortem.

Horse number	GI content taken	Diet	Age	Gender	Breed	Method of Euthanasia	Analysis samples used for
Horse 1	Cecum, right ventral colon, left ventral colon, and faeces	Haylage, readigrass, pasture nuts and at pasture	14 years old	Gelding	Warmblood	Somulose injection due to musculoskeletal problems	Fibre and starch analysis
Horse 2	Cecum, right ventral colon, left ventral colon, and faeces	Hay and at pasture	18 years old	Gelding	Warmblood	Somulose injection due to chronic lameness	Fibre and starch analysis
Horse 3	Cecum, right ventral colon, left ventral colon, and faeces	At pasture	22 years old	Gelding	Warmblood	Shot due to chronic lameness/laminitis	Fibre and starch analysis
Horse 4	Cecum, right ventral colon, left ventral colon, and faeces	At pasture	18 years old	Gelding	Tb x	Shot due to persistent lameness	Concordance study (M1)
Horse 5	Cecum, right ventral colon, left ventral colon, and faeces	At pasture	5 years old	Gelding	Tb x	Shot due to behavioural issues	Concordance study (M2)
Horse 6	Cecum, right ventral colon, left ventral colon, and faeces	At pasture	13 years old	Gelding	Tb x	Shot with sedation due to persistent lameness	Concordance study (M3)
Horse 7	Fresh faeces sampled gut models	At pasture and supplemented with alfafa and sugabeet.	12 years old	Mare	Paso fino	None – sampled *pre-mortem*	Repeatability study (M4 and M5)

Table S2: R^2 values for the regression models built with the SCFA/BCFA concentrations and counts for the prominent bacterial phyla, as an average of the three concordance study gut models. Where $R^2 > 0.5$ a *p* value showing the significance of this relationship is also stated below the R^2 value.

	Acetate	Propionate	Butyrate	Valerate	Isobutyrate
Firmicutes	V1 = 0.98 *p* = 0.09 V2 = 0.88 *p* = 0.23 V3 = 0.76 *p* = 0.33	V1 = 0.57 *p* = 0.45 V2 = 0.72 *p* = 0.36 V3 = 0.26	V1 = 0.64 *p* = 0.41 V2 = 0.13 V3 = 0.10	V1 = 0.26 V2 = 0.04 V3 = 0.02	V1 = 0.36 V2 = 0.05 V3 = 0.02
Bacteroidetes	V1 = 0.06 V2 < 0.01 V3 = 0.04	V1 = 0.54 *p* = 0.47 V2 = 0.04 V3 = 0.44	V1 = 0.48 V2 = 0.59 *p* = 0.44 V3 = 0.61 *p* = 0.43	V1 = 0.84 *p* = 0.26 V2 = 0.70 *p* = 0.37 V3 = 0.82 *p* = 0.28	V1 = 0.75 *p* = 0.34 V2 = 0.69 *p* = 0.37 V3 = 0.82 *p* = 0.28
Proteobacteria	V1 = 0.34 V2 = 0.61 *p* = 0.43 V3 = 0.39	V1 < 0.01 V2 = 0.41 V3 = 0.02	V1 = 0.01 V2 < 0.01 V3 < 0.01	V1 = 0.07 V2 = 0.01 V3 = 0.07	V1 = 0.03 V2 < 0.01 V3 = 0.06
Fusobacteria	V1 = 0.99 *p* < 0.01 V2 = 0.37 V3 = 0.21	V1 = 0.70 *p* = 0.37 V2 = 0.19 V3 < 0.01	V1 = 0.76 *p* = 0.33 V2 = 0.05 V3 = 0.04	V1 = 0.38 V2 = 0.11 V3 = 0.19	V1 = 0.49 V2 = 0.10 V3 = 0.18
Synegistetes	V1 = 0.47 V2 = 0.29 V3 = 0.34	V1 = 0.94 *p* = 0.16 V2 = 0.49 V3 = 0.83 *p* = 0.27	V1 = 0.90 *p* = 0.20 V2 = 0.99 *p* = 0.08 V3 = 0.94 *p* = 0.16	V1 = 0.99 *p* = 0.05 V2 = 0.99 *p* < 0.01 V3 = 0.99 *p* < 0.01	V1 = 0.99 *p* = 0.02 V2 = 0.99 *p* = 0.11 V3 = 0.99 *p* = 0.01