

Exploring the link between ruminal methane production and physiological changes in Japanese Black cattle during fattening

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Supplementary Table S1. Significant differences in rumen microbial MetaCyc pathway between the HME and LME groups identified using mixed model analysis (P <0.05).

MetaCyc Pathway (%)	HME	LME	Fold Change	SEM	P-value
GLYCOGENSYNTH-PWY	0.83	0.89	-1.08	0.009	<0.001
PWY-5505	0.54	0.68	-1.27	0.020	<0.001
PWY-7431	0.005	0.015	-3.20	0.001	0.001
P42-PWY	0.68	0.57	1.20	0.016	0.002
P261-PWY	0.03	0.02	1.71	0.002	0.002
ANAGLYCOLYSIS-PWY	0.88	0.92	-1.05	0.006	0.002
DAPLYSINESYN-PWY	0.64	0.71	-1.11	0.010	0.003
THISYN-PWY	0.63	0.52	1.21	0.017	0.003
ARO-PWY	0.82	0.87	-1.05	0.007	0.003
ARGSYNBSUB-PWY	0.61	0.69	-1.12	0.011	0.003
COMPLETE-ARO-PWY	0.87	0.91	-1.05	0.007	0.003
PWY-5154	0.50	0.40	1.25	0.015	0.003
PWY-6467	0.37	0.28	1.35	0.015	0.004
PWY0-1319	0.85	0.90	-1.05	0.007	0.004
PWY-5667	0.85	0.90	-1.05	0.007	0.004
PWY-1269	0.42	0.33	1.27	0.014	0.004
GLUTORN-PWY	0.58	0.64	-1.11	0.009	0.004
RHAMCAT-PWY	0.35	0.25	1.42	0.016	0.004
PWY-7323	0.70	0.57	1.22	0.020	0.004
ARGSYN-PWY	0.64	0.69	-1.08	0.008	0.005
NAGLIPASYN-PWY	0.41	0.31	1.34	0.017	0.005
PWY-7400	0.64	0.69	-1.07	0.008	0.006
PWY-5695	0.81	0.74	1.10	0.012	0.006
SER-GLYSYN-PWY	0.95	0.88	1.08	0.011	0.006
PWY-5100	0.86	0.91	-1.06	0.010	0.006
PWY-6749	0.02	0.03	-1.82	0.002	0.006
PWY-7663	0.82	0.90	-1.10	0.013	0.006
P161-PWY	0.45	0.54	-1.18	0.014	0.007
PWY-5973	0.84	0.92	-1.09	0.013	0.007
PHOSLIPSYN-PWY	0.82	0.85	-1.05	0.007	0.007
POLYISOPRENSYN-PWY	0.61	0.52	1.17	0.015	0.008
COLANSYN-PWY	0.54	0.48	1.12	0.010	0.009
P441-PWY	0.23	0.28	-1.20	0.008	0.009
FERMENTATION-PWY	0.23	0.30	-1.29	0.012	0.010

Supplementary Table S2. Significant differences in methane emissions, rumen fermentation characteristics, blood metabolites, and amino acid levels between the HME and LME groups identified using mixed model analysis (P <0.05).

Parameter	HME	LME	Fold Change	SEM	P-value
Rumen fermentation					
CH ₄ (L/day)	273.80	214.41	1.28	6.58	<0.001
CH ₄ /DMI (L/day)	37.20	28.84	1.29	1.01	<0.001
Ammonia (mg/dL)	14.55	10.39	1.40	0.79	0.002
C ₂ /C ₃	2.81	2.38	1.18	0.09	0.009
C ₃ (%)	21.85	25.60	-1.17	0.73	0.011
C ₄ (%)	14.78	11.58	1.28	0.53	0.014
Blood metabolites					
BHBA (μmol/L)	696.44	465.78	1.50	32.51	0.007
Total ketone body (μmol/L)	716.17	487.44	1.47	36.01	0.014
ALP (U/L)	234.06	273.67	-1.17	11.86	0.049
Blood amino acids (nmol/mL)					
Valine	227.18	282.91	-1.25	7.88	0.002
Phenylalanine	60.22	69.01	-1.15	1.30	0.003
Threonine	62.43	74.48	-1.19	2.08	0.005
Leucine	134.12	163.60	-1.22	4.90	0.009
Lysine	105.49	120.96	-1.15	2.81	0.010
Serine	77.44	89.57	-1.16	1.90	0.011
Tyrosine	56.11	64.18	-1.14	1.63	0.014
Histidine	49.24	53.49	-1.09	0.74	0.016
Isoleucine	106.04	124.12	-1.17	3.59	0.020
Methionine	30.06	33.18	-1.10	0.79	0.031
Ornithine	57.67	66.77	-1.16	2.09	0.031

DMI, dry matter intake; Emmean, estimated marginal means; HME, group of high methane-emission cattle; LME, group of low methane-emission cattle; SEM, standard error of the mean; C₂/C₃, acetate and propionate ratio; C₃, propionate; C₄, butyrate; BHBA, beta-hydroxybutyric acid; ALP, alkaline phosphatase.

Supplementary Table S3. Top 50 differentially expressed hepatic genes between the HME and LME groups identified using mixed model analysis (P <0.05).

Hepatic genes	HME	LME	Fold Change	SEM	P-value
GSTP1	2778.90	5071.03	-1.82	347.18	< 0.001
IGDCC4	111.47	53.78	2.07	8.68	< 0.001
RHOJ	1745.05	1266.23	1.38	68.78	< 0.001
RAB15	13.91	25.67	-1.85	2.17	0.0011
PTRH2	386.66	467.42	-1.21	21.13	0.0011
TMSB4X	15247.32	19512.26	-1.28	1136.08	0.0014
CCDC88B	115.16	175.40	-1.52	13.53	0.0015
DUSP8	50.65	131.91	-2.60	12.61	0.0016
OSCP1	120.94	82.37	1.47	5.30	0.0018
RASGRP2	300.39	446.90	-1.49	27.98	0.0018
PLLP	877.92	707.85	1.24	25.53	0.0023
SGPL1	6444.18	4557.28	1.41	261.39	0.0023
ATMIN	1678.68	1362.35	1.23	51.43	0.0023
IFI27L2	252.51	372.85	-1.48	20.39	0.0024
SYT7	161.50	283.12	-1.75	21.40	0.0024
IGSF5	1599.75	1323.77	1.21	41.50	0.0025
TG	27.99	54.29	-1.94	3.79	0.0025
C1orf53	236.26	352.65	-1.49	23.99	0.0028
ASCL1	219.98	109.73	2.00	23.29	0.0029
ARPC4	2139.97	2439.58	-1.14	57.92	0.0029
FLYWCH2	321.09	442.83	-1.38	20.52	0.0030
CREG1	19189.22	12743.34	1.51	1126.67	0.0031
CKB	5320.66	8829.93	-1.66	566.50	0.0034
IFI27	19092.79	7382.84	2.59	2810.50	0.0034
CD5L	19263.24	23680.73	-1.23	669.19	0.0036
GTSE1	23.75	46.78	-1.97	3.99	0.0037
ARHGAP30	505.84	619.24	-1.22	18.67	0.0037
RNF6	445.59	336.13	1.33	21.75	0.0038
MYO1F	560.38	694.34	-1.24	23.89	0.0040
MKX	43.69	15.16	2.88	3.99	0.0042
NHSL1	1221.35	929.41	1.31	49.44	0.0043
NUP210	2137.89	2789.49	-1.30	122.74	0.0044
BCOR	544.49	439.96	1.24	22.73	0.0044

PKMYT1	26.03	38.58	-1.48	1.87	0.0045
KLF11	2317.68	1819.49	1.27	80.81	0.0046
CMTM7	295.06	385.52	-1.31	20.77	0.0047
CPQ	789.64	514.51	1.53	36.47	0.0049
ACOT8	939.18	1218.44	-1.30	45.14	0.0049
CSTF1	747.75	624.29	1.20	22.51	0.0050
PSMF1	3695.72	3059.11	1.21	140.65	0.0051
RASGEF1B	2100.43	1575.98	1.33	88.52	0.0051
GDF10	44.92	65.17	-1.45	3.81	0.0051
ABCB9	47.47	81.02	-1.71	5.69	0.0052
ANXA9	363.78	492.34	-1.35	24.67	0.0054
HAPLN3	453.37	295.94	1.53	36.39	0.0054
PPP1R7	636.88	828.09	-1.30	35.64	0.0054
SLC1A1	1874.81	1138.51	1.65	139.78	0.0055
NUF2	31.49	58.55	-1.86	5.34	0.0056
ADGRA3	1750.76	1512.89	1.16	66.89	0.0057
KNR1	188667.49	222669.81	-1.18	5298.12	0.0059

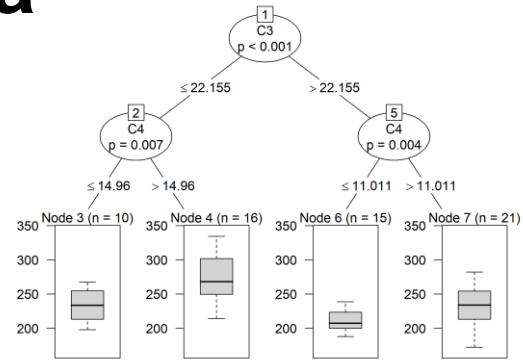
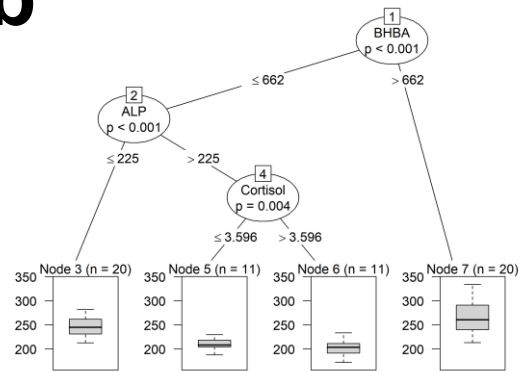
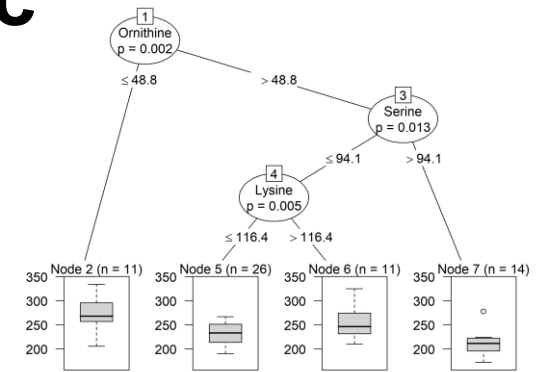
Supplementary Table S4. Absolute, estimated and predicted CH₄ and RME of individual cattles and group classification based on RME.

DMI, dry matter intake; RME, residual methane emission; HME, high methane emission group (n=6); LME, low methane emission group (n=6)

Cattle number	Absolute CH ₄ (L/d)			Estimated CH ₄ (L/d)	Estimated DMI (kg/d)	Daily gain ¹	Predicted CH ₄ (L/d)	RME ²	Group classification
	T1	T2	T3						
5032	203.6	172.2	203.4	205.9	7.38	0.805	240.1	-34.2	LME
5713	197.7	191.8	212.3	211.1	7.27	0.679	239	-27.8	LME
477	201.2	223.5	217.9	228.4	7.97	0.788	246	-17.6	LME
1116	222.8	187.8	228.7	220.5	7	0.832	236.2	-15.7	LME
943	246.4	229.6	190.1	226.7	7.26	0.564	238.9	-12.2	-
755	213.9	210.3	233.5	226.5	7.18	0.736	238	-11.5	LME
1094	259.9	224.4	231.0	237.4	7.97	0.746	246	-8.5	LME
2277	240.6	210.8	254.2	235.2	7.62	0.838	242.5	-7.3	-
452	240.2	208.3	191.1	220.5	6.04	0.645	226.6	-6	-
5935	229.5	229.6	238.5	231.9	7.15	0.777	237.7	-5.8	-
444	213.1	256.1	196.0	225.8	6.45	0.598	230.7	-4.9	-
4352	231.9	206.9	261.5	236.4	6.99	0.7	236.1	0.2	-
3676	205.5	206.6	309.3	239.5	7.13	0.752	237.5	2	-
4393	268.0	212.9	245.7	241.1	7.15	0.661	237.8	3.3	-
449	261.1	220.3	251.5	243.9	7.19	0.709	238.1	5.8	-
1292	254.4	244.6	278.1	251.8	7.84	0.845	244.7	7.1	HME
1614	262.7	267.3	234.1	249.4	6.91	0.636	235.3	14.1	HME
2271	288.2	263.3	241.8	257.9	7.3	0.782	239.2	18.7	HME
5521	276.5	267.4	282.1	263.8	6.97	0.723	235.9	27.8	HME
1167	234.6	325.0	334.2	278.8	7.83	0.75	244.5	34.3	HME
789	252.9	294.0	327.0	276.6	7.22	0.77	238.5	38.1	HME

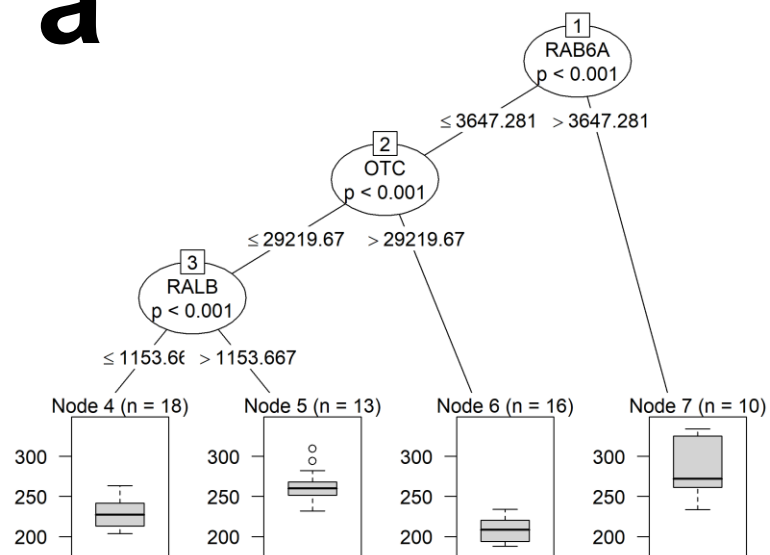
¹ Cattle numbers 943, 452, and 444 were excluded from group classification as their data points fell below the threshold of one standard deviation below the mean ($\mu - 1\sigma$).

²RME = Predicted CH₄ - Estimated CH₄.

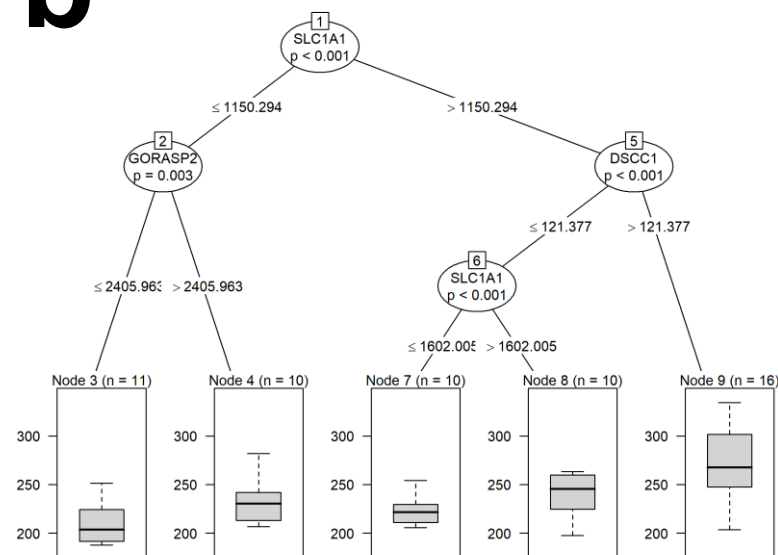
a**b****c**

Supplementary Figure 1. Linear mixed-effect model regression trees for methane emission (L/day), using (a) rumen fermentation characteristics, (b) blood metabolites, and (c) blood amino acids as independent variables. C₃, propionate; C₄, butyrate; BHBA, beta-hydroxybutyric acid; ALP, alkaline phosphatase.

a



b



Supplementary Figure 2. Linear mixed-effect model regression trees for methane emission (L/day). Methane emission (L/day) was used as a dependent variable and hepatic genes **(a)** selected using mixed effect model analysis and **(b)** those with 100% prevalence were used as independent variables. DSCC1, DNA replication and sister chromatid cohesion 1; GORASP2, golgi reassembly-stacking protein 2; OTC, ornithine transcarbamylase; RAB6A, Ras-related protein Rab-6A; RALB, Ras-related protein Ral-B; SLC1A1, solute carrier family 1 member 1 (glutamate transporter)