Supplementary Information

NMR-based metabolomics for simultaneously evaluating multiple determinants of primary beef quality in Japanese Black cattle

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Supplementary Table S1. ^{1}H and ^{13}C signal assignments of the compounds detected in D_2O extracts

Compound	Abbreviation	Assignment	Chemical shift (ppm)	
			^{1}H (multiplicity)	^{13}C
Amino acids				
alanine	Ala	α-**CH**	3.77	53.30
		β-**CH$_3$**	1.48 (d) *	18.89
		COOH		178.60
anserine	Ans	**CH**-2, ring	8.23	139.13
		C-4, ring		133.13
		CH-5, ring	7.09 (s)	123.27
		N–**CH$_3$**	3.77 (s)	35.93
carnitine		α-**CH$_2$**	2.42	45.72
		β-**CH**	4.57	66.70
		γ-**CH$_2$**	3.42	72.79
		N(**CH$_3$**)$_3$	3.21 (s) *	56.77
		COOH		180.40
carnosine	Car	β-alanyl α-C**H$_2$**	2.67 (m) *	
		β-alanyl β-C**H$_2$**	3.21	
		Histidyl α-C**H**	4.47 (m)	
		Histidyl β-**CH$_2$**	3.09	29.99
		CH-2, ring	8.18 (s)	137.08
		C-4, ring		133.95
		CH-5, ring	7.11 (s)	119.85
		COOH		179.35
		CONH		174.42
creatine	Cr	α-**CH$_2$**	3.92 (s)	56.57
		N–**CH$_3$**	3.03 (s) *	39.65
		COOH		177.43
		NH=**C**(NH$_2$) –N		159.89
glutamic acid	Glu	β-**CH$_2$**	2.05 (m)	29.70
		γ-**CH$_2$**	2.35 (m) *	36.07
		α-CH$_2$–**C**OOH		177.19
		γ-CH$_2$–**C**OOH		183.81
glutamine	Gln	β-C**H$_2$**	2.13 (m)	
		γ-C**H$_2$**	2.45 (m) *	
		COOH		176.74
		CONH$_2$		180.40

(to be continued)

Supplementary Table S1 (continued)

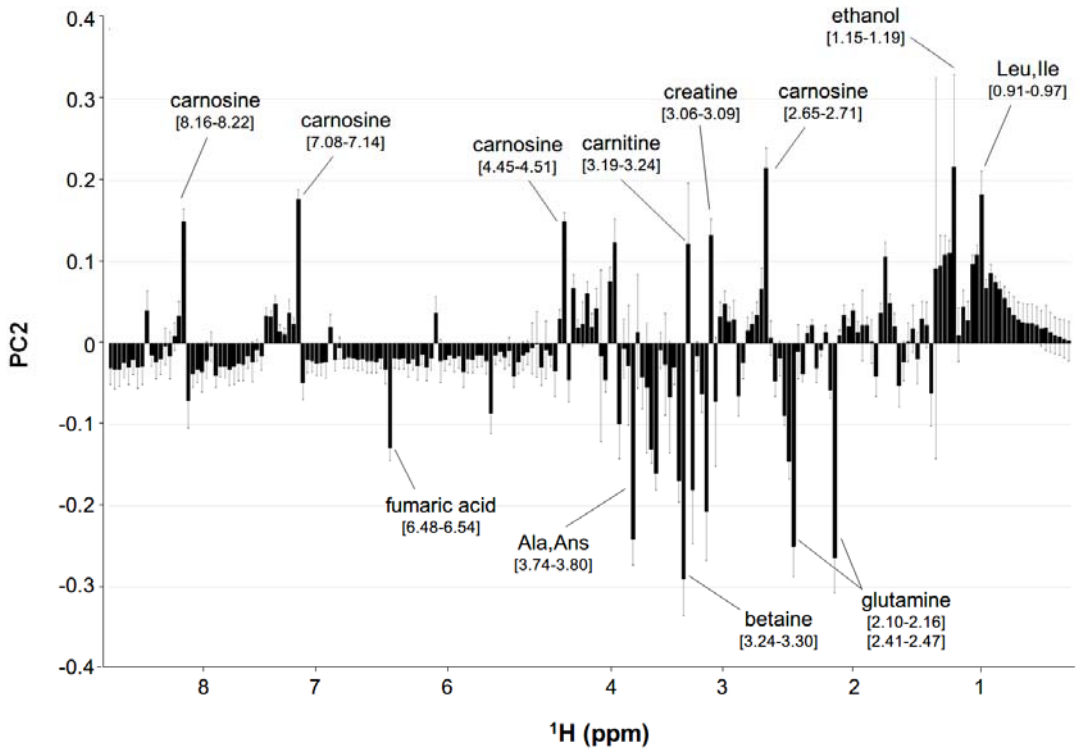
Compound	Abbreviation	Assignment	Chemical shift (ppm)	
			^{1}H (multiplicity)	^{13}C
glycine	Gly	α-**CH$_2$**	3.55	44.09
		COOH		175.17
isoleucine	Ile	α-**CH**	3.66	62.42
		β-**CH**	1.97	38.69
		γ-**C**H$_2$		27.22
		γ'-C**H$_3$**	1.00 (d) *****	
		δ-**CH$_3$**	0.93	13.59
		COOH		177.43
leucine	Leu	α-**CH**	3.73	56.17
		β-**CH$_2$**	1.70 (m) *****	42.59
		γ-**CH**	1.69 (m) *****	26.96
		δ-**CH$_3$**	0.94	23.68
		δ'-**CH$_3$**	0.96	24.78
		COOH		178.35
phenylalanine	Phe	β-C**H$_2$**	3.12, 3.27	
		C-1, ring		137.90
		CH-2,6, ring	7.32 (d) *****	131.78
		CH-3,5, ring	7.42 (dd)	131.65
		CH-4, ring	7.38 (t)	130.28
tyrosine	Tyr	**C**-1		129.61
		CH-2,6, ring	7.18 (d)	133.50
		CH-3,5, ring	6.88 (d) *****	118.55
		CH-4		157.91
valine	Val	α-**CH**	3.60 (d) *****	63.04
		β-**CH**	2.27	31.98
		γ-**CH$_3$**	0.97 (d)	19.42
		γ'-**CH$_3$**	1.03 (d)	20.50
Sugars				
α-glucose	α-Glc	**CH**-1	5.23 (d) *****	94.67
		CH-2	3.53	74.04
		CH-3	3.71	75.30
		CH-4	3.40	72.29
		CH-5	3.83	74.06
		CH$_2$-6	3.77, 3.84	63.24

(to be continued)

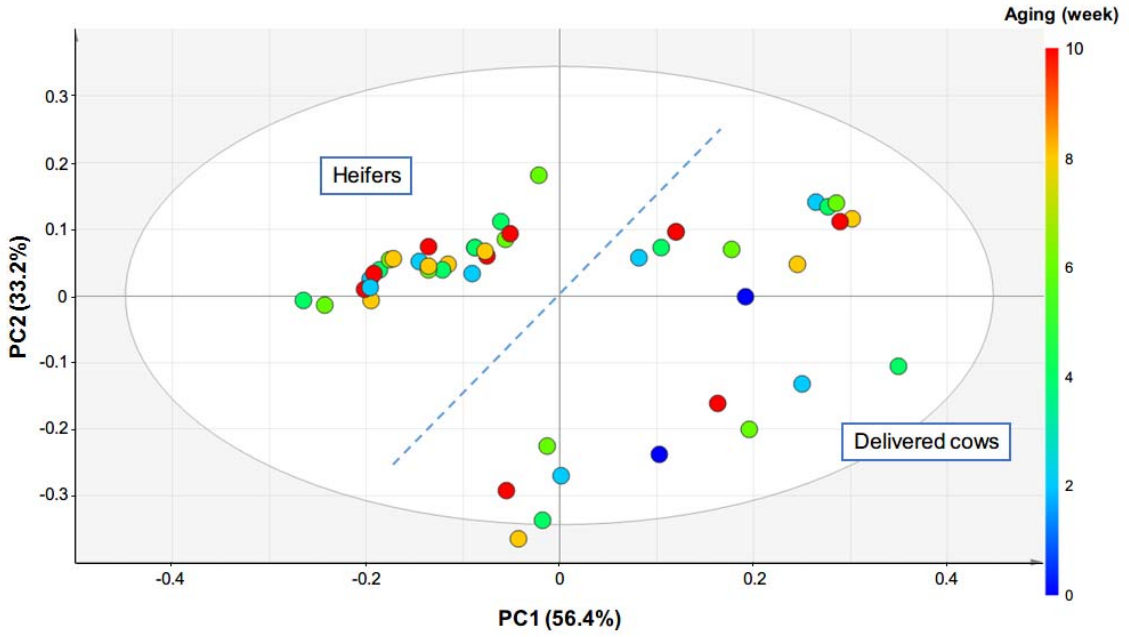
Supplementary Table S1 (continued)

Compound	Abbreviation	Assignment	Chemical shift (ppm)	
			^{1}H (multiplicity)	^{13}C
β-glucose	β-Glc	**CH**-1	4.64 (d) *	98.57
		CH-2	3.24	76.84
		CH-3	3.47	72.29
		CH-4	3.40	72.29
		CH-5	3.47	78.55
		CH$_2$-6	3.72, 3.90	63.42
Purine derivatives				
inosine	HxR	**CH**-1'	6.09 (d) *	91.12
		C**H**-2	8.23	
		CH-8	8.34 (s)	142.88
inosine monophosphate	IMP	C**H**-1'	6.14 (d) *	
		C**H**-2	8.22	
		C**H**-8	8.58 (s)	
hypoxanthine	Hx	C**H**-2	8.2 (s)	
		C**H**-8	8.19	
Organic acids				
acetic acid	AcOH	**CH$_3$**	1.93 (s) *	25.53
		COOH		183.81
fumaric acid		C**H**=C**H**	6.51 (s) *	
		COOH		177.38
lactic acid		α-**CH**	4.11 (q)	71.23
		β-**CH$_3$**	1.32 (d) *	22.90
		COOH		185.28
Alcohol				
ethanol	EtOH	**CH$_3$**	1.17 (t) *	19.45
		CH$_2$	3.65	60.13
glycerol		**CH$_2$**-1	3.55 (m)	65.16
		CH-2	3.78 (m)	74.74
		CH$_2$-3	3.66 (m) *	65.16
Other compounds				
betaine		N(**CH$_3$**)$_3$	3.25 (s) *	56.17
		CH$_2$	3.90 (s)	68.93
		COOH		172.02
choline		N(**CH$_3$**)$_3$	3.19 (s)	56.45

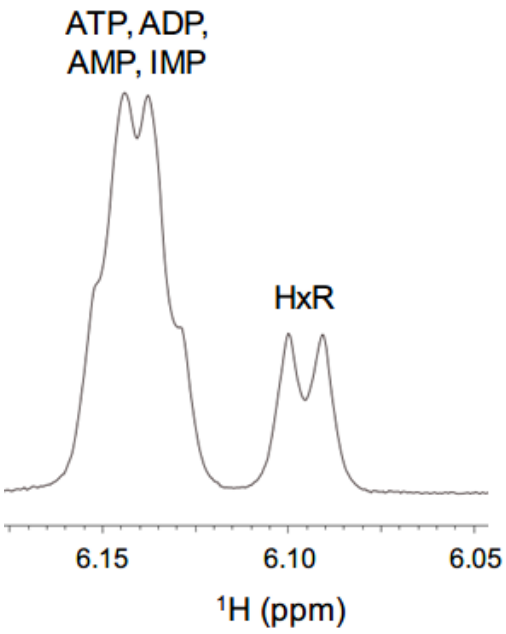
*Proton signals were chosen for the quantitative analysis.



Supplementary Figure S1. Loading plot for PC2 of the score plot that was generated using the ^{1}H NMR spectra of the D_2O extracts of ribeye samples with different aging duration. The PCA score plot is shown in Fig. 4A. Buckets with high loading values are labeled by the assigned compound names. The square brackets represent the chemical shift range of each spectral bucket.



Supplementary Figure S2. PCA score plot of the $CDCl_3$ extracts of ribulose samples with different aging duration (0, 2, 4, 6, 8 and 10 weeks). Ribulose samples are derived from heifers and delivered cows. The heifer samples are separated from the delivered cow in the score plot, and the individual regions are divided by a dashed line.



Supplementary Figure S3. ^{1}H NMR spectrum of an equimolar mixture of nucleotides (ATP, ADP, AMP and IMP) and HxR. The signals observed near 6.10 and 6.15 ppm were derived from the ^{1}H attached to the 1' position of the ribose ring. The nucleotides signals overlapped.