

Supplementary Material

Table S1. ^1H NMR chemical shifts (δ) and multiplicity of metabolites in bovine serum run in deuterated water (D_2O). Clearly observed resonances are indicated in bold text. s, singlet; d, doublet; dd, doublet of a doublet; m, multiplet; t, triplet. The right two columns show the direction of the relationship with serum β -hydroxybutyrate (BHBA) and non-esterified fatty acid (NEFA) concentrations determined by colorimetric assays. * = tentative identification.

	Metabolite	Chemical shift (δ) and multiplicity	BHBA	NEFA
1	cholate*	0.70 (m) , 0.91 (m), 0.96 (m), 1.43 (m), 1.87 (m), 2.10 (m), 2.22 (m), 3.65 (m), 4.06 (t)	-	↓
2	LDL/VLDL	0.86 (m) , 1.25 (m)	↑	↑
3	leucine	0.94 (d) , 0.95 (d) , 1.66 (m), 1.66 (m), 1.73 (m), 3.72 (m)	-	↑
4	isoleucine	0.93 (t) , 1.00 (d) , 1.24 (m), 1.45 (m), 1.45 (m), 3.66 (d)	-	↓
5	valine	0.98 (d) , 1.03 (d) , 2.26 (m), 3.60 (d)	↓	↓
6	β -hydroxybutyrate	1.20 (d) , 2.31 (m) , 2.41 (m) , 4.16 (m)	↑	↑
7	lactate	1.31 (d) , 4.31 (q)	↓	↓
8	alanine	1.46 (d) , 3.77 (q)	↓	↓
9	acetate	1.9 (s)	↑	↓
10	N-acetyl glycoprotein	2.03 (m)	↑	↑
11	pyruvate	2.46 (s) , 7.65 (s)	-	↓
12	citrate	2.52 (d) , 2.66 (d)	-	-
13	creatine	3.02 (s) , 3.92 (s)	↑	↓
14	phosphocreatine	3.03 (s) , 3.93 (s)	-	-
15	dimethyl sulfone	3.14 (s)	↓	-
16	choline	3.19 (s) , 3.50 (m), 4.05 (m)	-	-
17	phosphocholine	3.21 (s) , 3.58 (t), 4.17 (m)	↑	↑
18	betaine	3.25 (s) , 3.89 (s)	↑	-
19	methanol	3.34 (s)		
20	glucose	3.23 (dd) , 3.40 (m) , 3.46 (m) , 3.52 (dd) , 3.73 (m) , 3.82 (m) , 3.89 (dd) , 4.63 (d) , 5.22 (d)	↓	↓
21	glycine	3.50 (s)	↑	↑
22	β -glucose	4.63 (d)	↓	↓
23	α -glucose	5.22 (d)	↓	↓
24	3-phenyllactate*	2.87 (dd) , 3.09 (dd), 4.26 (dd), 7.31 (m) , 7.39 (m)	-	-
25	hippurate	3.96 (d), 7.54 (m) , 7.62 (m) , 7.83 (dd)	-	-
26	formate	8.44 (s)	-	-

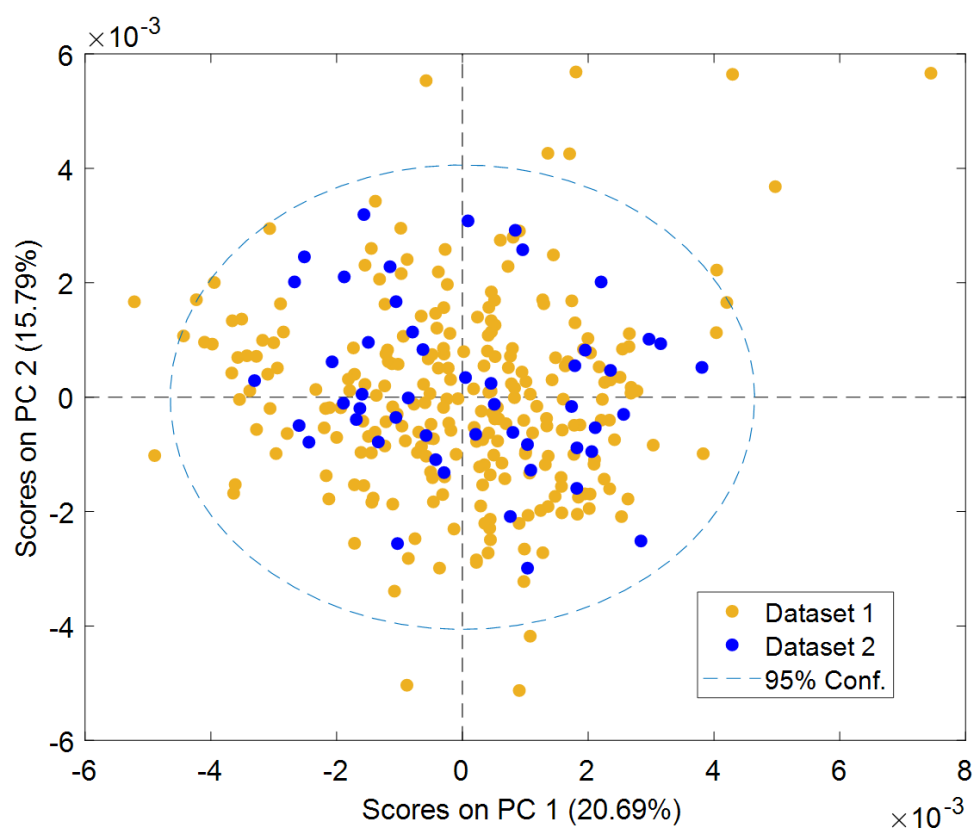
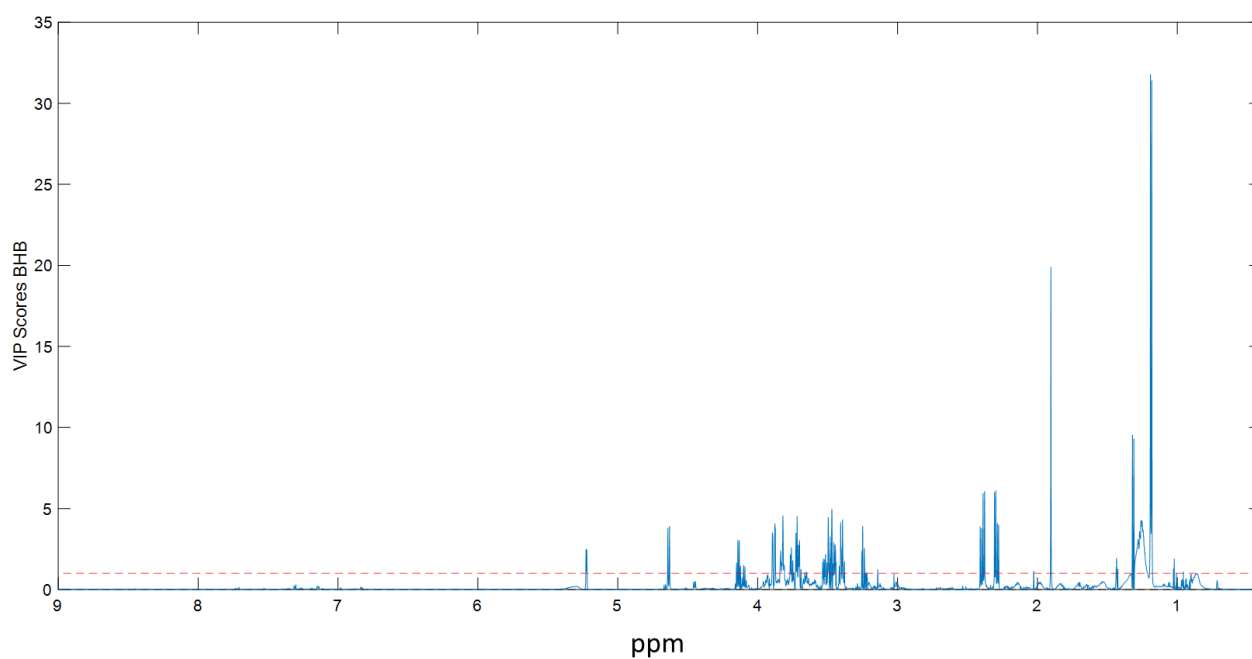


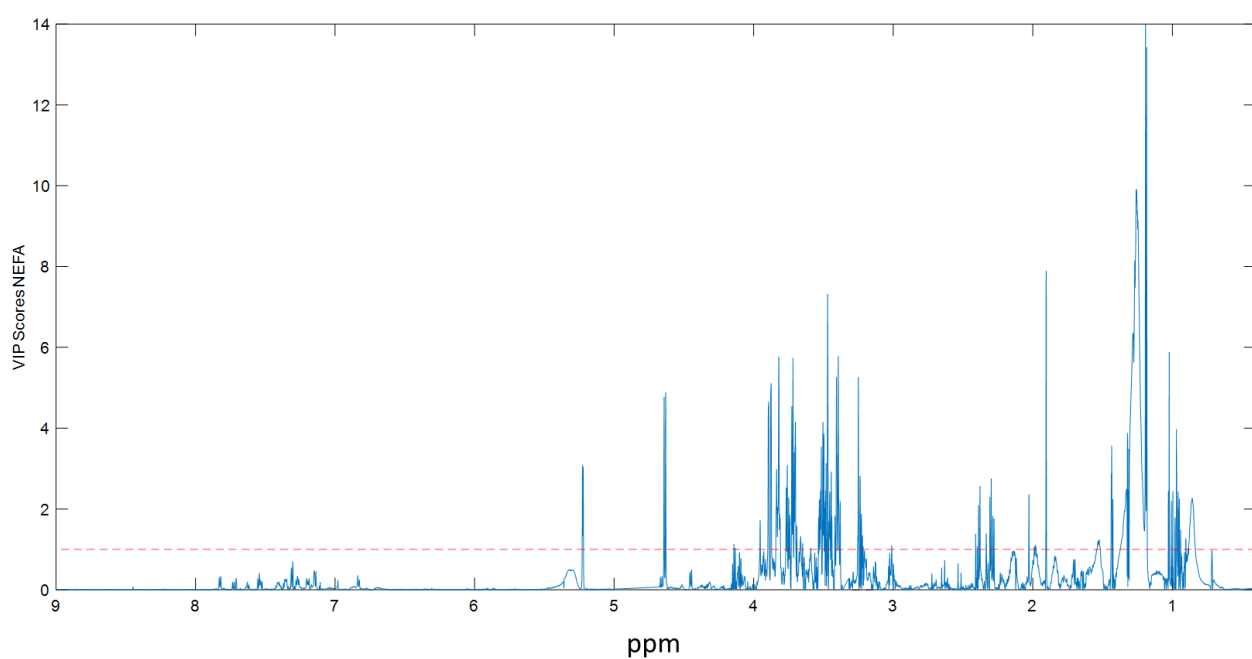
Figure S1. Results of PCA of ^1H NMR spectra of serum obtained from 298 dairy cows in early lactation from the Ellinbank research farm (Dataset 1, $N = 248$) and a commercial dairy farm in Tasmania (Dataset 2, $N = 50$).

Table S2. Results of ANOVA-simultaneous component analysis (ASCA) of ^1H NMR spectra of bovine serum ($N = 298$). Effect describes the relative influence of each variable (Herd, Age and days in milk (DIM)) on each spectra. P-value is derived from permutation testing (1000 iterations).

Variable	PCs	Effect	P-Value
Herd	1	0.47	0.33
Age	10	4.72	0.04
DIM	20	10.17	0.09
Residuals	-	86.06	-



(a)



(b)

Figure S2. Variable importance of projection (VIP) scores derived from orthogonal partial least squares (OPLS) regression of ^1H NMR spectra of serum obtained from 298 dairy cows in early lactation, against (a) BHBA concentration and (b) NEFA concentration.