Metabolomics of the Mammary Gland related to Milk Production.

Supporting Information

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Supplementary Table S1. The milk metabolites detected either by Liquid chromatography–mass spectrometry (LC-MS), or by nuclear magnetic resonance (NMR), or by both.

No.	Milk	Function	Detection by		Integrated region in NMR	C.V.
1	Acetyl-carnitine	Lipid metabolism	LC-MS	NMR	2.149-2.146; 3.200-3.189	
2	Acetate	Energy metabolism		NMR	1.931-1.919	
3	Acetone	Lipid Metabolism		NMR	2.243-2.234	
4	Acetylcholine	Energy metabolism	LC-MS			34
5	cis-Aconitate	Energy metabolism		NMR	5.729-5.709	
6	Alanine	AAs metabolism		NMR	1.500-1.474	
7	Arginine	AAs metabolism /Urea Cycle	LC-MS			23
8	Aspartate	AAs metabolism	LC-MS			18
9	β-hydroxybutyrate	Lipid metabolism		NMR	1.203-1.194; 1.192-1.186	
10	Butyrate	Lipid metabolism		NMR	2.167-2.160; 2.154-2.150; 1.588-1.538	
11	Carnitine	Lipid metabolism	LC-MS			20
12	Choline	Lipid metabolism	LC-MS	NMR	3.210-3.200	
13	Citrate	Energy/Lipid metabolism		NMR	2.722-2.641; 2.581-2.508	
14	Citrulline	AAs metabolism/Urea cycle	LC-MS			12
15	Creatine	Protein degradation		NMR	3.044-3.034	
16	Creatinine	Protein degradation		NMR	3.049-3.044	
17	CMP	Nucleotide metabolism	LC-MS	NMR	7.986-7.957; 6.007-5.983	
18	Ethanolamine	Lipid metabolism		NMR	3.145-3.139	
19	Fucose	Lipid metabolism		NMR	1.250-1.242	
20	Formate	Energy metabolism		NMR	8.467-8.452	
21	Fumarate	Energy metabolism		NMR	6.538-6.517	
22	Galactose-1-P	Lactose synthesis		NMR	5.525-5.486	
23	Glucose-1-P	Lactose synthesis		NMR	5.475-5.447	
24	Glutamate	AAs metabolism	LC-MS	NMR	2.145-2.142; 2.376-2.338	
25	Glutamine	AAs metabolism	LC-MS			20
26	Glycine	AAs metabolism	LC-MS			17
27	GPC	Lipid metabolism		NMR	3.242-3.227	

28	Hippurate	AAs/Energy metabolism		NMR	7.860-7.822; 7.666-7.623; 7.585-7.533	
29	Hydroxyproline	AAs metabolism	LC-MS			17
30	α-Ketoglutarate	Energy metabolism	LC-MS	NMR	3.027-2.999	
31	Kynurenine	AAs metabolism	LC-MS			24
32	Lactose	Lactose synthesis		NMR	5.263-5.219; 4.507-4.415; 3.185-3.171	
33	Lactate	Energy Metabolism		NMR	1.346-1.321	
34	Leucine	AAs metabolism	LC-MS			16
35	Lysine	AAs metabolism	LC-MS			24
36	Met (O)	AAs metabolism	LC-MS			13
37	Nac-Gla/Nac-Glu	Lipid metabolism		NMR	5.283-5.266; 5.219-5.202; 2.067-2.054	
38	Nac-NA	Lipd-metabolism		NMR	2.049-2.039	
39	Isoleucine	AAs metabolism	LC-MS			17
40	Orotate	Nucleotide metabolism		NMR	6.208-6.189	
41	Pantothenate	Energy metabolism	LC-MS			30
42	Phosphocholine	Lipid metabolism		NMR	3.227-3.213	
43	Phenylalanine	AAs metabolism	LC-MS			14
44	Phosphocreatine	AAs metabolism		NMR	3.055-3.050	
45	Proline	AAs metabolism	LC-MS			23
46	Serine	AAs metabolism	LC-MS			15
47	TMAO	AAs metabolism		NMR	3.273-3.263	
48	UDP-Nac-Gal	Lipid metabolism		NMR	8.084-8.040; 5.417-5.389; 2.082-2.074;	
49	UDP-Nac-Glu	Lipid metabolism		NMR	8.168-8.099; 5.376-5.344; 2.074-2.066	
50	Urea	AAs metabolism/Urea Cycle		NMR	5.884-5.732	
51	Uridine	Nucleotide metabolism	LC-MS	NMR	7.893-7.863	
52	Threonine	AAs metabolism	LC-MS			22
53	Tryptophan	AAs metabolism	LC-MS			18
54	Tyrosine	AAs metabolism	LC-MS			16
55	Valine	AAs metabolism	LC-MS	NMR	1.043-1.036	

Abbreviations: CMP, cytidine monophosphate; GPC, Glycerophosphocholine; Glu-1-P, glucose-1-phosphate; Gal-1-P, galactose-1-phosphate; HYP, hydroxyproline; Met (O), methionine sulfoxide; Nac-Gal, N-acetyl-galactosamine; Nac-Glu, N-acetyl-glucosamine; Nac-NA, N-acetyl-neuraminic acid; TMAO, trimethylamine N-oxide; UDP-Nac-Gal, uridine diphosphate-N-acetyl-galactosamine; UDP-Nac-Glu, uridine diphosphate-N-acetyl-glucosamine. C.V. (LC-MS) = Coefficient of Variation in % for MS data where no NMR data are available.

The metabolites in table S1 were identified based on our own library of standards (NMR/ LC-MS) except for N-acetyl-glucosamine, N-acetyl-neuraminic acid, N-acetyl-galactosamine, uridine diphosphate-N-acetyl-galactosamine and uridine diphosphate-N-acetyl-glucosamine where the identification was based on strong correlation between resonances observed in our 87 NMR datasets, NMR splitting patterns with coupling constants, chemical shifts of the resonances and the strong identity to the resonances (chemical shifts and coupling constants) of these molecules in the HMDB database.

Table S2. The number, milk yield, body weight, and energy balance (EB) of dairy cows with two dry period length (DPL, 0-day or 30-day).

	Number	Milk yield[1]	Body weight	EB[1]
Cows with 0-day DPL	60	28.1±7.0 kg	662.5±73.0 kg	-133.4±162.8
Cows with 30-day DPL	27	32.0±6.9 kg	663.5±65.6 kg	347.6±181.9

[1] Milk yield ($P = 0.02$) and EB in kJ/kg $^{0.75}$.d ($P < 0.01$) are different between two groups (0-day DPL and 30-day DPL).