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Preliminary Metabolomic Analysis of Goat Milk from Different Breeds Using Mass Spectrometry

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***Abstract*— Metabolomics facilitates the identification of numerous metabolites in a sample with mass less than 1 kD. The purpose of this study was to determine the effect of feed on the metabolites in raw goat milk samples obtained from two breeds namely Saanen and Shami that were collected from a farm in Selangor and Negeri Sembilan, Malaysia, respectively. The types of feed given to the goats were recorded. The fat in the samples were removed via centrifugation before analysis using liquid chromatography quadrupole time of flight mass spectrometry (LC-QToF-MS). From the chromatograms obtained the presence of veterinary drug residue, di- and tri-peptides, short and long-chain fatty acids, some components from plants, and insecticide residue were detected using HMP, KEGG, LMP, and METLIN databases identification. It is expected that through metabolomics study it may be possible to formulate the feed and determine the breed that give good quality milk.**

***Keywords*— Metabolomics, goat milk, LC-QToF-MS, databases.**

I. Introduction

In the last 20 years scientists begin to realise that a simultaneous compound or metabolite identification method would assist in confirming health and physiological status of an organism. Metabolites represent a diverse group of low-molecular-weight structures including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols and carbohydrates [1]. Metabolomics aims to detect, identify, and quantify a total population of low molecular weight compounds to gain functional information in a biological system [2].

Milk is a complex biological fluid secreted for neonate nourishment and development. Milk contains water, lipids, carbohydrates, proteins, vitamins, minerals, and smaller metabolites. Changes in chemical composition of milk affect nutritional, safety, and technological properties of the milk [3]. In addition to the normal nutritional composition, goat milk contains other metabolites that have functional properties and may be unique to type of feed and breed [4], [5]. Sundekilde [6] suggested that metabolomics approach can be used to establish biomarkers in bovine milk as a diagnostic tool for determining quality and technological properties. Metabolites as biomarkers for specific biochemical pathways will indicate different physiological status of the organism [2].

In addition, coupling chromatography to mass spectrometry (MS) offers an excellent solution to complex mixture analyses and has been extensively used in metabolomics. Chromatographic separation of metabolites prior to MS analyses has several advantages: i) reduces matrix effects and ionization suppression, ii) separates isomers, iii) provides additional and orthogonal data (i.e. retention time/factor/index) valuable for metabolite annotation, and iv) allows for more accurate quantification of individual metabolites [7].

Nevertheless it has now become relatively routine to comprehensively compare the levels of thousands of metabolite peaks in one sample group to another in an untargeted manner. This approach, called untargeted metabolomics, has the potential to implicate unexpected pathways with a unique phenotype or disease process [8].

Reaves [9] coined out that one of the uses of metabolomics data is to pick out a few interesting compounds for further study. This was the further emphasized by [10], whereby he classified a high quality milk as having low somatic cell count (SCC), low standard plate count (SPC), no human pathogen, and no antibiotic residues. Hence, this preliminary effort aims at profiling the metabolites of goat milk in selected farms in some parts of Peninsular Malaysia.

II. Materials and Methods

A. Milk collection and sample preparation

The method of [11] was followed with modification. Goat milk was collected from a farm in Negeri Sembilan and Selangor and the freshly collected milk samples were immediately cooled in ice and appropriate care was observed to prevent any possible cross-contamination. The individually milked samples from different goats (n = 4) were pooled, collected in sterile bottles and transported in an icebox at temperature to the Faculty of Science and Technology, Universiti Sains Islam Malaysia (USIM), then immediately frozen at -20°C until further analysis. Aseptic techniques were applied, wherein all the equipment were pre-sterilized prior to analysis.

Goat milk was thawed and centrifuged at 3,000 x g for 10 min using centrifuge (Novil) at 4°C to remove high molecular weight compounds. The supernatant were then collected and analyses were done on the same day.

B. Instrumentation

Untargeted metabolomics profile of three goat milk samples (1 = Saanen species, 2 = Shami species) were carried out using LC-QToF-MS (LCMS iFunnel Q-TOF) at Agro Biotechnology Institute (ABI), MOSTI, MARDI, Serdang. Specifications include column type: RRHD Zorbax Eclipse Plus C18; 1.8µ x 2.1mm x 100mm with temperature of 23°C, injection volume of 1.00 µL, flow rate of 0.300 mL/min, acquisition mode applied a minimum range of 100m/z to a maximum of 1700m/z.

An Agilent G6550A Accurate Mass QTOF was used to obtain the MS data. The mobile phases used were highly purified water + 0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). The flow rate was set up at 0.6 mL/min with 30 min total run time. The prepared samples were placed into the LCMS autosampler. The injection volume of sample was 10 μL. The samples were run at gradient 5% B (0-0.5 min); 30% B (0.5-13 min); and 95% B (13–22.0 min). Analysis was performed in positive ion mode with the following settings:-capillary voltage: 3500 V; nozzle voltage: 1000 V; fragmentor voltage: 175 V; nebulizer pressure (N): 35 psi; drying gas: 5L/min at 350°C, and sheath gas: 11 L/min at 350°C. The mass range was at 100–1700 m/z.

C. Database Annotation

For compound identification, several databases were referred to including KEGG, HMDB, LMP, and METLIN that were all from Mass Hunter Qualitative analysis software, Agilent Technologies.

III. Results and Discussions

Metabolite levels, although present at low concentrations, can provide information of biochemical status in response to environments or genetic manipulation. These components can have a profound impact on the development and maintenance of metabolic, immunological and physiological processes [2], [12].

Figures 1 and 2 show the extracted compound chromatogram (ECC) of goat milk from both Saanen and Shami breeds at the end of the analysis period (30 min), respectively. The ECC profiles of goat milk were distinct with dissimilar chromatogram patterns suggestive of different breeds. Reference [2] specified that metabolomic analysis could detect changes in the metabolites as a result of genetic influence or feed given [13], [14].

The distinctive feed given to both breeds was barli sprout to Saanen, and indigofera *spp*. and palm leaves to Shami breeds. The other feed given were quite similar at any other goat farms namely napier leaves and brans.

Table I listed out the known compounds detected, from the smallest to the highest mass present in milk from Saanen (only). However, the overall mass spectrometry analysis identified compounds with masses ranging from 0.099 kD to 19.6 kD and 0.098 kD to 18.8 kD for Saanen (22117 compounds) and Shami (20679 compounds) species, respectively. The ranges above than 1 kD indicate the poor sample preparation for metabolomic analysis. The metabolites were grouped into several categories namely plant secondary metabolites, drugs, naturally occurring metabolites, environmental contaminants, microbial metabolism in diverse environments including microbial secondary metabolites, and 'others' for easy classification purposes (Table II). Some microbial metabolites were noticed due to both long cold storage and the milking management practices of the milk samples obtained as supported by [15] and [16], correspondingly. Other compounds detected were drug metabolites such as pinacidil, spiramycin 3, deschlorobenzoyl indomethacin, methotrimeprazine sulfoxide and environmental contaminants (trichlorfon). Trichlorfon is an organophasphate which has adverse effects on health [17], [18].

The list of 20 highest concentrations of known compounds detected present in milk from Saanen and Shami are shown in Table II. It was observed that although the milk samples were from different breeds, some similarities existed. Such compounds include asp-phe-arg, maltose, pantothenic acid, 3-butyrl propionic acid, GPEtn(18:1(9Z)/0:0), and, neu5Ac alpha2-6Galbeta1-4Glcbeta-Sp., all of which were categorized as naturally occurring metabolites.

In addition, [19] indicated that valine and glycine were specific to goat milk, talose and malic acid to cow milk, and hydroxyglutaric acid to pasteurized samples via a gas chromatography-mass spectrometry (GCMS). It was noted that LCMS detected amino acids in the form of di- and tripeptides compared to GCMS that can identify individual amino acids [19] envisaging the beneficial effects towards health. For lipids, by-products of metabolism of polyunsaturated fatty acids (PUFA) generated hydroxy fatty acids, oxo fatty acids, conjugated fatty acids, and partially saturated *trans*-fatty acids as intermediates [20].

IV. Conclusions

Preliminary study of metabolite profiling of milk by mass spectrometry is able to generate the high quality data needed for further qualitative analysis. Goat milk has compounds related to health benefits in the form of conjugates.

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APPENDIX

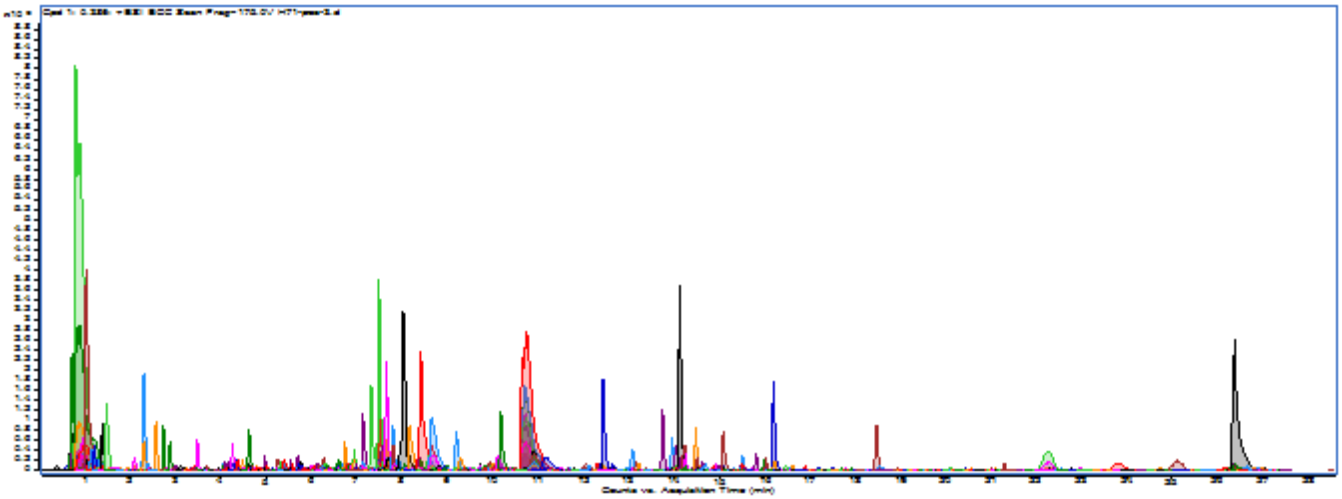

Fig. 1: Extracted Compound Chromatogram (ECC) of Goat Milk from Saanen Species.

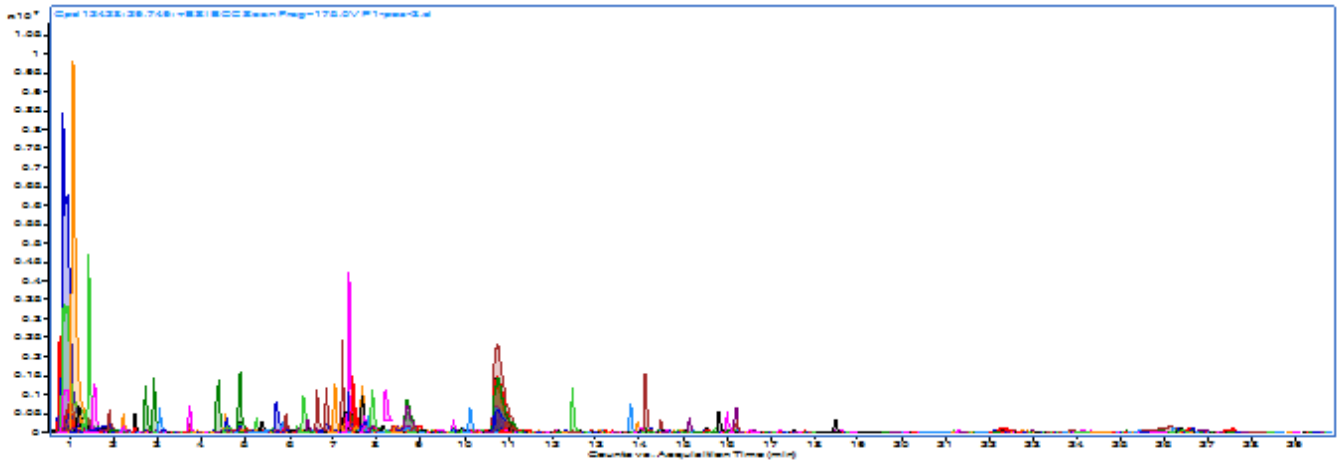

Fig. 2: Extracted Compound Chromatogram (ECC) of Goat Milk from Shami Species.

TABLE I
PARTIAL SUMMARY (QUALITATIVE) OF THE IDENTIFIED COMPOUNDS USING LC-QTOF-MS DETECTED IN MILK FROM SAANEN THE REFERRED DATABASE.

Molecular Mass (kD)	Retention Time (Min)	Putative Identification	Empirical Formula	METLIN Database
100.0891	26.314	4-Methylpentanal	C6 H12 O	6155
105.0793	14.138	3-Methylamino-1,2-propandiol	C4 H11 N O2	44718
112.0278	0.914	Uracil	C4 H4 N2 O2	258
118.0635	1.427	a-hydroxyisovalerate	C5 H10 O3	3751
120.0575	2.621	4-Hydroxystyrene	C8 H8 O	7012
122.0373	4.627	Benzoic acid	C7 H6 O2	1297
133.0739	16.218	L-O-Methylthreonine	C5 H11 N O3	44769

135.0685	3.488	N-Acetylarylamine	C8 H9 N O	6107
136.0391	1.094	Hypoxanthine	C5 H4 N4 O	83
136.0528	1.32	Phenylacetic acid	C8 H8 O2	129
139.0269	1.602	4-Nitrophenol	C6 H5 N O3	4100
140.0473	1.32	2-METHOXYRESORCINOL	C7 H8 O3	44540
141.0431	1.032	2-Aminomuconate 6-semialdehyde	C6 H7 N O3	3248
144.0786	1.272	3-butyrl propionic acid	C7 H12 O3	35708
144.0791	1.042	Hydroxycyclohexane-carboxylic Acid	C7 H12 O3	44761
144.0793	0.861	3-butyrl propionic acid	C7 H12 O3	35708
145.1107	1.144	N-METHYLISOLEUCINE	C7 H15 N O2	44542
145.1108	0.899	2R-aminoheptanoic acid	C7 H15 N O2	35932
148.0523	7.59	p-HYDROXYCINNAMALDEHYDE	C9 H8 O2	44643
149.0482	8.168	N-Acetyl-p-benzoquinonimine	C8 H7 N O2	578
149.1056	1.38	Trolamine	C6 H15 N O3	43365
151.0494	1.364	8-Hydroxyadenine	C5 H5 N5 O	5527
152.0333	1.181	Xanthine	C5 H4 N4 O2	82
158.0948	0.98	3-Oxovalproic acid	C8 H14 O3	2990
161.0843	3.488	Indole-3-ethanol	C10 H11 N O	6932
163.0633	5.061	3-Methyldioxyindole	C9 H9 N O2	7024
163.0634	3.487	4-(3-Pyridyl)-3-butenoic acid	C9 H9 N O2	6236
164.0475	1.321	o-Coumaric acid	C9 H8 O3	306
164.1202	13.97	cis-Jasmone	C11 H16 O	36077
165.0651	1.075	1-Methylguanine	C6 H7 N5 O	3778
166.0496	2.095	3-Methylxanthine	C6 H6 N4 O2	2820
166.0636	2.307	Atrolactic acid	C9 H10 O3	5462
170.1307	14.491	2-decylenic acid	C10 H18 O2	34719
178.0628	20.694	8S-hydroxy-2-Decene-4,6-diynoic acid	C10 H10 O3	35309
184.0888	5.906	Benzhydrol	C13 H12 O	1761
197.0693	4.626	L-DOPA	C9 H11 N O4	42
200.1414	17.7	2R-hydroxy-10-undecenoic acid	C11 H20 O3	35681
203.1172	1.041	Isopentenyladenine	C10 H13 N5	6612
205.0747	3.487	deschlorobenzoyl Indomethacin	C11 H11 N O3	829
210.0378	1.159	D-Saccharic acid	C6 H10 O8	3343
211.0848	5.366	3-O-Methyl-L-DOPA	C10 H13 N O4	967
213.1004	1.354	N-(3-oxo-hexanoyl)-homoserine lactone	C10 H15 N O4	36733
214.1572	8.963	3R-hydroxy-5Z-dodecenoic acid	C12 H22 O3	35609
214.1572	22.39	12-hydroxy-10-dodecenoic acid	C12 H22 O3	35534
216.1366	14.489	Undecanedioic acid	C11 H20 O4	5846
219.1107	1.186	Pantothenic Acid	C9 H17 N O5	241
220.0713	6.737	Trifluoromethylphenylpropanediol	C10 H11 F3 O2	1958
228.1476	2.824	Ile Pro	C11 H20 N2 O3	23858
228.148	3.125	1-L-Leucyl-L-Proline	C11 H20 N2 O3	4134
228.1728	17.844	10-keto tridecanoic acid	C13 H24 O3	35736
230.1634	3.419	Val Ile	C11 H22 N2 O3	23923
230.2251	12.033	2,2,9,9-tetramethyl-undecan-1,10-diol	C14 H30 O2	36474

232.1056	2.011	N2-Succinyl-L-ornithine	C9 H16 N2 O5	6075
232.1423	1.294	Thr Ile	C10 H20 N2 O4	23987
232.1425	1.833	Ethylenediamine-N,N'-di-a-butyric acid	C10 H20 N2 O4	2589
239.066	5.793	Acyclovir (8-hydroxy-9-(2-hydroxythoxymethyl)guanine	C8 H9 N5 O4	791
240.2093	26.223	6-pentadecenoic acid	C15 H28 O2	35234
241.1316	1.525	1,2-Propanediol, 3-[[2-(2-methoxyphenoxy)ethyl]amino]	C12 H19 N O4	1573
242.1883	24.746	14-hydroxy-12-tetradecenoic acid	C14 H26 O3	35544
244.1676	6.427	2-methyl-dodecanedioic acid	C13 H24 O4	35968
244.1788	3.881	Leu Leu	C12 H24 N2 O3	23804
244.2044	14.138	2-Hydroxymyristic acid	C14 H28 O3	6579
245.1639	4.263	Pinacidil	C13 H19 N5	1941
246.104	2.556	Met Pro	C10 H18 N2 O3 S	23664
246.1473	4.082	3-Hydroxydodecanedioic acid	C12 H22 O5	5402
248.1265	2.926	8-CYCLOPENTYLTHEOPHYLLINE	C12 H16 N4 O2	44461
249.0169	1.248	Alendronate	C4 H13 N O7 P2	818
250.0524	7.857	Silver sulfadiazine	C10 H10 N4 O2 S	2433
252.1148	2.04	Cimetidine	C10 H16 N6 S	1755
253.0817	5.815	Monapterin	C9 H11 N5 O4	5801
253.1319	1.146	4-(2-hydroxy-3-isopropyl-aminopropyl)benzoic scid	C13 H19 N O4	1354
254.1267	2.621	MIDODRINE	C12 H18 N2 O4	44344
255.922	0.719	Trichlorfon	C4 H8 Cl3 O4 P	2920
256.1043	12.207	Dinorpromazine	C15 H16 N2 S	2142
258.0885	1.429	4'-Hydroxyfenoprofen	C15 H14 O4	2690
260.0504	13.971	Tiaprofenic acid	C14 H12 O3 S	2844
260.1376	1.198	Glu Leu	C11 H20 N2 O5	44684
260.1735	1.265	carisoprodol	C12 H24 N2 O4	3947
261.1581	1.555	Pinacidil-N-Oxide	C13 H19 N5 O	1942
262.0583	0.806	7-Carboxynalidixic acid	C12 H10 N2 O5	1412
262.0802	1.216	Asp Glu	C9 H14 N2 O7	23953
264.1475	3.759	Phe Val	C14 H20 N2 O3	24030
264.1477	4.338	2H-Indol-2-one, 1,3-dihydro-4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-	C14 H20 N2 O3	1944
266.1267	2.074	Phe Thr	C13 H18 N2 O4	23662
266.2249	14.488	8-heptadecynoic acid	C17 H30 O2	35138
267.0973	8.217	Vidarabine	C10 H13 N5 O4	3020
267.0975	7.206	Zidovudine	C10 H13 N5 O4	3066
268.0848	14.047	2,4-Imidazolidinedione, 5-(7-oxabicyclo[4.1.0]hepta-2,4-dien-3-yl)-5-phenyl-	C15 H12 N2 O3	1853
268.1536	1.177	Leu His	C12 H20 N4 O3	23983
268.2404	17.706	9-heptadecylenic acid	C17 H32 O2	34747
270.085	1.604	Chloramphenicol alcohol	C11 H14 N2 O6	638
270.2196	23.371	3-keto palmitic acid	C16 H30 O3	35745
271.1645	1.514	Pro Arg	C11 H21 N5 O3	23777
272.2355	16.203	4-hydroxy palmitic acid	C16 H32 O3	35426
273.2671	20.115	C16 Sphinganine	C16 H35 N O2	41556
276.1213	7.254	4'-Hydroxytrimethoprim	C13 H16 N4 O3	2962
276.1717	14.044	cyclandelate	C17 H24 O3	3970

278.1268	2.765	Tyr Pro	C14 H18 N2 O4	23901
278.1636	5.664	Phe Leu	C15 H22 N2 O3	23831
278.2251	13.086	9,12,14-octadecatrienoic acid	C18 H30 O2	34823
280.1421	4.027	Tyr Val	C14 H20 N2 O4	23784
280.2407	16.786	13E,17-octadecadienoic acid	C18 H32 O2	34967
282.0186	0.727	2-Naphthaleneacetic acid, 6-(sulfooxy)-	C12 H10 O6 S	1381
282.2561	21.022	5-octadecylenic acid	C18 H34 O2	34752
284.1624	4.884	Dihydroartemisinin	C15 H24 O5	1119
284.2357	17.894	2-oxo-heptadecanoic acid	C17 H32 O3	35798
286.2151	15.503	Hexadecanedioic acid	C16 H30 O4	5642
288.1475	11.491	N-(3-Indolylacetyl)-L-isoleucine	C16 H20 N2 O3	34533
288.2301	22.298	10,16-dihydroxy-palmitic acid	C16 H32 O4	35689
289.1533	6.609	4-Amino-6,7-dimethoxy-2-(1-piperazinyl)quinazoline	C14 H19 N5 O2	2460
290.1885	14.519	8-hydroxy-17-octadecene-10,12-diynoic acid	C18 H26 O3	35647
294.1217	2.048	Phe Glu	C14 H18 N2 O5	23833
294.1583	3.548	Ile Tyr	C15 H22 N2 O4	23691
294.1584	4.218	Tyr Ile	C15 H22 N2 O4	23905
294.2196	15.517	12-oxo-9-octadecynoic acid	C18 H30 O3	35849
296.1008	2.021	Asp Tyr	C13 H16 N2 O6	23882
296.1191	4.577	Met Phe	C14 H20 N2 O3 S	23682
296.1263	13.402	Vomitoxin	C15 H20 O6	41207
296.2352	20.887	13S-hydroxy-9E,11Z-octadecadienoic acid	C18 H32 O3	35636
297.1078	2.094	1-Methylguanosine	C11 H15 N5 O5	6324
297.2667	17.366	(Z)-N-(2-hydroxyethyl)hexadec-7-enamide	C18 H35 N O2	3715
298.1418	7.242	Idebenone Metabolite (Benzenehexanoic acid, 2,5-dihydroxy-3,4-dimethoxy-6-methyl-)	C15 H22 O6	772
298.2146	14.805	8E-Heptadecenedioic acid	C17 H30 O4	35999
298.2491	18.572	8R-hydroxy-9Z-octadecenoic acid	C18 H34 O3	35650
300.2669	15.1	DL-11-hydroxy stearic acid	C18 H36 O3	35441
301.0618	7.859	3-Hydroxy-7-aminoclonazepam	C15 H12 Cl N3 O2	1886
302.2091	14.448	10-hydroxy-hexadecan-1,16-dioic acid	C16 H30 O5	36003
303.1795	2.121	Thr Ile Ala	C13 H25 N3 O5	17758
307.0996	1.398	Cys Trp	C14 H17 N3 O3 S	23806
309.1321	4.189	Ser Phe Gly	C14 H19 N3 O5	16806
309.1688	1.417	Tyr Lys	C15 H23 N3 O4	23796
310.2138	14.082	16-hydroperoxy-9Z,12,14E-octadecatrienoic acid	C18 H30 O4	35359
311.1844	1.852	Pro Val Pro	C15 H25 N3 O4	21539
311.2826	26.275	(±)13-Azaprostanoic acid	C19 H37 N O2	43397
312.2301	16.302	8,13-dihydroxy-9,11-octadecadienoic acid	C18 H32 O4	35506
312.2302	15.328	9-hydroxy-10-oxo-12-octadecenoic acid	C18 H32 O4	35510
314.2459	15.131	Octadecanedioic acid	C18 H34 O4	5748
316.2618	12.121	7,8-dihydroxy stearic acid	C18 H36 O4	35465
317.1947	2.348	Val Leu Ser	C14 H27 N3 O5	17706
317.2932	12.175	Phytosphingosine	C18 H39 N O3	7066
320.1339	1.14	Asn Ser Thr	C11 H20 N4 O7	17468
321.1792	7.922	Phe Arg	C15 H23 N5 O3	23741

325.2259	14.216	10-nitro,9Z,12Z-octadecadienoic acid	C18 H31 N O4	35951
326.1339	6.214	His Gly Asn	C12 H18 N6 O5	18496
326.2082	13.596	2,3-dinor-11b-PGF2alpha	C18 H30 O5	36093
326.2093	13.563	2,3-Dinor-8-iso-PGF2alpha	C18 H30 O5	36202
326.2102	14.52	2R-hydroperoxy-9Z,12Z,15Z-octadecatrienoic acid	C18 H30 O5	36034
327.1431	1.19	Pro Pro Asp	C14 H21 N3 O6	19777
328.1419	5.026	Phe Tyr	C18 H20 N2 O4	23928
328.2251	12.944	9S,11R,15S-trihydroxy-2,3-dinor-13E-prostaenoic acid-cyclo[8S,12R]	C18 H32 O5	36203
330.1675	5.651	Iridodial glucoside	C16 H26 O7	41180
330.2405	12.662	9,10,13-trihydroxy-11-octadecenoic acid	C18 H34 O5	35508
330.2424	14.489	9,12,13-trihydroxy-10-octadecenoic acid	C18 H34 O5	35509
330.2771	20.932	1-hexadecanoyl-sn-glycerol	C19 H38 O4	3855
332.2563	15.316	18-hydroxy-9S,10R-dihydroxy-stearic acid	C18 H36 O5	36012
335.1842	6.201	Leu Phe Gly	C17 H25 N3 O4	17301
339.1546	12.164	Ser His Pro	C14 H21 N5 O5	20096
339.1907	2.089	Ala Ile His	C15 H25 N5 O4	15676
341.2933	13.028	N-Acetylsphingosine	C20 H39 N O3	411
342.116	2.977	Maltose	C12 H22 O11	413
342.2043	9.125	2,3-dinor, 6-keto-PGF1alpha	C18 H30 O6	36156
342.2044	14.412	2,3-Dinor-TXB2	C18 H30 O6	3829
342.2773	20.27	Eicosanedioic acid	C20 H38 O4	35987
343.2479	5.551	Val Ile Leu	C17 H33 N3 O4	15845
343.3085	20.211	Dihydroceramide C2	C20 H41 N O3	43402
344.1548	0.879	Methotrimeprazine sulfoxide	C19 H24 N2 O2 S	1192
344.2046	4.17	Asn Val Ile	C15 H28 N4 O5	19356
344.2172	13.087	Ile Gly Arg	C14 H28 N6 O4	19906
344.2563	19.902	sn-1,2-Dioctanoylglycerol	C19 H36 O5	414
345.1551	2.601	Thr Pro Glu	C14 H23 N3 O7	18357
345.19	1.425	Glu Val Val	C15 H27 N3 O6	19647
345.2261	5.845	Leu Leu Thr	C16 H31 N3 O5	15718
346.2352	19.928	9,10-dihydroxy-Octadecanedioic acid	C18 H34 O6	35985
346.2353	13.971	9-hydroperoxy-12,13-dihydroxy-10-octadecenoic acid	C18 H34 O6	35355
347.0632	1.072	Zidovudine monophosphate	C10 H14 N5 O7 P	3068
347.1689	2.903	Thr Asp Ile	C14 H25 N3 O7	20490
348.1649	6.213	Asp Lys Ser	C13 H24 N4 O7	22423
348.2008	2.455	Lys Thr Thr	C14 H28 N4 O6	18228
348.2513	12.23	Sativic acid	C18 H36 O6	35482
348.2517	12.969	Dodecyl glucoside	C18 H36 O6	34493
349.1749	13.967	His Pro Pro	C16 H23 N5 O4	16950
349.2001	6.502	Ile Ala Phe	C18 H27 N3 O4	15688
351.1893	6.761	His Val Pro	C16 H25 N5 O4	20213
352.1884	13.99	Idebenone Metabolite (QS-10)	C19 H28 O6	758
353.1218	2.081	Asp Tyr Gly	C15 H19 N3 O7	23386
354.2395	14.161	13,14-dihydro-15-keto-PGF2alpha	C20 H34 O5	36105
354.277	22.734	1-(9Z,12Z-octadecadienoyl)-rac-glycerol	C21 H38 O4	4252

355.3452	26.149	N-(2-hydroxyethyl)icosanamide	C22 H45 N O2	3723
356.2562	22.568	13,14-dihydro-PGF2alpha	C20 H36 O5	36150
356.2927	15.823	1-(11E-octadecenoyl)-rac-glycerol	C21 H40 O4	4250
356.3278	16.236	13-hydroxy-docosanoic acid	C22 H44 O3	35576
358.2717	14.946	9,13-dihydroxy-12-ethoxy-10-octadecenoic acid	C20 H38 O5	35499
359.2053	2.223	Glu Leu Val	C16 H29 N3 O6	19672
360.1321	8.169	NITRENDIPINE	C18 H20 N2 O6	44354
360.178	3.585	Trp Val Gly	C18 H24 N4 O4	18336
361.1302	1.215	Asp Met Pro	C14 H23 N3 O6 S	21062
365.1945	6.199	Ile Ala Tyr	C18 H27 N3 O5	19800
366.19	2.485	Lys Tyr Gly	C17 H26 N4 O5	22856
367.1377	2.248	Gly Tyr Glu	C16 H21 N3 O7	19325
367.3086	14.078	PGE2alpha dimethyl amine	C22 H41 N O3	36173
368.1642	13.973	3-beta-hydroxyandrost-5-en-17-one sulfate	C19 H28 O5 S	4095
368.1643	13.171	Testosterone sulfate	C19 H28 O5 S	3558
368.181	8.478	Asn Val His	C15 H24 N6 O5	18456
368.2171	12.158	Pro Pro Arg	C16 H28 N6 O4	21182
370.1524	6.176	N-[(diphenylmethoxy)acetyl]-Glutamine	C20 H22 N2 O5	2322
370.1968	12.887	Lys Ser His	C15 H26 N6 O5	20296
370.2327	13.437	Arg Val Pro	C16 H30 N6 O4	18734
370.3083	19.143	Docosanedioic acid	C22 H42 O4	35989
371.14	22.565	Levobunolol sulfate	C17 H25 N O6 S	935
372.2522	12.633	8,8a-Deoxyoleandolide	C20 H36 O6	40994
372.3249	16.464	13,14-dihydroxy-docosanoic acid	C22 H44 O4	35577
373.1071	4.611	Cys Asp His	C13 H19 N5 O6 S	19299
373.1746	2.429	His Ala Phe	C18 H23 N5 O4	15997
374.2824	14.865	3beta-Hydroxychol-4-en-24-oic Acid	C24 H38 O3	42785
379.1044	0.802	Asp Met Asp	C13 H21 N3 O8 S	18369
382.1969	12.606	Asn His Leu	C16 H26 N6 O5	20737
382.343	12.87	24-hydroxy-10Z-tetracosenoic acid	C24 H46 O3	35581
383.1415	7.857	N-acetyllactosamine	C14 H25 N O11	4231
384.2122	14.312	Thr His Lys	C16 H28 N6 O5	19678
384.231	14.136	9a-Fluoroallotetrahydrocortisol	C21 H33 F O5	2717
384.359	18.438	24-hydroxy-tetracosanoic acid	C24 H48 O3	35579
388.1632	10.844	Nisoldipine	C20 H24 N2 O6	1551
388.2309	4.621	Glu Ile Lys	C17 H32 N4 O6	16755
388.2438	11.38	Arg Leu Thr	C16 H32 N6 O5	22752
390.1511	6.209	Loganin	C17 H26 O10	41146
390.1763	1.343	Asp Lys Glu	C15 H26 N4 O8	21250
390.2776	12.586	12beta-Hydroxy-3-oxo-5beta-cholan-24-oic Acid	C24 H38 O4	42745
391.2465	8.053	Leu Ile Phe	C21 H33 N3 O4	16864
393.1898	5.086	Phe Ile Asp	C19 H27 N3 O6	21641
394.1497	4.97	Phe Asn Asp	C17 H22 N4 O7	23445
398.1916	13.066	Asp His Lys	C16 H26 N6 O6	22066
400.0169	1.173	Octulose-1,8-bisphosphate	C8 H18 O14 P2	157

400.2072	12.832	Arg Pro Glu	C16 H28 N6 O6	18852
402.0963	6.212	4-O-Demethyl-13-dihydroadriamycinone	C20 H18 O9	664
402.261	8.381	Lys Gln Lys	C17 H34 N6 O5	22767
402.2959	22.093	Lys Lys Lys	C18 H38 N6 O4	18451
404.0019	1.637	Uridine diphosphate (UDP)	C9 H14 N2 O12 P2	97
404.2058	1.364	Flunarizine	C26 H26 F2 N2	2731
405.2268	14.042	Lisinopril	C21 H31 N3 O5	1009
407.1798	4.582	Asn Gln Phe	C18 H25 N5 O6	20970
407.2047	2.205	Leu Glu Phe	C20 H29 N3 O6	15774
408.2857	14.462	3beta,6alpha,7beta-Trihydroxy-5beta-cholan-24-oic Acid	C24 H40 O5	42672
408.2874	16.041	3beta,6beta,7alpha-Trihydroxy-5beta-cholan-24-oic Acid	C24 H40 O5	42673
410.1431	1.33	Tyr Asn Asp	C17 H22 N4 O8	17163
410.1741	14.876	ROTENONIC ACID, METHYL ETHER	C24 H26 O6	44195
412.1918	18.877	17alpha,20beta-Hydroxyprogesterone sulfate	C21 H32 O6 S	3562
412.3903	21.11	2-hydroxy-hexacosanoic acid	C26 H52 O3	35584
414.2272	10.031	Pro Trp Leu	C22 H30 N4 O4	16187
416.1585	8.421	Dehydronimodipine	C21 H24 N2 O7	1543
417.1642	2.148	Phe His Asp	C19 H23 N5 O6	20877
417.235	1.9	Arg Lys Asp	C16 H31 N7 O6	17233
418.1845	5.696	Val Asp Trp	C20 H26 N4 O6	23096
418.3081	25.607	(24S)-1alpha,24-dihydroxy-22-oxavitamin D3 / (24S)-1alpha,24-dihydroxy-22-oxacholecalciferol	C26 H42 O4	41998
420.2476	2.279	Val Arg Phe	C20 H32 N6 O4	19820
420.3202	13.378	(5Z)-4,4-difluorovitamin D3 / (5Z)-4,4-difluorocholecalciferol	C27 H42 F2 O	42074
422.0821	0.881	alpha,alpha'-Trehalose 6-phosphate	C12 H23 O14 P	3529
423.2	4.065	Tyr Glu Ile	C20 H29 N3 O7	15868
424.2543	4.669	His Arg Ile	C18 H32 N8 O4	7641
427.029	1.231	Zidovudine diphosphate	C10 H15 N5 O10 P2	3069
432.1527	6.536	BMPN-benzoic acid glucuronide	C21 H24 N2 O8	682
436.2055	26.78	Asp Phe Arg	C19 H28 N6 O6	17786
437.17	14.377	7-Hydroxydoxazosin	C22 H23 N5 O5	2456

TABLE II
IDENTIFIED METABOLITES WITH SCORE ABOVE 60, AND FIRST 20 HIGHEST CONCENTRATIONS OF METABOLITES IN RAW MILK FROM SHAMI AND SAANEN.

Breeds	***Shami***	***Saanen***
Drugs	Pinacidil Spiramycin 3 Deschlorobenzoyl Indomethacin -	Pinacidil Spiramycin 3 Deschlorobenzoyl Indomethacin Methotrimeprazine sulfoxide
Microbial metabolism in diverse environments including microbial secondary metabolites	Allantoic acid 3-Methylxanthine - Isopentenyladenine - -	- 3-Methylxanthine Atrolactic acid Isopentenyladenine Benzoic acid D-Saccharic acid
Environmental contaminants	Trichlorfon	Trichlorfon
Naturally occurring metabolites	Asp Phe Arg Selenocysteine Maltose Pantothenic acid 3-butyrl propionic acid GPEtn(18:1(9Z)/0:0) GPGroP(16:0/18:1(9Z)) Neu5Ac alpha2-6Galbeta1-4Glcbeta-Sp 1,4-Methylimidazoleacetic acid	Asp Phe Arg - Maltose Pantothenic acid 3-butyrl propionic acid GPEtn(18:1(9Z)/0:0) - Neu5Ac alpha2-6Galbeta1-4Glcbeta-Sp -
Plant secondary metabolites	4-Hydroxystyrene	-
Others	3-Hydroxydodecanedioic acid (*Disorder*) - - - Laminaribiose (*plant saccharide*) 5’guanylate diphosphate (*Purine metabolism in microbes*)	- 13S-hydroxy-9E,11Z-octadecadienoic acid (*Human skin tanning*) 13,14-dihydro-15-keto-PGF2 alpha (*hormone*) 9,12,13-trihydroxy-10-octadecenoic acid (*Anti-stress substance/antifungal*) - -