**Urinary Metabolomics Fingerprinting Around Parturition Identifies Metabolites that Differentiate Lame Dairy Cows from Healthy Ones**

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**Animal journal**

**Supplementary Material S1**

*Compound identification and quantification*

To determine concentration of amino acids (AAs), acylcarnitines (ACs), biogenic amines (BAs), glycerophospholipids, sphingolipids, and hexose (90-95% glucose) in the urine samples, a targeted quantitative metabolomics approach was applied using a combination of direct injection and tandem mass spectrometry (DI-MS/MS) with a reverse-phase liquid chromatography and tandem mass spectrometry (LC-MS/MS) kit.

The kit (AbsoluteIDQ 180) is a commercially available assay from BIOCRATES Life Science AG (Innsbruck, Austria). This kit assay is used in combination with an ABI 4000 Q-Trap (Applied Biosystems/MDS Sciex, Foster City, CA) mass spectrometer, and can be used for the targeted identification and quantification of up to 180 different endogenous metabolites from six analyte groups including AAs, ACs, BAs, glycerophospholipids, sphingolipids, and hexose. Amino acids and BAs were analyzed by a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method, whereas all other metabolites were quantified by DI-MS/MS.

The method combined the derivatization and extraction of analytes with the selective mass spectrometric detection using multiple reaction monitoring (MRM) pairs as described previously by Bouatra *et al.,* (2013). Isotope-labeled internal standards and other internal standards were integrated into a kit plate filter for metabolite quantification. The AbsoluteIDQ 180 kit contains a 96 deep-well plate with a filter plate, along with sealing tape and reagents and solvents used to prepare the plate assay. The first 14 wells (1 blank, 3 zero samples, 7 standards, and 3 quality control samples) in the kit were used for quality control and standardization, with the remaining 82 being available for urine sample analysis.

Urine samples were left to thaw on ice and then vortexed and centrifuged at 13 000 × g at 4 °C for 3 min. Ten μL of supernatant were loaded onto the center of the kit’s filter substrate on the upper 96-well kit plate and dried under a stream of nitrogen using Zanntek Analytical Evaporator (Glas-Col, Terre Haute, IN, USA). Subsequently, 20 μL of a 5% solution of phenyl-isothiocyanate solution was added for derivatization and after 20 min incubation, the filter spots were dried using the same evaporator. Extraction of metabolites was achieved by adding 300 μL methanol containing 5 mM ammonium acetate. The extracts were obtained by centrifugation using Sorvall Evolution RC Superspeed Centrifuge (Fisher Scientific, Toronto, ON, Canada) into the lower 96 deep-well plate, followed by a dilution step with 600 μL of the MS running solvent in the kit. Mass spectrometric analysis of extracts was conducted on an ABI 4000 Q-trap tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with a solvent delivery system. The Biocrates MetIQ software, which was included in the kit was used to control the entire assay workflow, from sample registration to automated calculation of metabolite concentrations to the export of data into other data analysis programs. A targeted profiling scheme was used to quantify all detectable metabolites using MRM, neutral loss, and precursor ion scans.

**Supplementary Material S2**

*Statistical analysis*

All metabolite concentrations measured from different analytical methods were normalized to each urine sample’s corresponding creatinine value (assuming a constant rate creatinine excretion for each urine sample) to compensate for variations in urine volume. The concentration of each metabolite is expressed under the unit of μM/mM creatinine.

Univariate analysis of continuous data was performed using Wilcoxon-Mann-Whitney (rank sum) test provided by R (Version 3.0.3, R Development Core Team, 2008). Statistical significance was declared at p < 0.05. All metabolomics data were performed by the MetaboAnalyst software (Xia *et al*., 2015). Recommended statistical procedures for metabolomics analysis were followed according to previously published protocols (Xia *et al.,* 2009). Metabolites that were frequently (>20%) below the limit of detection or with at least 20% missing values were removed from consideration. Otherwise, missing values were replaced by a value of one-half of the minimum positive value in the original data. Data normalization of metabolite concentration was done prior to statistical analysis and pathway analysis to create a Gaussian distribution (Xia *et al*., 2009). In this study, we used log-transformed and autoscaling metabolite values. To perform a standard cross-sectional 2-group study, we compared the group of healthy cows (control cows, CON) and the group of cows with lameness at each time point (-8, -4, disease diagnosis, +4, and +8 wks around calving) separately. Principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), quantitative enrichment analysis, and metabolic pathway analysis were performed via MetaboAnalyst. In the PLS-DA model, a VIP (variable importance in the projection) plot was used to rank the metabolites based on their importance in discriminating the lame group of cows from CON cows. Metabolites with the highest VIP values are the most powerful group discriminators. Typically, VIP values > 1 are significant and VIP values > 2 are highly significant. A 2 000 permutation test was implemented to validate the reliability of the model because it used random resampling of lame and CON cows to determine the probability that lame and CON groups were a result of chance (Xia *et al.,* 2011).

Biomarker profiles and the quality of the biomarker sets were determined using receiver-operator characteristic (ROC) curves as calculated by MetaboAnalyst 3.0 (Xia *et al.*, 2015). Paired sensitivity and false-positive ratios (1-specificity) at different classification decision boundaries were calculated. A ROC curve is plotted with sensitivity values on the Y-axis and the corresponding false-positive rates (1-specificity) on the X-axis. ROC curves are often summarized into a single metric known as the area under the ROC curve (AUC), which indicates the accuracy of a test for correctly distinguishing one group such as lame cows from CON ones. If all positive samples are ranked before negative ones, the AUC is 1.0, which indicates a perfectly discriminating test. The 95% confidence interval (CI) and p values were calculated. A permutation test was conducted for each ROC curve at different time points with 1 000 permutations. A rough guide for assessing the utility of a biomarker set based on its AUC is 0.9~1.0 = excellent; 0.8~0.9 = good; 0.7~0.8 = fair; 0.6~0.7 = poor; 0.5~0.6 = fail.

**Supplementary Table S1.** *Prepartum diet for dry cows*

|  |  |
| --- | --- |
| **Item** | **Close-Up diet (CUD)** |
| Ingredient | %, DM |
| Alfalfa hay | 10.0 |
| Barley silage | 60.0 |
| CUD grain | 30.0 |
| Nutrient composition of CUD grain mix | % amount per kg |
| Ruminant TM Pak 1 | 0.2775 |
| Selenium 1000 mg/kg (UNscr FineCr) 2 | 0.2 |
| Custom TM Complex Premix 3 | 0.33 |
| Vitamin A/D3-1000-200 4 | 0.006 |
| Barley grain, rolled | 39.5815 |
| Flo-bond mycotoxin binder | 0.5 |
| Limestone | 3.7 |
| Magnesium chloride | 1.645 |
| Mag Ox-56% 5 | 0.54 |
| Scale Molasses (60:40) | 2.5 |
| Canola meal | 17.0 |
| Vitamin E 50% Ads 6 | 0.18 |
| Soybean hulls, ground | 33.0 |
| Salt | 0.54 |

1 Ruminant TM Pak: a premix containing cobalt, copper, iodine, manganese, and zinc.

2 Selenium 1 000 mg/kg (UNscr FineCr) – Selenium supplement

3 Custom TM complex premix: a custom product supplying organic sources of cobalt, copper, manganese, and zinc.

4 Vitamin A/D3-1 000-2003: Vitamin A acetate (retinyl acetate) and Vitamin D3 (cholecalciferol).

5 Mag Ox 56%: 56% magnesium guarantee.

6 Vitamin E 50% Ads contains 226 800 IU of Vitamin E per pound.

**Supplementary Table S2*.*** *Ingredients of high grain ration fed to cows*

*during early lactation*

|  |  |
| --- | --- |
| **Item** | **Early Lactation Diet** |
| Ingredient % of DM | %, DM |
| Alfalfa Hay | 9.59 |
| Barley Silage | 30.24 |
| Alfalfa Silage | 9.64 |
| High 16% dairy ration | 50.5 |
| Nutrient composition of dairy ration | % amount per kg |
| ADE Vit Pak-30 Natural E 1 | 0.05 |
| Ruminant TM Pak 2 | 0.11 |
| Selenium, 1 000 mg/kg (UNscr FineCr) | 0.07 |
| Custom TM Complex premix 3 | 0.07 |
| AminoShure-L 4 | 0.33 |
| Blood meal | 3.50 |
| Barley grain, rolled | 39.91 |
| Barley grain, ground | 27.50 |
| Di-calcium phosphate 21% | 1.00 |
| Vitamin D-10 000 KIU/kg 5 | 0.02 |
| Diamond V XPC 6 | 0.13 |
| Dairy Xtract 7 | 0.02 |
| Energizer RP10 | 2.75 |
| Limestone | 1.70 |
| Mag Ox-56% 8 | 0.43 |
| Scale Molasses (60:40) | 1.25 |
| Nutri A-Z C Dry 9 | 0.10 |
| Amino Plus (High bypass soy) 10 | 8.00 |
| Vitamin E 50% Ads 11 | 0.01 |
| Soy bean meal-47.5% | 1.25 |
| Sodium bicarbonate | 0.80 |
| Salt | 0.51 |
| Poultry-Tallow | 0.50 |
| Biotin 2%-Rovimix H-2 12 | 0.01 |
| Wheat distillers grain (50:50) | 10.00 |

1 ADE Vit Pak-30 Natural E: a premix containing vitamins A, D3, and E.

2 Ruminant TM Pak: a premix containing cobalt, copper, iodine, manganese, and zinc.

3 Custom TM complex premix: a custom product supplying organic sources of cobalt, copper, manganese, and zinc.

4 AminoShure-L: hydrogenated vegetable oil, and L-lysine monohydrochloride (Halchemix, Port Perry, ON, Canada).

5 Vitamin D-10 000 KIU/kg: Vitamin D supplememnt

6 Diamond V XPC: concentrated yeast (Diamond V Mills, Cedar Rapids, IA, USA).

7 Dairy Xtract: Feed supplement

8 Mag Ox 56%: 56% magnesium guarantee.

9 Nutri A-Z C Dry

10 Amino Plus: a high by-pass soy meal.

11 Vitamin E 50% Ads contains 226 800 IU of Vitamin E per pound.

12 DSM Nutritional Products (Parsippany, NJ, USA).

Supplementary Table S3. Concentration (µM) of Urine Metabolites (Mean (SEM)) in Healthy Control and Pre-Lame/Lame Cows as Determined by DI/LC-MS/MS1 for Prepartum Time Points and the Week of Disease Diagnosis.

|  | **-8 weeks before parturition** | | | |  | **-4 weeks before parturition** | | | |  | **week of disease diagnosis*2*** | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Metabolite** | **LAM*3*** | **CON*4*** | ***p*-value** | **LAM/CON** |  | **LAM** | **CON** | ***p*-value** | **LAM/CON** |  | **LAM** | **CON** | ***p*-value** | **LAM/CON** |
| **Acylcarnitines** | | | | | | | | | | | | | | |
| C0 | 0.763 (0.347) | 0.843 (0.267) | 0.295 | Down |  | 0.681 (0.447) | 0.863 (0.517) | 0.083 | Down |  | 0.544 (0.065) | 0.848 (0.499) | 0.054 | Down |
| C10 | 0.029 (0.010) | 0.024 (0.009) | 0.267 | Up |  | 0.029 (0.006) | 0.027 (0.017) | 0.324 | Up |  | 0.033 (0.018) | 0.030 (0.020) | 0.657 | Up |
| C10:1 | 0.046 (0.035) | 0.025 (0.008) | 0.011\* | Up |  | 0.060 (0.061) | 0.028 (0.009) | 0.026\* | Up |  | 0.037 (0.008) | 0.025 (0.012) | 0.008\* | Up |
| C10:2 | 0.016 (0.005) | 0.010 (0.004) | 0.028\* | Up |  | 0.014 (0.006) | 0.010 (0.007) | 0.046\* | Up |  | 0.011 (0.001) | 0.012 (0.007) | 0.836 | Down |
| C12 | 0.082 (0.101) | 0.028 (0.017) | 0.139 | Up |  | 0.037 (0.038) | 0.024 (0.020) | 0.614 | Up |  | 0.133 (0.133) | 0.053 (0.036) | 0.072 | Up |
| C12-DC | 0.020 (0.009) | 0.016 (0.005) | 0.332 | Up |  | 0.028 (0.026) | 0.016 (0.003) | 0.594 | Up |  | 0.024 (0.014) | 0.016 (0.005) | 0.017\* | Up |
| C12:1 | 0.050 (0.051) | 0.063 (0.038) | 0.355 | Down |  | 0.050 (0.059) | 0.069 (0.068) | 0.457 | Down |  | 0.062 (0.034) | 0.086 (0.072) | 0.7 | Down |
| C14 | 0.007 (0.006) | 0.007 (0.002) | 0.295 | Up |  | 0.011 (0.009) | 0.007 (0.005) | 0.355 | Up |  | 0.009 (0.002) | 0.007 (0.005) | 0.039\* | Up |
| C14:1 | 0.003 (0.002) | 0.003 (0.001) | 0.745 | Up |  | 0.005 (0.004) | 0.003 (0.002) | 0.062 | Up |  | 0.006 (0.001) | 0.004 (0.002) | 0.013\* | Up |
| C14:1-OH | 0.006 (0.003) | 0.003 (0.001) | 0.139 | Up |  | 0.005 (0.004) | 0.003 (0.002) | 0.196 | Up |  | 0.005 (0.002) | 0.003 (0.002) | 0.023\* | Up |
| C14:2 | 0.003 (0.001) | 0.002 (0.001) | 0.79 | Up |  | 0.003 (0.002) | 0.003 (0.002) | 0.355 | Up |  | 0.003 (0.002) | 0.003 (0.002) | 0.176 | Up |
| C14:2-OH | 0.004 (0.002) | 0.003 (0.001) | 0.132 | Up |  | 0.010 (0.009) | 0.004 (0.005) | 0.083 | Up |  | 0.010 (0.007) | 0.006 (0.006) | 0.083 | Up |
| C16 | 0.008 (0.002) | 0.004 (0.002) | <0.001\* | Up |  | 0.007 (0.002) | 0.004 (0.002) | <0.001\* | Up |  | 0.007 (0.003) | 0.004 (0.002) | 0.023\* | Up |
| C16-OH | 0.005 (0.001) | 0.002 (0.001) | 0.001\* | Up |  | 0.004 (0.001) | 0.003 (0.002) | 0.046\* | Up |  | 0.004 (0.002) | 0.003 (0.002) | 0.091 | Up |
| C16:1 | 0.012 (0.005) | 0.007 (0.003) | 0.011\* | Up |  | 0.013 (0.008) | 0.007 (0.005) | 0.016\* | Up |  | 0.009 (0.003) | 0.008 (0.004) | 0.324 | Up |
| C16:1-OH | 0.004 (0.002) | 0.002 (0.001) | 0.085 | Up |  | 0.003 (0.003) | 0.002 (0.001) | 0.876 | Up |  | 0.002 (0.001) | 0.002 (0.001) | 0.597 | Up |
| C16:2 | 0.004 (0.002) | 0.002 (0.001) | 0.08 | Up |  | 0.004 (0.002) | 0.002 (0.001) | 0.003\* | Up |  | 0.003 (0.000) | 0.002 (0.001) | 0.003\* | Up |
| C16:2-OH | 0.005 (0.002) | 0.004 (0.001) | 0.097 | Up |  | 0.007 (0.004) | 0.004 (0.001) | 0.004\* | Up |  | 0.004 (0.001) | 0.004 (0.001) | 0.25 | Up |
| C18 | 0.003 (0.002) | 0.002 (0.000) | 0.246 | Up |  | 0.003 (0.002) | 0.002 (0.000) | 0.074 | Up |  | 0.003 (0.002) | 0.002 (0.001) | 0.162 | Up |
| C18:1 | 0.005 (0.002) | 0.004 (0.001) | 0.141 | Up |  | 0.006 (0.006) | 0.003 (0.001) | 0.925 | Up |  | 0.004 (0.002) | 0.004 (0.002) | 0.499 | Up |
| C18:1-OH | 0.006 (0.002) | 0.004 (0.001) | 0.036\* | Up |  | 0.007 (0.004) | 0.004 (0.001) | 0.042\* | Up |  | 0.006 (0.002) | 0.005 (0.002) | 0.01\* | Up |
| C18:2 | 0.007 (0.003) | 0.002 (0.001) | 0.011\* | Up |  | 0.004 (0.002) | 0.002 (0.001) | 0.009\* | Up |  | 0.007 (0.002) | 0.002 (0.002) | <0.001\* | Up |
| C2 | 0.082 (0.046) | 0.092 (0.038) | 0.268 | Down |  | 0.066 (0.009) | 0.111 (0.073) | 0.123 | Down |  | 0.078 (0.031) | 0.124 (0.099) | 0.196 | Down |
| C3 | 0.012 (0.006) | 0.013 (0.006) | 0.662 | Down |  | 0.012 (0.004) | 0.013 (0.008) | 0.79 | Down |  | 0.009 (0.004) | 0.014 (0.007) | 0.18 | Down |
| C3-DC (C4-OH) | 0.013 (0.006) | 0.020 (0.010) | 0.109 | Down |  | 0.018 (0.010) | 0.021 (0.013) | 0.7 | Down |  | 0.013 (0.003) | 0.025 (0.015) | 0.039\* | Down |
| C3-OH | 0.036 (0.016) | 0.010 (0.006) | 0.011\* | Up |  | 0.031 (0.015) | 0.010 (0.005) | 0.018\* | Up |  | 0.029 (0.012) | 0.013 (0.009) | 0.002\* | Up |
| C3:1 | 0.010 (0.005) | 0.021 (0.009) | 0.002\* | Down |  | 0.011 (0.006) | 0.022 (0.012) | 0.013\* | Down |  | 0.008 (0.002) | 0.027 (0.018) | <0.001\* | Down |
| C4 | 0.074 (0.036) | 0.080 (0.046) | 0.769 | Down |  | 0.062 (0.022) | 0.080 (0.052) | 0.533 | Down |  | 0.062 (0.050) | 0.030 (0.021) | 0.157 | Up |
| C4:1 | 0.023 (0.012) | 0.029 (0.012) | 0.335 | Down |  | 0.019 (0.005) | 0.033 (0.015) | 0.002\* | Down |  | 0.021 (0.013) | 0.037 (0.025) | 0.176 | Down |
| C5 | 0.025 (0.014) | 0.019 (0.008) | 0.196 | Up |  | 0.017 (0.006) | 0.022 (0.016) | 0.929 | Down |  | 0.014 (0.005) | 0.017 (0.011) | 0.324 | Down |
| C5-M-DC | 0.032 (0.014) | 0.012 (0.005) | 0.019\* | Up |  | 0.027 (0.009) | 0.014 (0.009) | 0.001\* | Up |  | 0.034 (0.015) | 0.017 (0.011) | 0.003\* | Up |
| C5-OH (C3-DC-M) | 0.031 (0.016) | 0.023 (0.006) | 0.268 | Up |  | 0.030 (0.010) | 0.025 (0.015) | 0.19 | Up |  | 0.043 (0.016) | 0.035 (0.025) | 0.119 | Up |
| C5:1 | 0.034 (0.014) | 0.042 (0.013) | 0.221 | Down |  | 0.026 (0.011) | 0.044 (0.022) | 0.019\* | Down |  | 0.033 (0.005) | 0.042 (0.025) | 0.457 | Down |
| C5:1-DC | 0.007 (0.002) | 0.007 (0.002) | 0.836 | Down |  | 0.005 (0.003) | 0.008 (0.007) | 0.083 | Down |  | 0.009 (0.002) | 0.010 (0.006) | 0.79 | Down |
| C5-DC (C6-OH) | 0.014 (0.006) | 0.010 (0.004) | 0.031\* | Up |  | 0.013 (0.008) | 0.011 (0.006) | 0.836 | Up |  | 0.019 (0.006) | 0.015 (0.011) | 0.033\* | Up |
| C6:1 | 0.015 (0.003) | 0.012 (0.005) | 0.167 | Up |  | 0.015 (0.004) | 0.012 (0.006) | 0.083 | Up |  | 0.020 (0.006) | 0.012 (0.007) | 0.007\* | Up |
| C7-DC | 0.010 (0.003) | 0.007 (0.003) | 0.042\* | Up |  | 0.011 (0.006) | 0.007 (0.004) | 0.019\* | Up |  | 0.010 (0.003) | 0.007 (0.005) | 0.016\* | Up |
| C8 | 0.022 (0.007) | 0.018 (0.004) | 0.222 | Up |  | 0.024 (0.015) | 0.017 (0.003) | 0.876 | Up |  | 0.022 (0.005) | 0.017 (0.006) | 0.027\* | Up |
| C9 | 0.044 (0.016) | 0.038 (0.025) | 0.196 | Up |  | 0.050 (0.014) | 0.041 (0.021) | 0.35 | Up |  | 0.035 (0.010) | 0.030 (0.018) | 0.532 | Up |
| **Glycerophospholipids (Lysophosphatidylcholines)** | | | | | | | | | | | | | | |
| lysoPC a C16:0 | 0.072 (0.152) | 0.026 (0.015) | 0.159 | Up |  | 0.040 (0.084) | 0.089 (0.202) | 0.178 | Down |  | 0.017 (0.015) | 0.018 (0.025) | 0.686 | Down |
| lysoPC a C16:1 | 0.004 (0.003) | 0.005 (0.003) | 0.331 | Down |  | 0.005 (0.007) | 0.006 (0.003) | 0.056 | Down |  | 0.004 (0.003) | 0.006 (0.004) | 0.203 | Down |
| lysoPC a C17:0 | 0.006 (0.009) | 0.005 (0.002) | 0.097 | Up |  | 0.002 (0.002) | 0.005 (0.004) | 0.056 | Down |  | 0.002 (0.003) | 0.004 (0.002) | 0.023\* | Down |
| lysoPC a C18:0 | 0.044 (0.058) | 0.032 (0.039) | 0.929 | Up |  | 0.052 (0.077) | 0.035 (0.047) | 0.422 | Up |  | 0.022 (0.017) | 0.031 (0.031) | 0.533 | Down |
| lysoPC a C18:1 | 0.010 (0.016) | 0.006 (0.003) | 0.415 | Up |  | 0.009 (0.015) | 0.017 (0.029) | 0.097 | Down |  | 0.006 (0.006) | 0.007 (0.005) | 0.514 | Down |
| lysoPC a C18:2 | 0.004 (0.007) | 0.008 (0.005) | 0.016\* | Down |  | 0.006 (0.012) | 0.023 (0.041) | 0.011\* | Down |  | 0.010 (0.004) | 0.014 (0.015) | 0.305 | Down |
| lysoPC a C20:3 | 0.012 (0.005) | 0.014 (0.006) | 0.552 | Down |  | 0.018 (0.017) | 0.017 (0.006) | 0.398 | Up |  | 0.014 (0.004) | 0.019 (0.010) | 0.128 | Down |
| lysoPC a C20:4 | 0.004 (0.004) | 0.002 (0.002) | 0.158 | Up |  | 0.004 (0.003) | 0.009 (0.020) | 0.572 | Down |  | 0.005 (0.009) | 0.004 (0.004) | 0.575 | Up |
| lysoPC a C28:1 |  |  |  |  |  | 0.003 (0.008) | 0.004 (0.006) | 0.046\* | Down |  | 0.004 (0.004) | 0.003 (0.002) | 0.475 | Up |
| **Glycerophospholipids (Phosphatidylcholines)** | | | | | | | | | | | | | | |
| PC aa C24:0 | 0.004 (0.004) | 0.005 (0.002) | 0.301 | Down |  | 0.007 (0.011) | 0.005 (0.003) | 0.199 | Up |  | 0.005 (0.003) | 0.005 (0.002) | 0.686 | Down |
| PC aa C28:1 | 0.012 (0.005) | 0.009 (0.003) | 0.065 | Up |  | 0.011 (0.009) | 0.011 (0.010) | 0.434 | Up |  | 0.010 (0.004) | 0.008 (0.002) | 0.21 | Up |
| PC aa C30:0 | 0.011 (0.007) | 0.010 (0.003) | 0.511 | Up |  | 0.021 (0.029) | 0.015 (0.008) | 0.398 | Up |  | 0.008 (0.004) | 0.009 (0.003) | 0.077 | Down |
| PC aa C30:2 | . | . | . | . |  | 0.000 (0.001) | 0.001 (0.001) | 0.08 | Down |  | 0.001 (0.001) | 0.001 (0.001) | 0.827 | Up |
| PC aa C32:0 | 0.004 (0.006) | 0.004 (0.003) | 0.199 | Up |  | 0.006 (0.011) | 0.025 (0.055) | 0.125 | Down |  | 0.005 (0.004) | 0.004 (0.005) | 0.136 | Up |
| PC aa C32:1 | 0.004 (0.008) | 0.003 (0.002) | 0.222 | Up |  | 0.003 (0.004) | 0.015 (0.034) | 0.056 | Down |  | 0.001 (0.001) | 0.003 (0.003) | 0.02\* | Down |
| PC aa C32:2 | 0.004 (0.003) | 0.007 (0.006) | 0.085 | Down |  | 0.003 (0.006) | 0.011 (0.012) | 0.011\* | Down |  | 0.004 (0.001) | 0.006 (0.003) | 0.159 | Down |
| PC aa C32:3 | . | . | . | . |  | 0.001 (0.001) | 0.002 (0.003) | 0.975 | Down |  | 0.000 (0.000) | 0.002 (0.005) | 0.346 | Down |
| PC aa C34:1 | 0.023 (0.047) | 0.036 (0.071) | 0.219 | Down |  | 0.053 (0.122) | 0.194 (0.762) | 0.139 | Down |  | 0.018 (0.021) | 0.042 (0.085) | 0.457 | Down |
| PC aa C34:2 | 0.049 (0.106) | 0.090 (0.203) | 0.072 | Down |  | 0.067 (0.153) | 0.432 (1.700) | 0.055 | Down |  | 0.017 (0.019) | 0.094 (0.188) | 0.157 | Down |
| PC aa C34:3 | 0.003 (0.004) | 0.006 (0.015) | 0.831 | Down |  | 0.003 (0.007) | 0.016 (0.060) | 0.102 | Down |  | 0.001 (0.001) | 0.006 (0.013) | 0.211 | Down |
| PC aa C34:4 | 0.002 (0.001) | 0.003 (0.002) | 0.657 | Down |  | 0.004 (0.006) | 0.004 (0.007) | 0.784 | Down |  | 0.002 (0.002) | 0.004 (0.004) | 0.243 | Down |
| PC aa C36:0 | 0.036 (0.020) | 0.027 (0.011) | 0.036\* | Up |  | 0.056 (0.068) | 0.033 (0.020) | 0.73 | Up |  | 0.030 (0.013) | 0.022 (0.005) | 0.305 | Up |
| PC aa C36:1 | 0.015 (0.028) | 0.018 (0.044) | 0.79 | Down |  | 0.012 (0.025) | 0.035 (0.110) | 0.295 | Down |  | 0.004 (0.004) | 0.017 (0.045) | 0.447 | Down |
| PC aa C36:2 | 0.031 (0.062) | 0.058 (0.139) | 0.324 | Down |  | 0.025 (0.059) | 0.175 (0.633) | 0.022\* | Down |  | 0.007 (0.008) | 0.059 (0.129) | 0.107 | Down |
| PC aa C36:3 | 0.022 (0.047) | 0.022 (0.048) | 0.235 | Down |  | 0.016 (0.035) | 0.103 (0.395) | 0.139 | Down |  | 0.007 (0.012) | 0.028 (0.055) | 0.196 | Down |
| PC aa C36:4 | 0.024 (0.051) | 0.031 (0.064) | 0.196 | Down |  | 0.018 (0.042) | 0.293 (1.238) | 0.031\* | Down |  | 0.007 (0.007) | 0.031 (0.057) | 0.429 | Down |
| PC aa C36:5 | 0.003 (0.006) | 0.003 (0.007) | 0.059 | Down |  | 0.002 (0.003) | 0.025 (0.102) | 0.082 | Down |  | 0.002 (0.002) | 0.003 (0.006) | 0.784 | Down |
| PC aa C36:6 | . | . | . | . |  | 0.001 (0.001) | 0.002 (0.004) | 0.572 | Down |  | 0.001 (0.001) | 0.001 (0.000) | 0.334 | Up |
| PC aa C38:0 | 0.006 (0.008) | 0.004 (0.001) | 0.826 | Up |  | 0.005 (0.005) | 0.009 (0.016) | 0.159 | Down |  | 0.003 (0.001) | 0.003 (0.002) | 0.733 | Up |
| PC aa C38:1 | 0.005 (0.005) | 0.003 (0.001) | 0.222 | Up |  | 0.001 (0.003) | 0.005 (0.010) | 0.059 | Down |  | 0.003 (0.002) | 0.002 (0.002) | 0.112 | Up |
| PC aa C38:3 | 0.009 (0.019) | 0.006 (0.004) | 0.056 | Up |  | 0.008 (0.013) | 0.051 (0.125) | 0.199 | Down |  | 0.005 (0.007) | 0.008 (0.015) | 0.162 | Down |
| PC aa C38:4 | 0.027 (0.044) | 0.022 (0.043) | 0.355 | Up |  | 0.017 (0.022) | 0.110 (0.433) | 1 | Down |  | 0.015 (0.008) | 0.027 (0.043) | 0.573 | Down |
| PC aa C38:5 | 0.018 (0.039) | 0.010 (0.020) | 0.26 | Up |  | 0.006 (0.011) | 0.041 (0.164) | 0.648 | Down |  | 0.004 (0.004) | 0.010 (0.020) | 0.952 | Down |
| PC aa C38:6 | 0.005 (0.005) | 0.017 (0.042) | 0.715 | Down |  | 0.005 (0.009) | 0.118 (0.490) | 0.048\* | Down |  | 0.002 (0.001) | 0.016 (0.037) | 0.196 | Down |
| PC aa C40:1 | 0.027 (0.014) | 0.031 (0.011) | 0.332 | Down |  | 0.043 (0.057) | 0.032 (0.009) | 0.048\* | Up |  | 0.015 (0.005) | 0.030 (0.012) | 0.001\* | Down |
| PC aa C40:2 | . | . | . | . |  | 0.002 (0.004) | 0.002 (0.005) | 0.247 | Down |  | 0.001 (0.001) | 0.002 (0.002) | 0.212 | Down |
| PC aa C40:3 | . | . | . | . |  | 0.001 (0.002) | 0.002 (0.006) | 0.976 | Down |  | 0.000 (0.000) | 0.001 (0.001) | 0.008\* | Down |
| PC aa C40:4 | 0.002 (0.003) | 0.002 (0.004) | 0.151 | Down |  | 0.002 (0.004) | 0.004 (0.012) | 0.077 | Down |  | 0.001 (0.002) | 0.003 (0.004) | 0.26 | Down |
| PC aa C40:5 | . | . | . | . |  | 0.000 (0.000) | 0.007 (0.026) | 0.248 | Down |  | 0.001 (0.001) | 0.002 (0.007) | 0.229 | Down |
| PC aa C40:6 | 0.029 (0.016) | 0.024 (0.009) | 0.975 | Up |  | 0.051 (0.068) | 0.061 (0.106) | 0.826 | Down |  | 0.024 (0.007) | 0.020 (0.012) | 0.077 | Up |
| PC aa C42:0 | 0.002 (0.001) | 0.004 (0.002) | 0.011\* | Down |  | 0.011 (0.021) | 0.005 (0.003) | 0.125 | Up |  | 0.003 (0.000) | 0.003 (0.002) | 0.226 | Down |
| PC aa C42:1 | 0.002 (0.002) | 0.001 (0.001) | 0.976 | Up |  | 0.002 (0.003) | 0.002 (0.002) | 0.648 | Up |  | 0.000 (0.001) | 0.002 (0.003) | 0.041\* | Down |
| PC aa C42:2 | 0.010 (0.003) | 0.007 (0.004) | 0.093 | Up |  | 0.007 (0.009) | 0.007 (0.006) | 0.243 | Up |  | 0.004 (0.004) | 0.007 (0.005) | 0.039\* | Down |
| PC aa C42:4 | 0.002 (0.002) | 0.001 (0.000) | 0.198 | Up |  | 0.001 (0.001) | 0.001 (0.002) | 0.51 | Down |  | 0.002 (0.002) | 0.001 (0.001) | 0.25 | Up |
| PC aa C42:5 | 0.005 (0.004) | 0.002 (0.001) | 0.103 | Up |  | 0.008 (0.005) | 0.004 (0.003) | 0.11 | Up |  | 0.009 (0.005) | 0.003 (0.002) | 0.006\* | Up |
| PC aa C42:6 | 0.038 (0.026) | 0.023 (0.008) | 0.301 | Up |  | 0.051 (0.063) | 0.023 (0.008) | 0.826 | Up |  | 0.025 (0.010) | 0.018 (0.005) | 0.475 | Up |
| PC ae C30:0 | 0.010 (0.007) | 0.012 (0.004) | 0.178 | Down |  | 0.016 (0.016) | 0.011 (0.003) | 0.638 | Up |  | 0.009 (0.002) | 0.011 (0.004) | 0.277 | Down |
| PC ae C30:1 | 0.002 (0.003) | 0.001 (0.000) | 0.776 | Up |  | 0.004 (0.004) | 0.003 (0.004) | 0.549 | Up |  | 0.003 (0.001) | 0.001 (0.001) | <0.001\* | Up |
| PC ae C30:2 | 0.002 (0.002) | 0.011 (0.012) | 0.016\* | Down |  | 0.001 (0.002) | 0.012 (0.011) | 0.002\* | Down |  | 0.001 (0.001) | 0.016 (0.016) | 0.012\* | Down |
| PC ae C32:1 | 0.001 (0.002) | 0.001 (0.001) | 0.158 | Up |  | 0.000 (0.000) | 0.005 (0.012) | 0.077 | Down |  | 0.000 (0.000) | 0.001 (0.001) | 0.288 | Down |
| PC ae C32:2 | . | . | . | . |  | 0.001 (0.001) | 0.002 (0.005) | 0.616 | Down |  | 0.001 (0.001) | 0.001 (0.003) | 0.601 | Down |
| PC ae C34:0 | 0.001 (0.001) | 0.002 (0.002) | 0.361 | Down |  | 0.002 (0.004) | 0.003 (0.005) | 0.411 | Down |  | 0.002 (0.001) | 0.002 (0.002) | 1 | Down |
| PC ae C34:1 | 0.002 (0.003) | 0.002 (0.001) | 0.364 | Up |  | 0.005 (0.011) | 0.012 (0.025) | 0.019\* | Down |  | 0.001 (0.001) | 0.002 (0.003) | 0.112 | Down |
| PC ae C34:2 | 0.001 (0.001) | 0.003 (0.005) | 0.831 | Down |  | 0.002 (0.003) | 0.010 (0.034) | 0.48 | Down |  | 0.001 (0.002) | 0.003 (0.006) | 0.188 | Down |
| PC ae C34:3 | . | . | . | . |  | 0.001 (0.001) | 0.011 (0.028) | 0.014\* | Down |  | 0.001 (0.002) | 0.001 (0.002) | 0.924 | Down |
| PC ae C36:0 | 0.011 (0.013) | 0.009 (0.004) | 0.638 | Up |  | 0.016 (0.026) | 0.011 (0.005) | 0.097 | Up |  | 0.005 (0.003) | 0.009 (0.004) | 0.037\* | Down |
| PC ae C36:1 | 0.008 (0.013) | 0.004 (0.004) | 0.836 | Up |  | 0.008 (0.013) | 0.012 (0.041) | 0.494 | Down |  | 0.003 (0.002) | 0.006 (0.006) | 0.7 | Down |
| PC ae C36:2 | 0.004 (0.006) | 0.006 (0.008) | 0.088 | Down |  | 0.005 (0.008) | 0.017 (0.051) | 0.573 | Down |  | 0.002 (0.002) | 0.007 (0.010) | 0.041\* | Down |
| PC ae C36:3 | 0.003 (0.002) | 0.002 (0.004) | 0.285 | Up |  | 0.001 (0.001) | 0.006 (0.022) | 0.903 | Down |  | 0.002 (0.002) | 0.003 (0.005) | 0.523 | Down |
| PC ae C36:4 | 0.016 (0.018) | 0.004 (0.004) | 0.002\* | Up |  | 0.016 (0.018) | 0.020 (0.073) | 0.013\* | Down |  | 0.014 (0.010) | 0.004 (0.005) | 0.004\* | Up |
| PC ae C36:5 | 0.002 (0.002) | 0.001 (0.001) | 0.51 | Up |  | 0.003 (0.005) | 0.020 (0.050) | 0.059 | Down |  | 0.004 (0.005) | 0.002 (0.002) | 0.828 | Up |
| PC ae C38:0 | 0.026 (0.015) | 0.019 (0.006) | 0.178 | Up |  | 0.045 (0.060) | 0.023 (0.013) | 0.594 | Up |  | 0.022 (0.004) | 0.017 (0.008) | 0.007\* | Up |
| PC ae C38:1 | 0.001 (0.001) | 0.001 (0.003) | 0.784 | Down |  | 0.003 (0.006) | 0.003 (0.011) | 0.393 | Up |  | 0.000 (0.000) | 0.001 (0.002) | 0.013\* | Down |
| PC ae C38:2 | 0.003 (0.006) | 0.001 (0.001) | 0.511 | Up |  | 0.004 (0.009) | 0.006 (0.012) | 0.158 | Down |  | 0.001 (0.001) | 0.001 (0.001) | 0.076 | Down |
| PC ae C38:3 | 0.005 (0.009) | 0.001 (0.001) | 0.801 | Up |  | 0.005 (0.012) | 0.011 (0.026) | 0.082 | Down |  | 0.002 (0.001) | 0.002 (0.002) | 0.1 | Up |
| PC ae C38:4 | 0.005 (0.006) | 0.003 (0.004) | 0.543 | Up |  | 0.004 (0.007) | 0.016 (0.058) | 0.243 | Down |  | 0.002 (0.001) | 0.004 (0.005) | 0.952 | Down |
| PC ae C38:5 | 0.001 (0.001) | 0.002 (0.004) | 0.657 | Down |  | 0.004 (0.008) | 0.014 (0.059) | 0.143 | Down |  | 0.001 (0.002) | 0.003 (0.004) | 0.831 | Down |
| PC ae C38:6 | 0.009 (0.006) | 0.005 (0.003) | 0.054 | Up |  | 0.016 (0.008) | 0.013 (0.035) | 0.003\* | Up |  | 0.013 (0.010) | 0.005 (0.005) | 0.019\* | Up |
| PC ae C40:1 | 0.004 (0.005) | 0.002 (0.001) | 0.802 | Up |  | 0.005 (0.006) | 0.005 (0.010) | 0.975 | Down |  | 0.003 (0.003) | 0.002 (0.002) | 0.664 | Up |
| PC ae C40:2 | 0.002 (0.003) | 0.001 (0.001) | 0.449 | Up |  | 0.003 (0.003) | 0.006 (0.012) | 0.491 | Down |  | 0.001 (0.002) | 0.001 (0.001) | 0.334 | Up |
| PC ae C40:3 | 0.003 (0.003) | 0.001 (0.001) | 0.006\* | Up |  | 0.001 (0.002) | 0.005 (0.012) | 0.08 | Down |  | 0.001 (0.002) | 0.001 (0.001) | 0.975 | Up |
| PC ae C40:4 | 0.005 (0.006) | 0.006 (0.002) | 0.301 | Down |  | 0.006 (0.006) | 0.009 (0.012) | 0.125 | Down |  | 0.003 (0.002) | 0.006 (0.004) | 0.077 | Down |
| PC ae C40:5 | 0.001 (0.002) | 0.001 (0.000) | 0.635 | Up |  | 0.001 (0.003) | 0.008 (0.019) | 0.117 | Down |  | 0.000 (0.001) | 0.001 (0.001) | 0.532 | Down |
| PC ae C40:6 | 0.002 (0.003) | 0.002 (0.001) | 0.683 | Up |  | 0.002 (0.003) | 0.009 (0.021) | 0.056 | Down |  | 0.001 (0.001) | 0.001 (0.001) | 0.291 | Down |
| PC ae C42:0 | 0.035 (0.022) | 0.090 (0.043) | 0.006\* | Down |  | 0.063 (0.080) | 0.097 (0.097) | 0.046\* | Down |  | 0.025 (0.006) | 0.105 (0.088) | 0.001\* | Down |
| PC ae C42:1 | 0.004 (0.003) | 0.008 (0.004) | 0.03\* | Down |  | 0.004 (0.005) | 0.008 (0.007) | 0.055 | Down |  | 0.002 (0.001) | 0.008 (0.008) | 0.016\* | Down |
| PC ae C42:2 | 0.001 (0.001) | 0.001 (0.001) | 0.247 | Down |  | 0.002 (0.003) | 0.002 (0.003) | 0.483 | Down |  | 0.000 (0.000) | 0.002 (0.002) | 0.008\* | Down |
| PC ae C42:3 | . | . | . | . |  | 0.001 (0.002) | 0.002 (0.005) | 0.252 | Down |  | 0.000 (0.001) | 0.000 (0.000) | 0.064 | Down |
| PC ae C42:4 | . | . | . | . |  | 0.000 (0.000) | 0.001 (0.001) | 0.113 | Down |  | 0.000 (0.000) | 0.000 (0.000) | 0.917 | Up |
| PC ae C42:5 | 0.058 (0.045) | 0.073 (0.033) | 0.222 | Down |  | 0.080 (0.110) | 0.072 (0.022) | 0.048\* | Up |  | 0.036 (0.006) | 0.071 (0.037) | 0.006\* | Down |
| PC ae C44:3 | 0.006 (0.005) | 0.002 (0.002) | 0.157 | Up |  | 0.008 (0.005) | 0.003 (0.003) | 0.002\* | Up |  | 0.007 (0.003) | 0.002 (0.002) | 0.001\* | Up |
| PC ae C44:4 | 0.009 (0.006) | 0.006 (0.004) | 0.219 | Up |  | 0.009 (0.012) | 0.008 (0.006) | 0.196 | Up |  | 0.008 (0.003) | 0.008 (0.005) | 0.745 | Down |
| PC ae C44:5 | 0.005 (0.002) | 0.007 (0.003) | 0.246 | Down |  | 0.005 (0.006) | 0.007 (0.006) | 0.074 | Down |  | 0.002 (0.002) | 0.006 (0.002) | 0.007\* | Down |
| PC ae C44:6 | 0.002 (0.002) | 0.003 (0.002) | 0.125 | Down |  | 0.001 (0.001) | 0.003 (0.004) | 0.008\* | Down |  | 0.001 (0.001) | 0.002 (0.002) | 0.05\* | Down |
| **Sphingomyelins** | | | | | | | | | | | | | | |
| SM (OH) C14:1 | 0.001 (0.002) | 0.000 (0.000) | 0.353 | Up |  | 0.001 (0.002) | 0.011 (0.028) | 0.036\* | Down |  | 0.000 (0.001) | 0.000 (0.000) | 0.277 | Up |
| SM (OH) C16:1 | 0.002 (0.002) | 0.001 (0.001) | 0.22 | Up |  | 0.003 (0.005) | 0.007 (0.015) | 0.75 | Down |  | 0.001 (0.001) | 0.001 (0.001) | 0.554 | Down |
| SM (OH) C22:1 | . | . | . | . |  | 0.003 (0.005) | 0.021 (0.053) | 0.033\* | Down |  | 0.002 (0.003) | 0.003 (0.004) | 0.335 | Down |
| SM (OH) C22:2 | 0.002 (0.004) | 0.001 (0.001) | 0.353 | Up |  | 0.006 (0.010) | 0.023 (0.058) | 0.753 | Down |  | 0.001 (0.001) | 0.001 (0.001) | 0.112 | Up |
| SM (OH) C24:1 | 0.001 (0.001) | 0.001 (0.001) | 0.391 | Down |  | 0.003 (0.006) | 0.001 (0.003) | 0.332 | Up |  | 0.001 (0.001) | 0.001 (0.001) | 0.879 | Up |
| SM C16:0 | 0.028 (0.059) | 0.020 (0.037) | 0.095 | Up |  | 0.030 (0.070) | 0.134 (0.544) | 0.136 | Down |  | 0.010 (0.010) | 0.021 (0.042) | 0.7 | Down |
| SM C16:1 | 0.006 (0.010) | 0.003 (0.004) | 0.879 | Up |  | 0.005 (0.009) | 0.020 (0.080) | 0.521 | Down |  | 0.002 (0.001) | 0.003 (0.006) | 0.523 | Down |
| SM C18:0 | 0.008 (0.015) | 0.003 (0.002) | 0.33 | Up |  | 0.002 (0.003) | 0.039 (0.103) | 0.132 | Down |  | 0.003 (0.003) | 0.003 (0.004) | 0.596 | Up |
| SM C18:1 | . | . | . | . |  | 0.002 (0.004) | 0.018 (0.046) | 0.072 | Down |  | 0.001 (0.001) | 0.001 (0.002) | 0.513 | Down |
| SM C20:2 | 0.000 (0.000) | 0.000 (0.000) | 0.518 | Up |  | 0.000 (0.001) | 0.001 (0.002) | 0.227 | Down |  | 0.000 (0.000) | 0.000 (0.000) | 0.357 | Up |
| SM C22:3 | 0.001 (0.001) | 0.001 (0.001) | 0.3 | Down |  | 0.002 (0.002) | 0.003 (0.007) | 0.432 | Down |  | 0.002 (0.002) | 0.001 (0.001) | 0.152 | Up |
| SM C24:0 | 0.019 (0.025) | 0.009 (0.006) | 0.471 | Up |  | 0.019 (0.021) | 0.032 (0.066) | 0.826 | Down |  | 0.008 (0.001) | 0.008 (0.005) | 0.25 | Up |
| SM C24:1 | 0.004 (0.008) | 0.005 (0.006) | 0.109 | Down |  | 0.003 (0.006) | 0.089 (0.233) | 0.004\* | Down |  | 0.003 (0.002) | 0.006 (0.010) | 0.335 | Down |
| SM C26:0 | 0.001 (0.001) | 0.000 (0.000) | 0.117 | Up |  | 0.001 (0.002) | 0.001 (0.000) | 0.06 | Up |  | 0.001 (0.001) | 0.001 (0.001) | 0.779 | Up |
| SM C26:1 | 0.000 (0.000) | 0.000 (0.000) | 0.535 | Up |  | 0.000 (0.000) | 0.000 (0.000) | 0.064 | Down |  | 0.001 (0.001) | 0.000 (0.000) | 0.006\* | Up |
| **Hexose** | | | | | | | | | | | | | | |
| Hexose | 310 (351) | 168 (136) | 0.355 | Up |  | 165 (180) | 128 (107) | 0.745 | Up |  | 511 (496) | 317 (251) | 0.324 | Up |
| **Amino Acids** | | | | | | | | | | | | | | |
| Ala | 14.39 (5.35) | 20.81 (18.06) | 0.533 | Down |  | 12.46 (2.54) | 17.33 (13.32) | 0.533 | Down |  | 39.31 (41.45) | 39.38 (39.02) | 0.836 | Down |
| Arg | 2.62 (0.90) | 1.66 (0.93) | 0.016\* | Up |  | 2.01 (0.68) | 1.63 (1.03) | 0.399 | Up |  | 2.46 (0.95) | 2.23 (1.68) | 0.457 | Up |
| Asn | 4.99 (3.10) | 4.53 (1.93) | 0.882 | Up |  | 7.20 (7.70) | 4.86 (2.70) | 0.7 | Up |  | 5.43 (1.55) | 7.45 (6.64) | 0.657 | Down |
| Asp | 8.05 (2.89) | 5.40 (3.32) | 0.091 | Up |  | 7.86 (2.94) | 5.55 (4.45) | 0.039\* | Up |  | 10.85 (2.52) | 9.26 (6.95) | 0.095 | Up |
| Gln | 43.01 (17.06) | 34.29 (18.94) | 0.268 | Up |  | 47.21 (14.77) | 40.40 (24.75) | 0.139 | Up |  | 54.17 (9.60) | 42.71 (33.23) | 0.062 | Up |
| Glu | 10.07 (4.35) | 15.44 (14.16) | 0.573 | Down |  | 7.58 (4.41) | 13.19 (7.08) | 0.028\* | Down |  | 11.64 (3.40) | 15.73 (12.39) | 0.745 | Down |
| Gly | 21.78 (4.91) | 19.40 (11.21) | 0.388 | Up |  | 20.30 (5.17) | 32.12 (40.95) | 0.657 | Down |  | 55.17 (67.52) | 109.71 (137.55) | 0.388 | Down |
| His | 6.47 (2.70) | 6.29 (2.88) | 0.888 | Up |  | 9.67 (9.80) | 6.87 (4.54) | 0.7 | Up |  | 8.81 (3.05) | 10.47 (8.57) | 0.882 | Down |
| Ser | 12.37 (3.21) | 13.92 (7.96) | 0.976 | Down |  | 12.80 (2.82) | 15.16 (9.31) | 0.976 | Down |  | 23.47 (11.06) | 23.79 (15.28) | 0.882 | Down |
| Thr | 11.98 (4.43) | 10.85 (4.95) | 0.619 | Up |  | 11.06 (4.36) | 11.79 (7.55) | 0.976 | Down |  | 11.32 (4.43) | 11.14 (7.55) | 0.494 | Up |
| Tyr | 4.33 (1.26) | 2.86 (1.52) | 0.039\* | Up |  | 3.83 (1.26) | 3.02 (2.31) | 0.123 | Up |  | 4.21 (1.76) | 2.95 (2.21) | 0.054 | Up |
| **Biogenic Amines** | | | | | | | | | | | | | | |
| ADMA | 4.38 (1.62) | 3.58 (1.99) | 0.377 | Up |  | 2.79 (0.93) | 3.11 (2.35) | 0.79 | Down |  | 2.99 (2.63) | 1.53 (1.49) | 0.072 | Up |
| SDMA | 10.77 (12.14) | 4.54 (1.56) | 0.028\* | Up |  | 16.16 (23.74) | 5.07 (2.50) | 0.108 | Up |  | 11.80 (7.45) | 4.90 (3.69) | 0.001\* | Up |
| Carnosine | 1.67 (0.85) | 1.23 (0.47) | 0.106 | Up |  | 1.45 (0.48) | 1.43 (0.94) | 0.324 | Up |  | 1.28 (0.47) | 1.42 (1.32) | 0.7 | Down |

**1** Direct injection and tandem mass spectrometry with a reverse-phase liquid chromatography and tandem mass

spectrometry

2 Diagnosis of lame Holstein cows (n = 6) ranging from +1 week to +3 week postpartum.

3 n = 6 cases.

4 n = 20 cases.

\* Denotes significantly altered metabolites. Significance was declared at P < 0.05.

Abbreviations: LAM = Lameness; CON = Control; C12-DC = Dodecanedioylcarnitine; C5-M-DC = Methylglutarylcarnitine; C5:1-DC = Glutaconylcarnitine; C7-DC = Pimelylcarnitine; “aa” = signifies that fatty acids are bound at the sn-1 and the sn-2 position; “ae” = indicates that fatty acids are attached to the glycerol backbone via ester bonds; ADMA = Asymmetric Dimethylarginine; SDMA = Symmetric Dimethylarginine.

Supplementary Table S4. Concentration (µM) of Urine Metabolites (Mean (SEM)) in Healthy Control and Lame Cows as Determined by DI/LC-MS/MS1 for Postpartum Time Points.

|  | **+4 weeks after parturition** | | | |  | **+8 weeks after parturition** | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Metabolite** | **LAM*2*** | **CON*3*** | ***p*-value** | **LAM/CON** |  | **LAM** | **CON** | ***p*-value** | **LAM/CON** |
| **Acylcarnitines** | | | | | | | | | |
| C0 | 1.248 (0.831) | 1.200 (0.400) | 0.589 | Up |  | 0.908 (0.211) | 0.803 (0.183) | 0.38 | Up |
| C10 | 0.063 (0.026) | 0.043 (0.020) | 0.178 | Up |  | 0.048 (0.008) | 0.024 (0.006) | 0.30 | Up |
| C10:1 | 0.103 (0.074) | 0.034 (0.019) | 0.009\* | Up |  | 0.068 (0.021) | 0.018 (0.002) | 0.002 | Up |
| C10:2 | 0.027 (0.006) | 0.016 (0.006) | 0.011\* | Up |  | 0.027 (0.008) | 0.011 (0.003) | 0.001 | Up |
| C12 | 0.131 (0.078) | 0.063 (0.026) | 0.09 | Up |  | 0.110 (0.057) | 0.039 (0.021) | 0.026 | Up |
| C12-DC | 0.036 (0.012) | 0.027 (0.012) | 0.214 | Up |  | 0.035 (0.013) | 0.018 (0.005) | 0.015 | Up |
| C12:1 | 0.115 (0.045) | 0.066 (0.058) | 0.13 | Up |  | 0.075 (0.006) | 0.025 (0.008) | 0.50 | Up |
| C14 | 0.016 (0.008) | 0.008 (0.003) | 0.081 | Up |  | 0.013 (0.005) | 0.006 (0.001) | 0.015 | Up |
| C14:1 | 0.011 (0.006) | 0.006 (0.003) | 0.132 | Up |  | 0.011 (0.008) | 0.003 (0.001) | 0.048 | Up |
| C14:1-OH | 0.009 (0.003) | 0.006 (0.002) | 0.063 | Up |  | 0.012 (0.005) | 0.003 (0.001) | 0.011 | Up |
| C14:2 | 0.012 (0.015) | 0.003 (0.001) | 0.31 | Up |  | 0.006 (0.002) | 0.003 (0.001) | 0.005 | Up |
| C14:2-OH | 0.019 (0.018) | 0.012 (0.011) | 0.485 | Up |  | 0.014 (0.011) | 0.005 (0.001) | 0.102 | Up |
| C16 | 0.018 (0.010) | 0.005 (0.002) | 0.025\* | Up |  | 0.015 (0.004) | 0.003 (0.001) | 0.001 | Up |
| C16-OH | 0.011 (0.006) | 0.005 (0.002) | 0.043\* | Up |  | 0.009 (0.005) | 0.003 (0.001) | 0.056 | Up |
| C16:1 | 0.024 (0.009) | 0.017 (0.010) | 0.189 | Up |  | 0.024 (0.015) | 0.013 (0.005) | 0.124 | Up |
| C16:1-OH | 0.008 (0.002) | 0.003 (0.001) | 0.002\* | Up |  | 0.006 (0.003) | 0.002 (0.001) | 0.011 | Up |
| C16:2 | 0.008 (0.005) | 0.003 (0.001) | 0.047\* | Up |  | 0.007 (0.004) | 0.002 (0.001) | 0.004 | Up |
| C16:2-OH | 0.011 (0.004) | 0.006 (0.003) | 0.035\* | Up |  | 0.013 (0.007) | 0.004 (0.002) | 0.009 | Up |
| C18 | 0.008 (0.006) | 0.003 (0.001) | 0.009\* | Up |  | 0.007 (0.004) | 0.002 (0.001) | 0.018 | Up |
| C18:1 | 0.012 (0.010) | 0.004 (0.002) | 0.009\* | Up |  | 0.009 (0.004) | 0.003 (0.001) | 0.021 | Up |
| C18:1-OH | 0.013 (0.007) | 0.007 (0.004) | 0.064 | Up |  | 0.017 (0.006) | 0.005 (0.002) | 0.001 | Up |
| C18:2 | 0.010 (0.004) | 0.003 (0.002) | 0.003\* | Up |  | 0.014 (0.008) | 0.002 (0.001) | 0.019 | Up |
| C2 | 0.208 (0.163) | 0.247 (0.086) | 0.24 | Down |  | 0.119 (0.025) | 0.144 (0.057) | 0.354 | Down |
| C3 | 0.036 (0.026) | 0.031 (0.012) | 0.937 | Up |  | 0.026 (0.008) | 0.022 (0.009) | 0.411 | Up |
| C3-DC (C4-OH) | 0.048 (0.021) | 0.042 (0.016) | 0.624 | Up |  | 0.026 (0.009) | 0.030 (0.014) | 0.564 | Down |
| C3-OH | 0.079 (0.048) | 0.023 (0.006) | 0.034\* | Up |  | 0.075 (0.007) | 0.019 (0.007) | 0.24 | Up |
| C3:1 | 0.026 (0.022) | 0.046 (0.018) | 0.123 | Down |  | 0.015 (0.009) | 0.033 (0.014) | 0.026 | Down |
| C4 | 0.082 (0.069) | 0.063 (0.035) | 0.937 | Up |  | 0.044 (0.026) | 0.049 (0.033) | 0.937 | Down |
| C4:1 | 0.038 (0.023) | 0.054 (0.008) | 0.172 | Down |  | 0.038 (0.019) | 0.032 (0.009) | 0.487 | Up |
| C4:1-DC (C6) | 0.027 (0.017) | 0.020 (0.006) | 0.354 | Up |  | 0.012 (0.005) | 0.016 (0.004) | 0.234 | Down |
| C5 | 0.040 (0.027) | 0.030 (0.012) | 0.399 | Up |  | 0.025 (0.005) | 0.019 (0.010) | 0.18 | Up |
| C5-M-DC | 0.066 (0.036) | 0.023 (0.007) | 0.032\* | Up |  | 0.051 (0.018) | 0.017 (0.005) | 0.005 | Up |
| C5-OH (C3-DC-M) | 0.064 (0.032) | 0.045 (0.012) | 0.211 | Up |  | 0.050 (0.016) | 0.028 (0.005) | 0.021 | Up |
| C5:1 | 0.060 (0.038) | 0.070 (0.020) | 0.589 | Down |  | 0.038 (0.007) | 0.038 (0.007) | 0.96 | Down |
| C5:1-DC | 0.022 (0.008) | 0.016 (0.005) | 0.167 | Up |  | 0.013 (0.003) | 0.011 (0.003) | 0.291 | Up |
| C5-DC (C6-OH) | 0.025 (0.017) | 0.022 (0.006) | 0.709 | Up |  | 0.022 (0.007) | 0.014 (0.005) | 0.058 | Up |
| C6:1 | 0.028 (0.015) | 0.020 (0.004) | 0.262 | Up |  | 0.024 (0.009) | 0.015 (0.003) | 0.067 | Up |
| C7-DC | 0.019 (0.009) | 0.011 (0.005) | 0.112 | Up |  | 0.015 (0.003) | 0.007 (0.002) | 0 | Up |
| C8 | 0.044 (0.021) | 0.024 (0.005) | 0.07 | Up |  | 0.037 (0.008) | 0.016 (0.006) | 0.001 | Up |
| C9 | 0.055 (0.049) | 0.064 (0.033) | 0.485 | Down |  | 0.049 (0.044) | 0.041 (0.017) | 0.662 | Up |
| **Glycerophospholipids (Lysophosphatidylcholines)** | | | | | | | | | |
| lysoPC a C16:0 | 0.072 (0.063) | 0.011 (0.007) | 0.064 | Up |  | 0.041 (0.020) | 0.005 (0.002) | 0.002 | Up |
| lysoPC a C16:1 | 0.028 (0.036) | 0.008 (0.008) | 0.231 | Up |  | 0.020 (0.013) | 0.005 (0.005) | 0.039 | Up |
| lysoPC a C17:0 | 0.014 (0.017) | 0.006 (0.008) | 0.573 | Up |  | 0.006 (0.005) | 0.003 (0.002) | 0.212 | Up |
| lysoPC a C18:0 | 0.075 (0.048) | 0.026 (0.008) | 0.055 | Up |  | 0.043 (0.024) | 0.014 (0.004) | 0.031 | Up |
| lysoPC a C18:1 | 0.055 (0.101) | 0.008 (0.008) | 0.871 | Up |  | 0.017 (0.031) | 0.008 (0.006) | 0.687 | Up |
| lysoPC a C18:2 | 0.070 (0.108) | 0.022 (0.017) | 0.485 | Up |  | 0.025 (0.043) | 0.010 (0.011) | 0.936 | Up |
| lysoPC a C20:3 | 0.049 (0.053) | 0.014 (0.012) | 0.18 | Up |  | 0.028 (0.024) | 0.011 (0.004) | 0.394 | Up |
| lysoPC a C20:4 | 0.080 (0.136) | 0.005 (0.005) | 0.015\* | Up |  | 0.073 (0.138) | 0.002 (0.001) | 0.295 | Up |
| lysoPC a C28:1 | 0.017 (0.016) | 0.002 (0.002) | 0.069 | Up |  | 0.000 (0.000) | 0.001 (0.001) | 0.215 | Down |
| **Glycerophospholipids (Phosphatidylcholines)** | | | | | | | | | |
| PC aa C24:0 | 0.022 (0.021) | 0.005 (0.003) | 0.101 | Up |  | 0.013 (0.005) | 0.005 (0.002) | 0.008 | Up |
| PC aa C28:1 | 0.021 (0.018) | 0.008 (0.004) | 0.026\* | Up |  | 0.011 (0.003) | 0.010 (0.004) | 0.394 | Up |
| PC aa C30:0 | 0.046 (0.045) | 0.013 (0.006) | 0.015\* | Up |  | 0.049 (0.041) | 0.009 (0.004) | 0.066 | Up |
| PC aa C30:2 | 0.005 (0.006) | 0.002 (0.001) | 0.199 | Up |  | 0.007 (0.007) | 0.000 (0.000) | 0.056 | Up |
| PC aa C32:0 | 0.303 (0.595) | 0.001 (0.001) | 0.222 | Up |  | 0.140 (0.266) | 0.001 (0.001) | 0.065 | Up |
| PC aa C32:1 | 0.096 (0.182) | 0.001 (0.001) | 0.222 | Up |  | 0.030 (0.056) | 0.001 (0.001) | 0.044 | Up |
| PC aa C32:2 | 0.013 (0.017) | 0.004 (0.005) | 0.463 | Up |  | 0.012 (0.012) | 0.003 (0.002) | 0.131 | Up |
| PC aa C32:3 | 0.004 (0.009) | 0.000 (0.001) | 1 | Up |  | 0.000 (0.000) | 0.000 (0.000) | 0.088 | Down |
| PC aa C34:1 | 1.133 (2.307) | 0.005 (0.002) | 0.065 | Up |  | 0.505 (0.953) | 0.005 (0.004) | 0.18 | Up |
| PC aa C34:2 | 0.737 (1.465) | 0.009 (0.004) | 0.24 | Up |  | 0.375 (0.670) | 0.011 (0.014) | 0.31 | Up |
| PC aa C34:3 | 0.030 (0.059) | 0.000 (0.000) | 0.222 | Up |  | 0.020 (0.027) | 0.001 (0.001) | 0.222 | Up |
| PC aa C34:4 | 0.010 (0.010) | 0.013 (0.015) | 0.674 | Down |  | 0.005 (0.003) | 0.003 (0.004) | 0.322 | Up |
| PC aa C36:0 | 0.166 (0.223) | 0.032 (0.011) | 0.132 | Up |  | 0.078 (0.068) | 0.028 (0.007) | 0.041 | Up |
| PC aa C36:1 | 0.153 (0.306) | 0.001 (0.001) | 0.199 | Up |  | 0.070 (0.123) | 0.001 (0.001) | 0.378 | Up |
| PC aa C36:2 | 0.285 (0.556) | 0.004 (0.004) | 0.093 | Up |  | 0.136 (0.225) | 0.004 (0.002) | 0.065 | Up |
| PC aa C36:3 | 0.119 (0.228) | 0.003 (0.001) | 0.041\* | Up |  | 0.061 (0.102) | 0.001 (0.001) | 0.109 | Up |
| PC aa C36:4 | 0.338 (0.668) | 0.004 (0.003) | 0.149 | Up |  | 0.192 (0.369) | 0.002 (0.002) | 0.093 | Up |
| PC aa C36:5 | 0.046 (0.092) | 0.001 (0.001) | 0.213 | Up |  | 0.026 (0.051) | 0.001 (0.001) | 0.561 | Up |
| PC aa C36:6 | 0.004 (0.008) | 0.000 (0.001) | 1 | Up |  | 0.006 (0.005) | 0.000 (0.000) | 0.027 | Up |
| PC aa C38:0 | 0.053 (0.071) | 0.006 (0.003) | 0.093 | Up |  | 0.021 (0.025) | 0.002 (0.002) | 0.004 | Up |
| PC aa C38:1 | 0.011 (0.012) | 0.003 (0.004) | 0.209 | Up |  | 0.005 (0.002) | 0.003 (0.001) | 0.036 | Up |
| PC aa C38:3 | 0.036 (0.068) | 0.003 (0.002) | 0.31 | Up |  | 0.023 (0.032) | 0.002 (0.001) | 0.015 | Up |
| PC aa C38:4 | 0.148 (0.263) | 0.005 (0.002) | 0.002\* | Up |  | 0.071 (0.098) | 0.005 (0.002) | 0.015 | Up |
| PC aa C38:5 | 0.140 (0.269) | 0.001 (0.002) | 0.871 | Up |  | 0.056 (0.103) | 0.002 (0.001) | 0.132 | Up |
| PC aa C38:6 | 0.058 (0.108) | 0.002 (0.001) | 0.378 | Up |  | 0.024 (0.042) | 0.005 (0.007) | 0.31 | Up |
| PC aa C40:1 | 0.058 (0.040) | 0.032 (0.013) | 0.18 | Up |  | 0.050 (0.016) | 0.023 (0.009) | 0.005 | Up |
| PC aa C40:2 | 0.005 (0.005) | 0.002 (0.002) | 0.197 | Up |  | 0.003 (0.003) | 0.001 (0.002) | 0.573 | Up |
| PC aa C40:3 | 0.007 (0.009) | 0.002 (0.002) | 0.226 | Up |  | 0.002 (0.001) | 0.000 (0.000) | 0.032 | Up |
| PC aa C40:4 | 0.008 (0.010) | 0.000 (0.000) | 0.104 | Up |  | 0.006 (0.009) | 0.001 (0.001) | 0.222 | Up |
| PC aa C40:5 | 0.014 (0.026) | 0.000 (0.000) | 0.253 | Up |  | 0.010 (0.020) | 0.000 (0.000) | 0.274 | Up |
| PC aa C40:6 | 0.090 (0.066) | 0.023 (0.013) | 0.054 | Up |  | 0.065 (0.032) | 0.018 (0.010) | 0.002 | Up |
| PC aa C42:0 | 0.015 (0.016) | 0.003 (0.003) | 0.093 | Up |  | 0.006 (0.004) | 0.004 (0.005) | 0.628 | Up |
| PC aa C42:1 | 0.004 (0.006) | 0.001 (0.001) | 0.871 | Up |  | 0.002 (0.002) | 0.000 (0.001) | 0.494 | Up |
| PC aa C42:2 | 0.010 (0.008) | 0.008 (0.004) | 0.476 | Up |  | 0.008 (0.006) | 0.007 (0.006) | 0.31 | Up |
| PC aa C42:4 | 0.003 (0.005) | 0.000 (0.001) | 0.326 | Up |  | 0.005 (0.006) | 0.001 (0.001) | 1 | Up |
| PC aa C42:5 | 0.011 (0.017) | 0.000 (0.001) | 0.056 | Up |  | 0.006 (0.007) | 0.002 (0.001) | 0.205 | Up |
| PC aa C42:6 | 0.067 (0.044) | 0.024 (0.014) | 0.015\* | Up |  | 0.042 (0.024) | 0.016 (0.007) | 0.042 | Up |
| PC ae C30:0 | 0.020 (0.009) | 0.012 (0.006) | 0.117 | Up |  | 0.014 (0.007) | 0.010 (0.003) | 0.202 | Up |
| PC ae C30:1 | 0.006 (0.007) | 0.002 (0.001) | 0.212 | Up |  | 0.006 (0.007) | 0.001 (0.001) | 0.065 | Up |
| PC ae C30:2 | 0.005 (0.007) | 0.002 (0.001) | 0.936 | Up |  | 0.001 (0.002) | 0.002 (0.002) | 0.166 | Down |
| PC ae C32:1 | 0.031 (0.058) | 0.000 (0.000) | 0.038\* | Up |  | 0.013 (0.020) | 0.000 (0.000) | 0.104 | Up |
| PC ae C32:2 | 0.016 (0.022) | 0.001 (0.001) | 0.222 | Up |  | 0.004 (0.007) | 0.000 (0.001) | 0.326 | Up |
| PC ae C34:0 | 0.008 (0.016) | 0.001 (0.001) | 0.688 | Up |  | 0.005 (0.003) | 0.000 (0.001) | 0.025 | Up |
| PC ae C34:1 | 0.030 (0.056) | 0.002 (0.002) | 0.468 | Up |  | 0.016 (0.023) | 0.001 (0.001) | 0.054 | Up |
| PC ae C34:2 | 0.236 (0.478) | 0.001 (0.001) | 0.051 | Up |  | 0.094 (0.170) | 0.001 (0.000) | 0.054 | Up |
| PC ae C34:3 | 0.274 (0.558) | 0.000 (0.000) | 0.104 | Up |  | 0.089 (0.173) | 0.000 (0.000) | 0.222 | Up |
| PC ae C36:0 | 0.019 (0.015) | 0.011 (0.006) | 0.233 | Up |  | 0.009 (0.007) | 0.007 (0.005) | 0.394 | Up |
| PC ae C36:1 | 0.037 (0.042) | 0.004 (0.004) | 0.115 | Up |  | 0.017 (0.018) | 0.002 (0.001) | 0.092 | Up |
| PC ae C36:2 | 0.038 (0.063) | 0.002 (0.002) | 0.375 | Up |  | 0.017 (0.023) | 0.003 (0.001) | 0.24 | Up |
| PC ae C36:3 | 0.058 (0.106) | 0.001 (0.001) | 0.054 | Up |  | 0.019 (0.029) | 0.002 (0.001) | 0.149 | Up |
| PC ae C36:4 | 0.094 (0.159) | 0.003 (0.002) | 0.002\* | Up |  | 0.069 (0.114) | 0.004 (0.004) | 0.093 | Up |
| PC ae C36:5 | 0.518 (1.065) | 0.001 (0.001) | 0.37 | Up |  | 0.259 (0.503) | 0.000 (0.000) | 0.222 | Up |
| PC ae C38:0 | 0.083 (0.049) | 0.014 (0.005) | 0.018\* | Up |  | 0.035 (0.006) | 0.016 (0.006) | 0 | Up |
| PC ae C38:1 | 0.030 (0.054) | 0.000 (0.001) | 0.104 | Up |  | 0.003 (0.003) | 0.000 (0.001) | 0.494 | Up |
| PC ae C38:2 | 0.009 (0.011) | 0.001 (0.001) | 0.151 | Up |  | 0.005 (0.004) | 0.000 (0.000) | 0.051 | Up |
| PC ae C38:3 | 0.013 (0.022) | 0.001 (0.001) | 0.104 | Up |  | 0.007 (0.009) | 0.001 (0.001) | 0.378 | Up |
| PC ae C38:4 | 0.017 (0.032) | 0.001 (0.001) | 0.051 | Up |  | 0.016 (0.029) | 0.001 (0.001) | 0.375 | Up |
| PC ae C38:5 | 0.132 (0.264) | 0.001 (0.000) | 0.054 | Up |  | 0.046 (0.089) | 0.001 (0.001) | 0.494 | Up |
| PC ae C38:6 | 0.233 (0.462) | 0.005 (0.003) | 0.065 | Up |  | 0.104 (0.185) | 0.006 (0.002) | 0.31 | Up |
| PC ae C40:1 | 0.008 (0.009) | 0.002 (0.001) | 0.19 | Up |  | 0.002 (0.002) | 0.001 (0.001) | 0.175 | Up |
| PC ae C40:2 | 0.007 (0.008) | 0.000 (0.001) | 0.025\* | Up |  | 0.002 (0.002) | 0.002 (0.004) | 0.687 | Up |
| PC ae C40:3 | 0.005 (0.005) | 0.000 (0.000) | 0.073 | Up |  | 0.005 (0.003) | 0.001 (0.001) | 0.02 | Up |
| PC ae C40:4 | 0.007 (0.004) | 0.004 (0.004) | 0.262 | Up |  | 0.005 (0.005) | 0.004 (0.002) | 0.725 | Up |
| PC ae C40:5 | 0.010 (0.019) | 0.000 (0.000) | 0.326 | Up |  | 0.001 (0.001) | 0.000 (0.000) | 0.182 | Up |
| PC ae C40:6 | 0.020 (0.034) | 0.001 (0.002) | 0.09 | Up |  | 0.010 (0.018) | 0.001 (0.001) | 0.678 | Up |
| PC ae C42:0 | 0.074 (0.030) | 0.059 (0.029) | 0.384 | Up |  | 0.063 (0.011) | 0.054 (0.022) | 0.36 | Up |
| PC ae C42:1 | 0.007 (0.009) | 0.005 (0.003) | 0.65 | Up |  | 0.007 (0.009) | 0.005 (0.003) | 0.81 | Up |
| PC ae C42:2 | 0.002 (0.001) | 0.000 (0.001) | 0.104 | Up |  | 0.002 (0.003) | 0.001 (0.001) | 0.432 | Up |
| PC ae C42:3 | 0.005 (0.009) | 0.000 (0.000) | 0.182 | Up |  | 0.002 (0.002) | 0.000 (0.000) | 0.104 | Up |
| PC ae C42:4 | . | . | . | . |  | . | . | . | . |
| PC ae C42:5 | 0.107 (0.054) | 0.072 (0.037) | 0.217 | Up |  | 0.094 (0.014) | 0.048 (0.018) | 0.001 | Up |
| PC ae C44:3 | 0.004 (0.004) | 0.002 (0.002) | 0.375 | Up |  | 0.006 (0.006) | 0.003 (0.002) | 0.222 | Up |
| PC ae C44:4 | 0.023 (0.015) | 0.008 (0.005) | 0.052 | Up |  | 0.016 (0.007) | 0.004 (0.001) | 0.008 | Up |
| PC ae C44:5 | 0.007 (0.002) | 0.007 (0.006) | 0.485 | Down |  | 0.004 (0.003) | 0.007 (0.003) | 0.11 | Down |
| PC ae C44:6 | 0.003 (0.002) | 0.001 (0.001) | 0.174 | Up |  | 0.003 (0.003) | 0.001 (0.001) | 0.186 | Up |
| **Sphingomyelins** | | | | | | | | | |
| SM (OH) C14:1 | 0.006 (0.009) | 0.001 (0.001) | 0.158 | Up |  | 0.047 (0.090) | 0.000 (0.000) | 0.034 | Up |
| SM (OH) C16:1 | 0.013 (0.014) | 0.001 (0.002) | 0.103 | Up |  | 0.075 (0.144) | 0.000 (0.001) | 0.158 | Up |
| SM (OH) C22:1 | 0.021 (0.032) | 0.001 (0.001) | 0.295 | Up |  | 0.078 (0.150) | 0.000 (0.000) | 0.678 | Up |
| SM (OH) C22:2 | 0.009 (0.017) | 0.000 (0.001) | 0.326 | Up |  | 0.136 (0.270) | 0.000 (0.000) | 0.158 | Up |
| SM (OH) C24:1 | 0.003 (0.006) | 0.000 (0.001) | 0.532 | Up |  | 0.002 (0.001) | 0.001 (0.001) | 0.051 | Up |
| SM C16:0 | 0.195 (0.383) | 0.003 (0.003) | 0.078 | Up |  | 0.743 (1.327) | 0.002 (0.001) | 0.295 | Up |
| SM C16:1 | 0.013 (0.021) | 0.001 (0.002) | 0.213 | Up |  | 0.454 (0.905) | 0.001 (0.001) | 0.222 | Up |
| SM C18:0 | 0.079 (0.160) | 0.002 (0.001) | 0.871 | Up |  | 0.377 (0.639) | 0.000 (0.001) | 0.004 | Up |
| SM C18:1 | 0.025 (0.044) | 0.001 (0.002) | 0.326 | Up |  | 0.094 (0.180) | 0.000 (0.001) | 0.326 | Up |
| SM C20:2 | 0.004 (0.005) | 0.000 (0.001) | 0.158 | Up |  | 0.001 (0.001) | 0.001 (0.001) | 0.864 | Up |
| SM C22:3 | 0.002 (0.003) | 0.000 (0.001) | 0.532 | Up |  | 0.001 (0.002) | 0.000 (0.001) | 0.803 | Up |
| SM C24:0 | 0.071 (0.100) | 0.009 (0.005) | 0.485 | Up |  | 0.283 (0.494) | 0.007 (0.003) | 0.065 | Up |
| SM C24:1 | 0.093 (0.177) | 0.002 (0.002) | 0.336 | Up |  | 0.192 (0.281) | 0.000 (0.000) | 0.034 | Up |
| SM C26:0 | 0.001 (0.001) | 0.002 (0.004) | 0.655 | Down |  | 0.001 (0.003) | 0.001 (0.001) | 0.934 | Up |
| SM C26:1 | 0.002 (0.002) | 0.000 (0.000) | 0.182 | Up |  | 0.001 (0.003) | 0.000 (0.001) | 0.599 | Up |
| **Hexose** | | | | | | | | | |
| Hexose | 382.830 (336.870) | 340.847 (107.336) | 0.781 | Up |  | 430.284 (327.604) | 218.833 (102.644) | 0.31 | Up |
| **Amino Acids** | | | | | | | | | |
| Ala | 188.785 (209.329) | 95.228 (49.189) | 0.331 | Up |  | 105.595 (61.515) | 59.306 (42.245) | 0.16 | Up |
| Arg | 4.501 (1.933) | 4.301 (1.924) | 0.861 | Up |  | 4.985 (1.472) | 2.908 (1.206) | 0.026\* | Up |
| Asn | 9.027 (4.312) | 11.126 (3.717) | 0.388 | Down |  | 8.459 (3.039) | 5.631 (1.976) | 0.085 | Up |
| Asp | 45.491 (50.981) | 24.671 (22.303) | 0.394 | Up |  | 21.669 (11.599) | 14.594 (9.936) | 0.24 | Up |
| Gln | 48.138 (42.308) | 96.033 (48.197) | 0.097 | Down |  | 83.474 (60.152) | 44.943 (29.456) | 0.189 | Up |
| Glu | 78.674 (107.987) | 40.438 (24.117) | 0.937 | Up |  | 24.350 (15.137) | 20.500 (11.842) | 0.818 | Up |
| Gly | 136.038 (78.605) | 317.795 (141.928) | 0.015\* | Down |  | 124.453 (89.159) | 230.661 (185.570) | 0.235 | Down |
| His | 14.516 (4.916) | 23.086 (9.007) | 0.068 | Down |  | 18.432 (8.575) | 12.725 (4.527) | 0.18 | Up |
| Ser | 29.084 (10.712) | 44.854 (18.200) | 0.097 | Down |  | 35.051 (13.093) | 23.070 (5.752) | 0.067 | Up |
| Thr | 18.003 (9.361) | 25.282 (8.958) | 0.199 | Down |  | 23.430 (15.487) | 14.938 (10.030) | 0.286 | Up |
| Tyr | 7.801 (2.659) | 5.093 (1.891) | 0.07 | Up |  | 8.388 (2.337) | 3.071 (1.542) | 0.001\* | Up |
| **Biogenic Amines** | | | | | | | | | |
| ADMA | 1.258 (0.578) | 2.385 (1.596) | 0.153 | Down |  | 1.779 (1.098) | 2.568 (1.644) | 0.351 | Down |
| SDMA | 16.779 (17.815) | 8.368 (4.097) | 0.394 | Up |  | 18.089 (23.479) | 5.807 (1.339) | 0.132 | Up |
| Carnosine | 2.566 (1.101) | 2.866 (1.102) | 0.648 | Down |  | 2.916 (1.619) | 1.435 (0.396) | 0.076 | Up |

**1** Direct injection and tandem mass spectrometry with a reverse-phase liquid chromatography and tandem mass spectrometry.

2 n = 6 cases of lame Holstein cows.

3 n = 20 cases of healthy Holstein cows.

\* Denotes significantly altered metabolites. Significance was declared at P < 0.05.

Abbreviations: LAM = Lameness; CON = Control; C12-DC = Dodecanedioylcarnitine; C5-M-DC = Methylglutarylcarnitine; C5:1-DC = Glutaconylcarnitine; C7-DC = Pimelylcarnitine; “aa” = signifies that fatty acids are bound at the sn-1 and the sn-2 position; “ae” = indicates that fatty acids are attached to the glycerol backbone via ester bonds; ADMA = Asymmetric Dimethylarginine; SDMA = Symmetric Dimethylarginine.