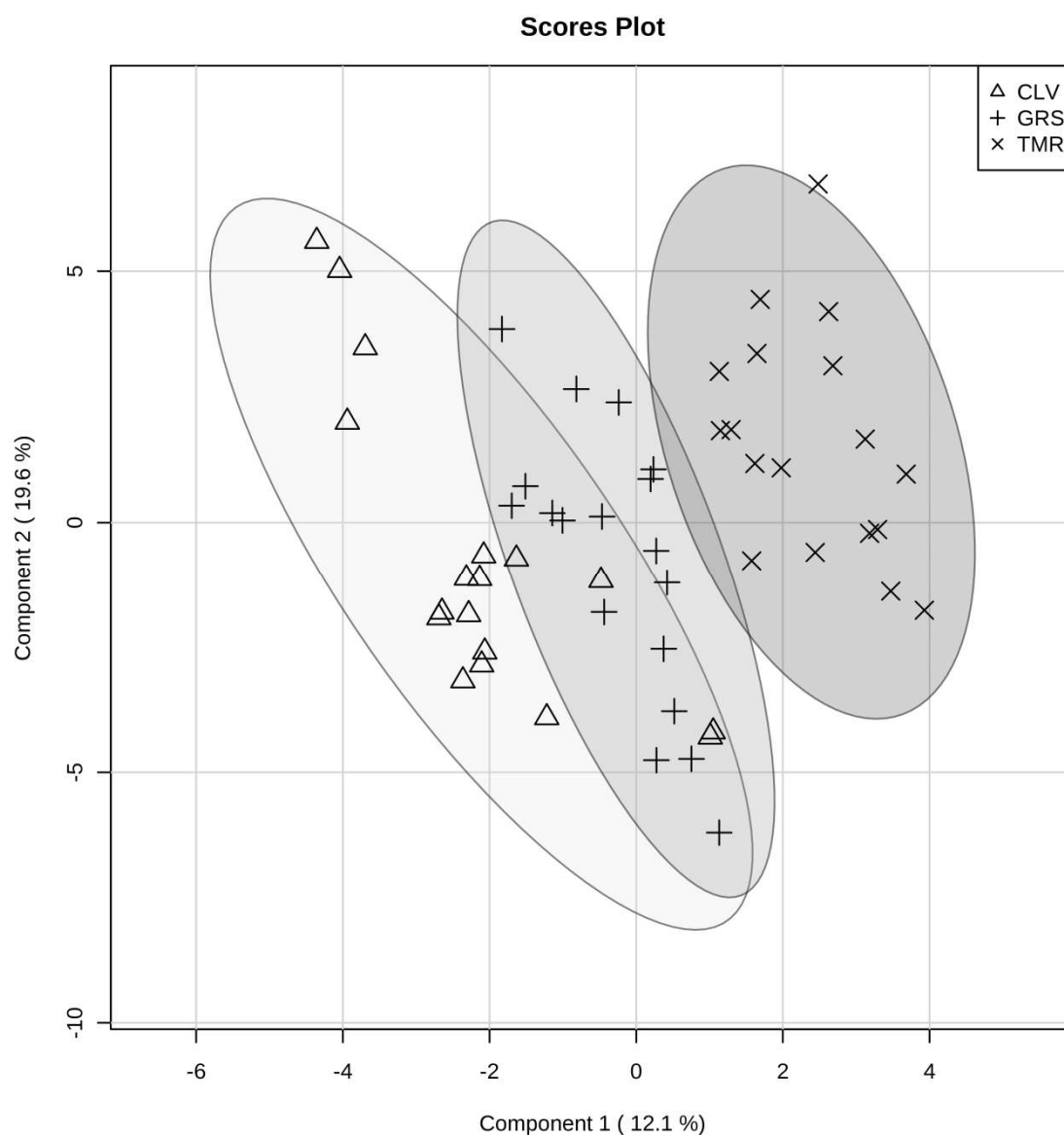


# Supplementary Materials: Pasture Feeding Changes the Bovine Rumen and Milk Metabolome

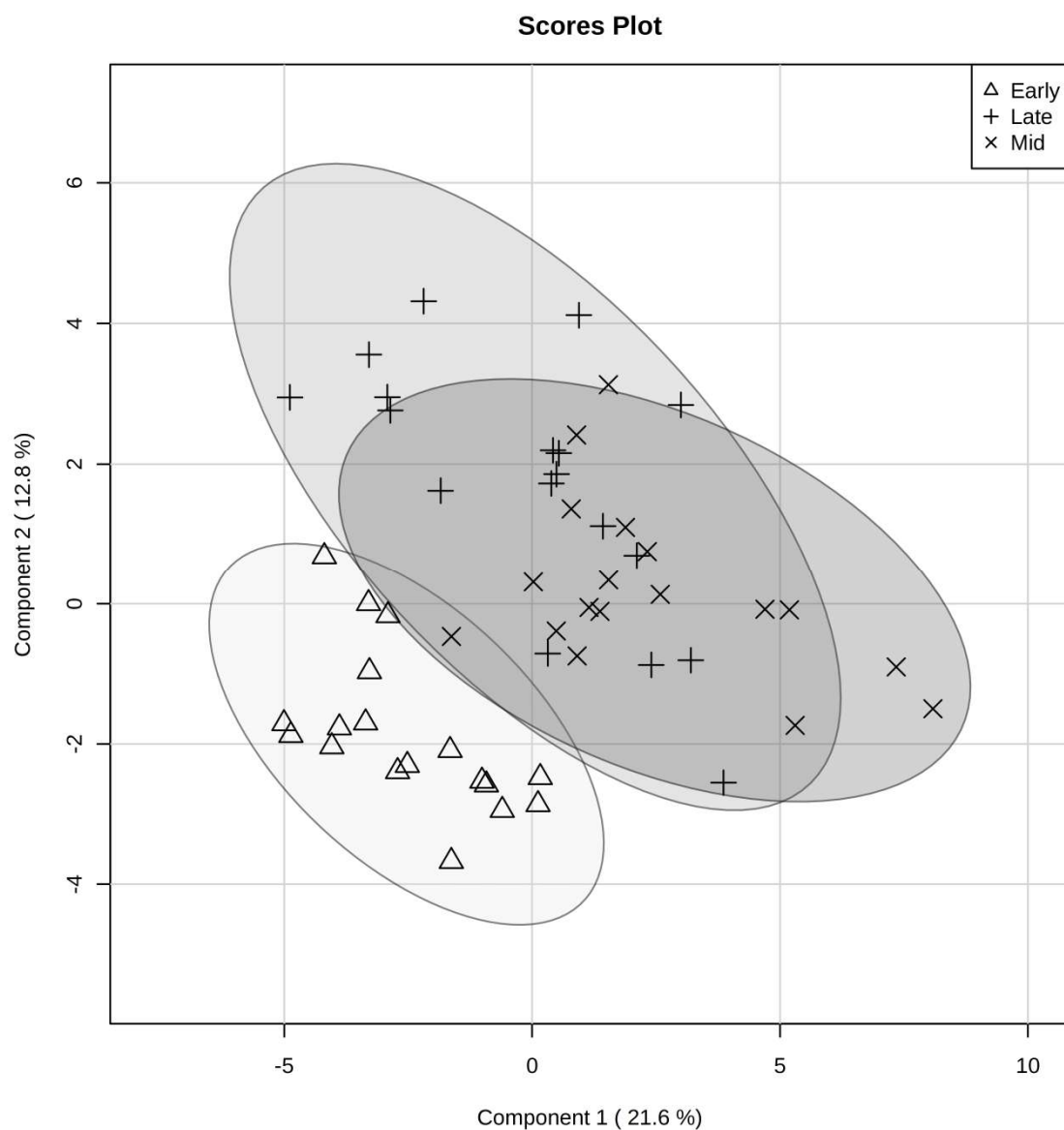
**Supplementary Table S1.** Average concentrations of rumen metabolites ( $\mu\text{M}$ ) measured in the rumen of lactating dairy cows fed diets consisting of total mixed ration (TMR), perennial ryegrass (GRS) or perennial ryegrass and white clover (CLV) throughout each stage of lactation early mid and late as determined by  $^1\text{H}$ -NMR.

|                              | Early-Lactation |           |           | Mid-Lactation |           |           | Late-Lactation |           |           | SEM    |
|------------------------------|-----------------|-----------|-----------|---------------|-----------|-----------|----------------|-----------|-----------|--------|
| Metabolite ( $\mu\text{M}$ ) | TMR             | GRS       | CLV       | TMR           | GRS       | CLV       | TMR            | GRS       | CLV       |        |
| 2-Hydroxyisovalerate         | 4.57            | 8.48      | 8.53      | 10.71         | 5.74      | 5.50      | 5.89           | 4.29      | 8.44      | 0.74   |
| 3-Hydroxybutyric acid        | 6.80            | 7.83      | 9.22      | 7.55          | 10.85     | 12.63     | 23.98          | 7.35      | 16.05     | 1.56   |
| 3-Hydroxyphenylacetic acid   | 23.01           | 27.90     | 22.28     | 36.44         | 26.53     | 27.88     | 20.68          | 12.80     | 18.39     | 1.20   |
| 3-Phenylpropionate           | 779.59          | 717.96    | 554.52    | 761.80        | 704.73    | 759.75    | 696.33         | 476.13    | 588.32    | 19.66  |
| 4-Aminobutyrate              | 22.71           | 49.42     | 36.95     | 36.29         | 50.38     | 56.43     | 50.16          | 56.67     | 52.93     | 3.56   |
| Acetic acid                  | 56,450.48       | 55,371.36 | 57,153.50 | 59,611.64     | 55,080.78 | 59,988.47 | 49,825.50      | 54,056.92 | 57,825.81 | 834.44 |
| Acetoin                      | 20.89           | 20.64     | 21.65     | 25.69         | 22.45     | 25.61     | 29.64          | 21.00     | 28.60     | 1.10   |
| Acetone                      | 6.14            | 8.42      | 6.62      | 9.86          | 8.84      | 14.48     | 10.00          | 8.34      | 15.01     | 0.61   |
| Adenine                      | 29.17           | 31.67     | 42.77     | 29.28         | 35.09     | 37.18     | 20.86          | 11.59     | 11.68     | 2.06   |
| Adenosine                    | 3.27            | 6.70      | 3.14      | 6.29          | 6.40      | 8.43      | 4.09           | 7.24      | 3.67      | 0.60   |
| Aspartate                    | 115.08          | 96.53     | 122.66    | 144.89        | 158.90    | 217.53    | 199.76         | 92.07     | 103.86    | 8.45   |
| Benzoic acid                 | 25.42           | 30.23     | 28.39     | 25.50         | 27.81     | 26.28     | 20.71          | 20.91     | 27.73     | 0.82   |
| Beta Alanine                 | 7.38            | 11.08     | 7.21      | 14.03         | 27.64     | 29.24     | 33.88          | 16.00     | 15.88     | 2.00   |
| Betaine                      | 9.76            | 4.38      | 3.98      | 7.73          | 8.03      | 4.07      | 3.23           | 2.83      | 3.35      | 0.96   |
| Butyrate                     | 11,884.27       | 15,544.54 | 13,741.89 | 13,676.94     | 14,568.74 | 16,162.84 | 12,194.68      | 12,622.88 | 14,281.63 | 352.66 |
| Cadaverine                   | 53.46           | 128.58    | 109.88    | 82.03         | 93.26     | 107.44    | 111.32         | 67.68     | 103.96    | 7.00   |
| Choline                      | 20.93           | 11.82     | 13.28     | 20.48         | 16.65     | 15.76     | 36.26          | 15.57     | 8.73      | 1.62   |
| <i>cis</i> -Aconitate        | 4.19            | 9.40      | 4.99      | 7.88          | 11.74     | 8.08      | 4.66           | 4.24      | 9.71      | 0.90   |
| Citric acid                  | 3.31            | 5.53      | 5.41      | 11.23         | 9.21      | 7.85      | 7.85           | 7.85      | 6.61      | 0.66   |
| Creatine                     | 7.71            | 6.76      | 7.08      | 9.90          | 8.13      | 6.92      | 8.46           | 5.90      | 6.79      | 0.66   |
| D-Glucose                    | 426.25          | 844.25    | 784.76    | 755.50        | 641.33    | 913.27    | 380.15         | 149.02    | 108.05    | 55.14  |
| Dimethyl sulfone             | 6.04            | 19.23     | 37.43     | 3.03          | 14.60     | 36.46     | 2.43           | 16.59     | 26.23     | 2.00   |
| Dimethylamine                | 1.68            | 3.14      | 2.08      | 8.06          | 1.82      | 9.18      | 3.02           | 1.97      | 3.13      | 0.96   |
| Dimethylglycine              | 7.72            | 7.09      | 4.91      | 15.24         | 4.68      | 8.22      | 19.87          | 1.88      | 8.60      | 2.07   |
| D-Maltose                    | 124.21          | 58.00     | 43.83     | 31.74         | 29.01     | 39.19     | 57.68          | 16.20     | 17.03     | 6.30   |
| Ethanol                      | 12.53           | 112.58    | 27.92     | 21.71         | 37.59     | 42.91     | 42.65          | 27.70     | 24.28     | 8.88   |
| Ethanolamine                 | 28.92           | 32.07     | 34.83     | 37.34         | 37.35     | 43.58     | 21.87          | 15.35     | 10.03     | 2.07   |
| Formate                      | 115.52          | 114.01    | 116.01    | 121.81        | 113.23    | 119.66    | 118.16         | 117.49    | 118.62    | 0.60   |
| Glycerol                     | 209.23          | 220.06    | 219.03    | 286.20        | 270.45    | 294.14    | 269.32         | 236.48    | 233.55    | 6.28   |

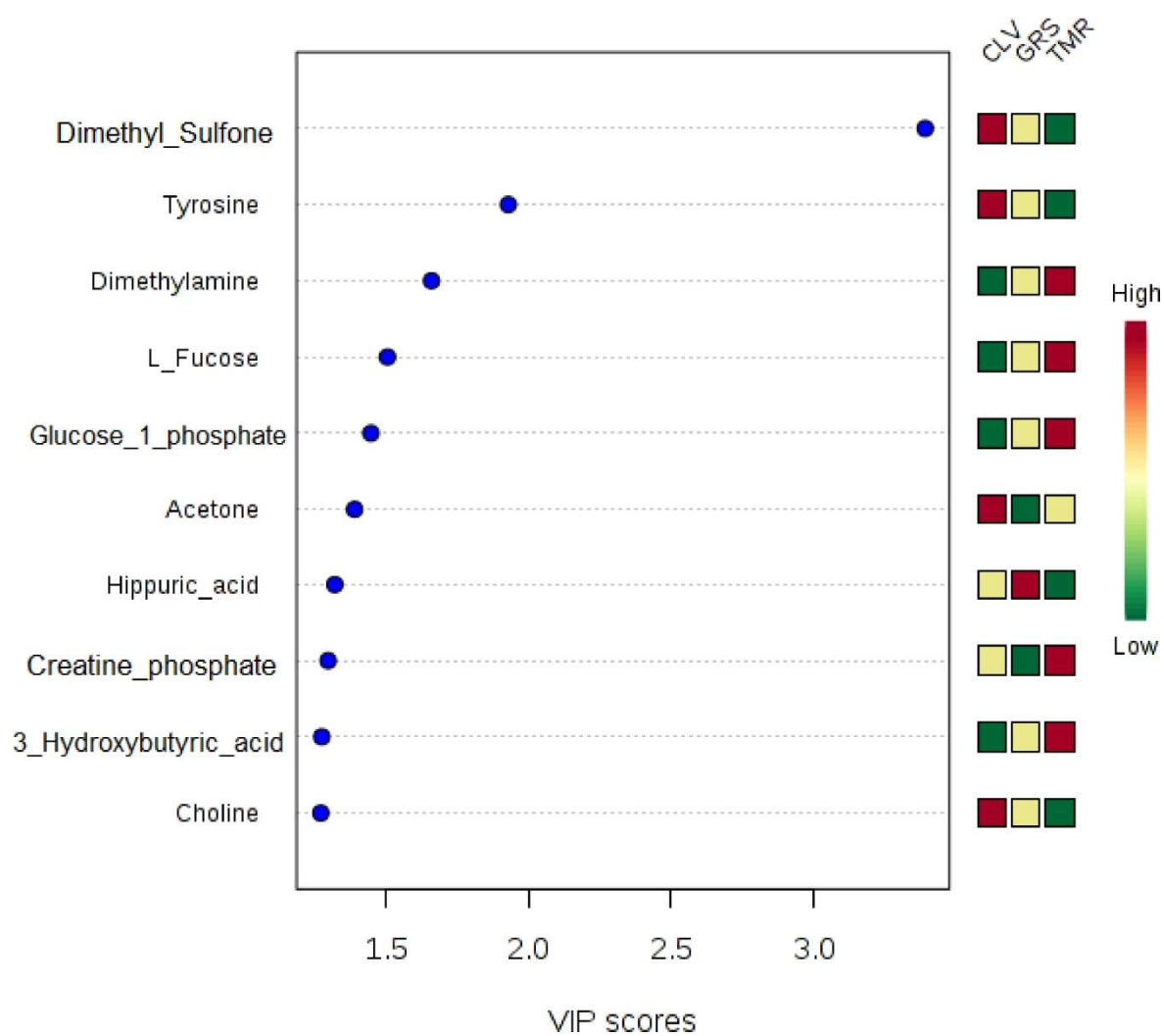
|                                    |           |           |           |           |           |           |           |           |           |        |
|------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--------|
| Glycine                            | 86.27     | 104.25    | 135.53    | 131.22    | 156.95    | 210.20    | 146.98    | 96.28     | 129.71    | 6.69   |
| Hypoxanthine                       | 161.40    | 169.98    | 213.58    | 190.70    | 203.38    | 213.12    | 161.39    | 104.28    | 111.55    | 7.38   |
| Inosine                            | 12.98     | 50.25     | 38.24     | 13.83     | 23.43     | 22.48     | 7.76      | 8.04      | 5.71      | 2.63   |
| Isobutyric acid                    | 808.14    | 745.82    | 775.18    | 818.37    | 859.50    | 1156.38   | 763.78    | 830.53    | 1061.15   | 21.81  |
| Isoleucine                         | 62.98     | 61.93     | 91.98     | 90.16     | 112.33    | 131.68    | 100.18    | 76.12     | 93.63     | 4.58   |
| Isopropanol                        | 16.71     | 23.59     | 26.03     | 19.85     | 26.48     | 63.61     | 17.92     | 16.67     | 36.89     | 2.57   |
| Isovaleric acid                    | 711.27    | 600.31    | 605.88    | 671.78    | 754.82    | 1187.49   | 667.28    | 748.80    | 1059.89   | 30.43  |
| L-Glutamic acid                    | 242.93    | 261.69    | 366.75    | 311.73    | 298.38    | 403.02    | 327.27    | 196.13    | 281.97    | 12.09  |
| L-Alanine                          | 139.00    | 189.28    | 211.52    | 206.31    | 231.38    | 306.74    | 195.89    | 148.08    | 198.38    | 8.72   |
| L-Histidine                        | 22.80     | 36.49     | 27.76     | 32.86     | 33.98     | 28.78     | 43.23     | 39.58     | 46.94     | 1.84   |
| L-Lactic acid                      | 14.46     | 20.63     | 18.28     | 26.22     | 18.61     | 36.30     | 51.30     | 48.99     | 27.90     | 4.02   |
| L-Leucine                          | 72.92     | 79.11     | 101.35    | 96.06     | 117.16    | 153.91    | 100.20    | 85.28     | 103.47    | 4.49   |
| L-Lysine                           | 143.95    | 170.81    | 176.06    | 202.61    | 240.94    | 323.59    | 102.05    | 109.71    | 118.18    | 12.56  |
| L-Phenylalanine                    | 42.16     | 43.90     | 50.09     | 58.58     | 68.03     | 78.82     | 53.72     | 40.00     | 44.38     | 2.53   |
| L-Proline                          | 77.17     | 67.54     | 95.11     | 86.45     | 107.08    | 161.58    | 89.36     | 66.88     | 68.31     | 6.43   |
| L-Threonine                        | 67.54     | 67.70     | 91.85     | 117.76    | 110.78    | 139.98    | 140.72    | 77.84     | 88.00     | 5.34   |
| Methanol                           | 10.76     | 9.68      | 11.35     | 10.78     | 10.37     | 13.90     | 19.68     | 9.01      | 9.08      | 1.17   |
| Methionine                         | 26.29     | 32.48     | 38.10     | 37.99     | 45.61     | 51.26     | 36.60     | 27.32     | 32.93     | 1.65   |
| Methylamine                        | 1.40      | 18.28     | 4.95      | 3.12      | 8.82      | 41.14     | 9.41      | 7.08      | 2.66      | 3.05   |
| Nicotinate                         | 24.98     | 35.88     | 42.51     | 31.36     | 30.60     | 37.12     | 20.97     | 19.21     | 28.18     | 1.33   |
| O-Hydroxyphenylacetic acid         | 15.75     | 12.81     | 11.63     | 20.66     | 14.62     | 21.82     | 15.73     | 11.82     | 13.53     | 0.62   |
| <i>p</i> -Cresol                   | 55.29     | 43.48     | 37.74     | 57.63     | 75.38     | 110.54    | 62.23     | 78.99     | 107.27    | 4.00   |
| Phenylacetate                      | 202.23    | 159.84    | 157.23    | 194.39    | 318.03    | 536.45    | 202.11    | 308.47    | 449.38    | 20.18  |
| <i>p</i> -Hydroxyphenylacetic acid | 17.39     | 15.03     | 14.67     | 16.88     | 15.52     | 12.45     | 19.29     | 13.32     | 14.19     | 0.62   |
| Propionate                         | 17,764.88 | 20,803.31 | 18,280.66 | 19,344.51 | 17,120.38 | 17,982.38 | 14,248.39 | 16,330.55 | 18,159.29 | 463.30 |
| Putrescine                         | 64.55     | 89.79     | 59.37     | 65.59     | 53.28     | 48.96     | 46.63     | 26.36     | 28.37     | 4.77   |
| Succinate                          | 48.88     | 40.93     | 43.84     | 64.01     | 82.49     | 107.18    | 257.82    | 100.28    | 121.76    | 11.31  |
| Trimethylamine                     | 1.94      | 1.64      | 1.35      | 2.10      | 2.67      | 13.55     | 13.35     | 1.25      | 1.30      | 1.33   |
| Tryptophan                         | 6.21      | 7.33      | 8.28      | 7.97      | 9.41      | 10.69     | 7.34      | 5.42      | 5.59      | 0.37   |
| Tyrosine                           | 32.46     | 33.49     | 47.99     | 48.98     | 57.79     | 72.48     | 51.37     | 39.38     | 47.68     | 2.38   |
| Uracil                             | 225.92    | 287.28    | 350.22    | 285.07    | 364.46    | 373.89    | 90.48     | 140.81    | 168.73    | 14.57  |
| Uridine                            | 5.86      | 12.35     | 9.84      | 7.33      | 6.15      | 4.83      | 9.02      | 3.97      | 11.75     | 0.97   |
| Valerate                           | 1,020.82  | 1,715.66  | 1,141.45  | 1,201.26  | 1,212.65  | 1,611.75  | 869.19    | 842.36    | 1,231.22  | 62.85  |
| Valine                             | 71.25     | 71.60     | 92.41     | 106.05    | 118.54    | 191.78    | 103.48    | 81.25     | 102.18    | 6.42   |



**Supplementary Figure S1.** Partial least square discriminant analysis (PLS-DA) score plot of the rumen metabolome of lactating dairy cows fed diets consisting of total mixed ration (TMR), perennial ryegrass (GRS) or perennial ryegrass and white clover (CLV) as determined by <sup>1</sup>H-NMR. The shaded ellipses represent the 95% confidence interval estimated from the scores.



**Supplementary Figure S2.** Score plot of the partial least square discriminant analysis (PLS-DA) examining the effect of stage of lactation on the rumen metabolome of lactating dairy cows fed separate diets collected throughout each stage of lactation early, mid and late, as determined by  $^1\text{H}$ -NMR. The shaded ellipses represent the 95% confidence interval estimated from the scores.



**Supplementary Figure 3.** Variable importance plot (VIP) which shows the compounds primarily responsible for separation of raw milk metabolomes from cows fed diets consisting of total mixed ration (TMR), perennial ryegrass (GRS) or perennial ryegrass and white clover (CLV) for the PLS-DA model.