

Table S1. The list of ruminal fluid metabolites significantly differences between the low crude protein diet and the normal crude protein diet

| Metabolites | Score | MZ | Retention time (s) | Ion mode | VIP | p-value ¹⁾ | Fold Change ²⁾ |
|--|-------|--------|--------------------|----------|-------|-----------------------|---------------------------|
| Yohimbine | 0.332 | 335.18 | 42.23 | NEG | 1.878 | 0.00 | 0.650 |
| Vincamine | 0.495 | 355.20 | 374.18 | POS | 1.616 | 0.04 | 0.719 |
| Phenylbutazone | 0.335 | 307.15 | 42.20 | NEG | 1.734 | 0.02 | 0.803 |
| Tamsulosin | 0.404 | 407.17 | 322.07 | NEG | 2.016 | 0.01 | 0.801 |
| 3-Phenylpropanoic acid | 0.879 | 149.06 | 94.20 | NEG | 2.271 | 0.00 | 0.693 |
| Vanylglycol | 0.420 | 165.05 | 305.52 | NEG | 1.902 | 0.02 | 0.605 |
| Gemfibrozil | 0.500 | 249.15 | 46.23 | NEG | 1.803 | 0.01 | 0.589 |
| Pyrocatechol | 0.478 | 152.07 | 117.62 | POS | 1.849 | 0.05 | 0.813 |
| Phentermine | 0.352 | 337.20 | 41.22 | POS | 1.682 | 0.04 | 0.761 |
| 12-Oxo-2,3-dinor-10,15-phytodienoic acid | 0.362 | 301.12 | 61.87 | NEG | 1.561 | 0.05 | 0.844 |
| 20-hydroxy-PGF2a | 0.610 | 369.22 | 140.14 | NEG | 1.822 | 0.03 | 0.833 |
| Hexadecanedioic acid | 0.484 | 345.23 | 139.01 | NEG | 2.040 | 0.02 | 0.826 |
| Pimelic acid | 0.872 | 159.07 | 328.85 | NEG | 1.871 | 0.02 | 0.824 |
| Azelaic acid | 0.986 | 187.10 | 306.54 | NEG | 1.870 | 0.02 | 0.811 |
| Suberic acid | 0.905 | 173.08 | 319.56 | NEG | 2.164 | 0.01 | 0.783 |
| Pindone | 0.450 | 229.09 | 157.72 | NEG | 2.000 | 0.01 | 0.771 |
| Adipic acid | 0.878 | 141.02 | 419.64 | NEG | 1.783 | 0.04 | 0.762 |
| Sebacic acid | 0.977 | 201.11 | 292.01 | NEG | 2.042 | 0.01 | 0.754 |
| 5-Hydroxyhexanoic acid | 0.881 | 131.07 | 210.91 | NEG | 2.019 | 0.00 | 0.657 |
| Caprylic acid | 0.448 | 159.10 | 154.75 | NEG | 2.107 | 0.00 | 0.630 |
| Stearoylcarnitine | 0.983 | 428.37 | 148.99 | POS | 1.841 | 0.03 | 1.991 |

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|--|-------|--------|--------|-----|-------|------|-------|
| Glycerol 1-myristate | 0.730 | 285.24 | 44.46 | POS | 1.888 | 0.01 | 1.459 |
| Exemestane | 0.303 | 373.09 | 261.28 | POS | 2.092 | 0.01 | 0.801 |
| Citramalic acid | 0.386 | 148.04 | 39.52 | POS | 1.352 | 0.04 | 0.757 |
| Stearidonic Acid | 0.845 | 277.22 | 86.09 | POS | 2.105 | 0.01 | 0.703 |
| 1-Palmitoyl-2-linoleoyl-sn-glycero-3-phosphate | 0.958 | 671.46 | 150.49 | NEG | 1.625 | 0.05 | 0.732 |
| 6k-PGF1alpha-d4 | 0.809 | 373.26 | 275.40 | NEG | 1.961 | 0.01 | 0.574 |
| 1-Stearoyl-sn-glycerol | 0.820 | 379.28 | 90.70 | NEG | 1.747 | 0.02 | 1.157 |
| DL-Homocysteine | 0.467 | 153.07 | 314.78 | POS | 1.830 | 0.04 | 0.894 |
| Arg-Met | 0.381 | 270.13 | 368.28 | POS | 1.874 | 0.03 | 0.873 |
| Met-Ser | 0.378 | 300.10 | 265.98 | POS | 1.801 | 0.03 | 0.829 |
| Vigabatrin | 0.473 | 171.11 | 347.85 | POS | 1.610 | 0.02 | 0.822 |
| His-Val | 0.372 | 318.16 | 314.77 | POS | 2.363 | 0.00 | 0.801 |
| gamma-L-Glu-epsilon-L-Lys | 0.411 | 339.16 | 339.96 | POS | 1.947 | 0.01 | 0.788 |
| Trans-4-Hydroxy-L-proline | 0.621 | 96.04 | 49.99 | POS | 1.850 | 0.04 | 0.784 |
| DL-a-Hydroxybutyric acid | 0.457 | 168.07 | 42.83 | POS | 1.886 | 0.02 | 0.772 |
| Scopoline | 0.633 | 156.10 | 48.97 | POS | 1.652 | 0.02 | 0.752 |
| N2-Acetyl-L-ornithine | 0.500 | 349.20 | 41.22 | POS | 2.319 | 0.00 | 0.742 |
| Phenylacetyl glycine | 0.498 | 176.07 | 301.79 | POS | 2.145 | 0.01 | 0.730 |
| N-Acetyl-L-tyrosine | 0.446 | 224.09 | 274.40 | POS | 1.952 | 0.02 | 0.710 |
| D-Alanyl-D-alanine (D-Ala-D-Ala) | 0.499 | 143.08 | 135.97 | POS | 1.937 | 0.02 | 0.702 |
| Hexanoyl glycine | 0.359 | 347.22 | 28.49 | POS | 1.872 | 0.01 | 0.695 |
| Argininosuccinic acid | 0.303 | 313.11 | 226.92 | POS | 1.622 | 0.02 | 0.682 |
| N1-Acetylspermine | 0.361 | 305.25 | 201.08 | POS | 1.929 | 0.01 | 0.673 |
| alpha-Ketocaproic acid | 0.417 | 259.12 | 322.32 | NEG | 1.405 | 0.05 | 0.687 |

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|------------------------|-------|--------|--------|-----|-------|------|-------|
| 3-Methylthiopropionate | 0.453 | 162.06 | 309.35 | POS | 1.812 | 0.03 | 0.815 |
| His-Ser | 0.496 | 243.11 | 330.86 | POS | 2.265 | 0.00 | 0.755 |
| His-Thr | 0.302 | 256.12 | 287.22 | POS | 1.709 | 0.03 | 0.753 |
| Pro-Trp | 0.497 | 284.13 | 323.48 | POS | 1.637 | 0.02 | 0.746 |
| Val-Phe | 0.402 | 325.18 | 398.44 | POS | 1.865 | 0.02 | 0.738 |
| Thr-Ser | 0.617 | 248.12 | 363.96 | POS | 2.083 | 0.01 | 0.720 |
| Ala-Lys | 0.914 | 218.15 | 444.30 | POS | 1.810 | 0.02 | 0.714 |
| Lys-Gly | 0.626 | 221.17 | 222.42 | POS | 1.874 | 0.04 | 0.418 |
| L-Sorbose | 0.311 | 161.04 | 348.65 | NEG | 1.827 | 0.01 | 0.768 |
| D-Mannitol 1-phosphate | 0.616 | 321.17 | 41.57 | NEG | 2.015 | 0.03 | 0.694 |
| 3-Hexanone | 0.500 | 121.07 | 47.66 | NEG | 1.762 | 0.03 | 0.560 |
| L-Threonate | 0.446 | 136.03 | 26.12 | POS | 1.866 | 0.03 | 1.068 |
| Glycerol | 0.698 | 171.01 | 379.58 | NEG | 2.072 | 0.01 | 0.812 |
| Allantoin | 0.444 | 158.04 | 79.43 | NEG | 1.736 | 0.04 | 0.824 |
| Temazepam | 0.316 | 316.09 | 294.27 | NEG | 1.911 | 0.05 | 0.702 |
| Saccharin | 0.339 | 183.00 | 65.36 | NEG | 2.070 | 0.00 | 0.656 |
| Ethosuximide | 0.378 | 162.05 | 46.15 | NEG | 1.943 | 0.04 | 0.516 |
| Pyridostigmine cation | 0.473 | 245.11 | 373.43 | POS | 1.657 | 0.04 | 0.865 |
| 5-Methoxyindoleacetate | 0.499 | 266.10 | 247.62 | POS | 1.873 | 0.04 | 0.812 |
| Benomyl | 0.369 | 290.14 | 377.48 | POS | 1.710 | 0.03 | 0.797 |
| Donepezil | 0.455 | 312.15 | 224.44 | POS | 2.031 | 0.01 | 0.749 |
| 4-Pyridoxic acid | 0.996 | 184.06 | 39.52 | POS | 1.575 | 0.02 | 0.722 |
| Oxindole | 0.964 | 134.06 | 40.18 | POS | 1.664 | 0.04 | 0.679 |
| Indoleacetic acid | 0.915 | 176.07 | 55.57 | POS | 2.209 | 0.01 | 0.739 |

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|---|-------|--------|--------|-----|-------|------|-------|
| Clomipramine | 0.480 | 260.10 | 266.10 | POS | 1.704 | 0.04 | 0.826 |
| Pyridoxine | 0.995 | 170.08 | 117.85 | POS | 1.711 | 0.03 | 0.663 |
| DL-3-Phenyllactic acid | 0.652 | 165.05 | 46.03 | NEG | 1.948 | 0.01 | 0.722 |
| Equol | 0.316 | 207.08 | 140.01 | POS | 1.640 | 0.04 | 0.857 |
| 2,5-Dihydroxycinnamic acid methyl ester | 0.394 | 236.09 | 214.37 | POS | 2.063 | 0.01 | 0.779 |
| norpropoxyphene | 0.320 | 290.20 | 280.32 | POS | 1.788 | 0.02 | 0.778 |
| Coumarin | 0.401 | 369.39 | 24.60 | POS | 1.940 | 0.04 | 0.575 |
| trans-cinnamate | 0.384 | 209.08 | 128.29 | POS | 2.150 | 0.01 | 0.829 |
| Adynerin | 0.797 | 515.30 | 119.27 | NEG | 1.847 | 0.02 | 0.870 |

MZ, mass-to-charge ratio; NEG, negative; POS, positive; VIP, variable importance in projection

¹)p -values were calculated according to Student's T-test (n=6).

²) If the fold change value is less than 1, it means that there is less metabolite in the low crude protein group than in the normal crude protein group.