***Animal***

**Supplementation of a clay mineral based product modulates plasma metabolomic profile and liver enzymes in cattle fed grain-rich diets**

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**Supplementary Table S1.** Analyzed chemical composition of forage, concentrate and the grain-rich diet fed to dairy cows during the intermittent subacute ruminal acidosis (SARA)-challenges.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Feedstuff | | |
| Variables1 | Forage2 | Concentrate3 | SARA-diet4 |
| % of dry matter (DM) unless otherwise stated | | |  |
| DM, % of fresh matter | 49.9 | 89.5 | 75.6 |
| Ash | 8.5 | 4.4 | 5.8 |
| CP | 12.9 | 16.3 | 15.1 |
| Ether extract | 1.6 | 3. | 2.5 |
| NDF | 54.0 | 15.8 | 29.2 |
| ADF | 34.5 | 7.4 | 16.9 |
| NFC | 23.0 | 60.5 | 47.4 |

1CP = crude protein at the duodenum; NDF = neutral detergent fiber; ADF = acid detergent fiber; NFC = 100 – (NDF % + CP % + ether extract % + ash %).  
2Consisted of 50% hay and 50% grass silage (DM-basis).  
3Contained 33% barley grain, 30% wheat, 16% rapeseed meal, 15% corn, 3.2% beet pulp, 1% mineral-vitamin premix (containing 13.5% Ca, 9% Mg, 5% P, 1.5% Na, 2100000 IU vitamin A/kg, 300000 IU vitamin D/kg, 7500 mg vitamin E/kg), 1% beet molasses, 0.5% calcium carbonate and 0.3% NaCl.  
4Contained 65% concentrate and 35% forage (DM-basis).

**Supplementary Table S2.** Influence of a clay mineral based product (CM) on the concentration of blood metabolites in dairy cows subjected to a subacute ruminal acidosis (SARA)-feeding phase with 65% concentrates for 1 wk. Only metabolites that were not affected by the feed additive are presented.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Metabolite1 | CON2 | CM | SEM | *P*-Value |
| Amino acids, µM |  |  |  |  |
| Alanine | 183 | 188 | 12.7 | 0.75 |
| Asparagine | 27.5 | 33.4 | 2.56 | 0.18 |
| Aspartate | 5.54 | 11.5 | 8.032 | 0.60 |
| Citrulline | 34.3 | 41.0 | 8.82 | 0.55 |
| Glutamine | 363 | 391 | 27.3 | 0.40 |
| Glutamate | 56.4 | 60.4 | 3.40 | 0.42 |
| Leucine | 221 | 260 | 23.76 | 0.17 |
| Ornithine | 84.3 | 93.0 | 9.04 | 0.50 |
| Phenylalanine | 45.5 | 57.9 | 16.70 | 0.60 |
| Proline | 79.0 | 90.3 | 7.87 | 0.30 |
| Serine | 99.5 | 111 | 8.64 | 0.35 |
| Tryptophan | 49.6 | 55.2 | 5.29 | 0.43 |
| Tyrosine | 42.7 | 48.7 | 4.41 | 0.35 |
| Valine | 335 | 395 | 38.8 | 0.21 |
|  |  |  |  |  |
| Biogenic Amines, µM |  |  |  |  |
| Acetylornithine |  |  |  |  |
| Symmetric dimethylarginine | 0.239 | 0.304 | 0.6292 | 0.94 |
| alpha-Aminoadipic acid | 1.86 | 1.74 | 0.249 | 0.69 |
| Kynurenine | 3.52 | 3.69 | 0.518 | 0.76 |
| Putrescine | 0.093 | 0.110 | 0.6117 | 0.98 |
| Sarcosine | 0.90 | 1.10 | 2.579 | 0.96 |
| Spermidine | 0.076 | 0.106 | 0.0181 | 0.26 |
| Serotonin | 1.33 | 1.63 | 0.376 | 0.56 |
| cis-4-Hydroxyproline | -176E-19 | -176E-19 | 0.3788 | 1.00 |
| trans-4-Hydroxyproline | 8.51 | 8.99 | 1.145 | 0.65 |
| Creatinine | 69.9 | 70.0 | 5.75 | 0.99 |
| Carnosine | 14.2 | 13.1 | 1.22 | 0.46 |
| Taurine | 46.5 | 52.0 | 4.77 | 0.41 |
|  |  |  |  |  |
| Acylcarnitines, µM |  |  |  |  |
| C0 | 18.8 | 21.6 | 1.86 | 0.20 |
| C3:1 | 0.016 | 0.019 | 0.0015 | 0.20 |
| C4:1 | 0.023 | 0.023 | 0.0020 | 1.00 |
| C5-DC (C6-OH) | 0.011 | 0.011 | 0.0017 | 1.00 |
| C5-M-DC | 0.023 | 0.023 | 0.0019 | 1.00 |
| C5-OH (C3-DC-M) | 0.036 | 0.038 | 0.0036 | 0.72 |
| C5:1-DC | 0.010 | 0.010 | 0.0019 | 1.00 |
| C6 (C4:1-DC) | 0.053 | 0.048 | 0.0031 | 0.26 |
| C6:1 | 0.026 | 0.025 | 0.0020 | 0.66 |
| C7-DC | 0.011 | 0.010 | 0.0020 | 0.65 |
| C8 | 0.084 | 0.078 | 0.0038 | 0.26 |
| C9 | 0.010 | 0.010 | 0.0025 | 1.00 |
| C10:1 | 0.070 | 0.070 | 0.0058 | 1.00 |
| C12-DC | 0.080 | 0.074 | 0.0040 | 0.28 |
| C12:1 | 0.055 | 0.061 | 0.0049 | 0.31 |
| C14 | 0.011 | 0.010 | 0.0014 | 0.46 |
| C14:1 | 0.018 | 0.020 | 0.0019 | 0.30 |
| C14:2 | 0.0075 | 0.0075 | 0.00165 | 1.00 |
| C16 | 0.014 | 0.016 | 0.0021 | 0.31 |
| C16:1 | 0.020 | 0.023 | 0.0013 | 0.17 |
| C16:2 | 0.0013 | -926E-19 | 0.3075 | 1.00 |
| C16:2-OH | 0.011 | 0.010 | 1.2747 | 1.00 |
| C18 | 0.0100 | 0.0113 | 0.06998 | 0.99 |
| C18:1 | 0.061 | 0.068 | 0.2229 | 0.98 |
| C18:1-OH | 0.0100 | 0.0100 | 1.5431 | 1.00 |
| C18:2 | 0.0025 | 0.0063 | 0.7066 | 1.00 |
|  |  |  |  |  |
| Lysophosphatidylcholines, µM | |  |  |  |
| |  | | --- | | lysoPC a C14:0 | | 4.82 | 4.98 | 0.549 | 0.83 |
| |  | | --- | | lysoPC a C16:0 | | 8.93 | 11.82 | 1.781 | 0.27 |
| lysoPC a C16:1 | 0.911 | 1.043 | 0.1499 | 0.53 |
| |  | | --- | | lysoPC a C17:0 | | 1.24 | 1.39 | 0.3099 | 0.71 |
| |  | | --- | | lysoPC a C18:0 | | 8.61 | 10.03 | 2.053 | 0.63 |
| |  | | --- | | lysoPC a C18:1 | | 6.47 | 6.97 | 1.4061 | 0.81 |
| |  | | --- | | lysoPC a C18:2 | | 7.50 | 7.81 | 1.904 | 0.90 |
| lysoPC a C20:3 | 1.29 | 1.22 | 0.318 | 0.84 |
| |  | | --- | | lysoPC a C20:4 | | 1.38 | 1.35 | 0.352 | 0.94 |
| |  | | --- | | lysoPC a C24:0 | | 0.636 | 0.600 | 0.0232 | 0.20 |
| |  | | --- | | lysoPC a C26:0 | | 0.066 | 0.046 | 0.0518 | 0.78 |
| lysoPC a C26:1 | 0.030 | 0.046 | 0.0810 | 0.89 |
| |  | | --- | | lysoPC a C28:0 | | 0.166 | 0.145 | 0.0274 | 0.54 |
| |  | | --- | | lysoPC a C28:1 | | 0.183 | 0.115 | 0.1695 | 0.78 |
|  |  |  |  |  |
| Phosphatidylcholines, µM |  |  |  |  |
| PC aa C24:0 | 0.039 | 0.051 | 0.1415 | 0.92 |
| PC aa C26:0 | 0.334 | 0.408 | 0.1580 | 0.74 |
| PC aa C28:1 | 0.660 | 0.700 | 0.3540 | 0.93 |
| PC aa C30:0 | 1.09 | 1.20 | 1.473 | 0.96 |
| PC aa C30:2 | 0.554 | 0.153 | 2.6334 | 0.92 |
| PC aa C32:0 | 1.88 | 1.98 | 1.290 | 0.96 |
| PC aa C32:1 | 1.77 | 2.03 | 0.237 | 0.12 |
| PC aa C32:2 | 1.98 | 2.17 | 0.302 | 0.39 |
| PC aa C32:3 | 5.12 | 5.83 | 2.459 | 0.82 |
| PC aa C34:1 | 14.2 | 19.7 | 4.55 | 0.30 |
| PC aa C34:3 | 4.43 | 5.60 | 1.000 | 0.40 |
| PC aa C34:4 | 2.17 | 2.39 | 0.120 | 0.41 |
| PC aa C36:0 | 1.78 | 1.71 | 0.473 | 0.91 |
| PC aa C36:1 | 20.3 | 25.6 | 4.76 | 0.37 |
| PC aa C36:2 | 46.5 | 52.0 | 8.41 | 0.64 |
| PC aa C36:3 | 20.9 | 24.7 | 3.30 | 0.42 |
| PC aa C36:5 | 2.23 | 2.82 | 0.808 | 0.58 |
| PC aa C36:6 | 1.33 | 1.45 | 0.111 | 0.42 |
| PC aa C38:0 | 0.593 | 0.603 | 0.137 | 0.96 |
| PC aa C38:1 | 1.29 | 1.38 | 0.369 | 0.79 |
| PC aa C38:3 | 16.4 | 18.9 | 2.72 | 0.46 |
| PC aa C38:4 | 15.9 | 20.0 | 2.63 | 0.27 |
| PC aa C40:1 | 0.190 | 0.200 | 0.0155 | 0.66 |
| PC aa C40:2 | 0.114 | 0.105 | 0.0167 | 0.70 |
| PC aa C40:3 | 1.58 | 1.47 | 0.379 | 0.79 |
| PC aa C40:4 | 4.71 | 5.40 | 1.003 | 0.58 |
| PC aa C40:5 | 9.94 | 12.7 | 1.652 | 0.24 |
| PC aa C40:6 | 3.19 | 3.99 | 0.423 | 0.18 |
| PC aa C42:0 | 0.036 | 0.038 | 0.0209 | 0.97 |
| PC aa C42:1 | 0.028 | 0.038 | 0.0223 | 0.84 |
| PC aa C42:2 | 0.069 | 0.064 | 0.0197 | 0.84 |
| PC aa C42:5 | 0.528 | 0.510 | 0.1409 | 0.87 |
| PC aa C42:6 | 0.306 | 0.314 | 0.0859 | 0.94 |
| PC ae C30:0 | 0.225 | 0.220 | 0.3332 | 0.99 |
| PC ae C30:1 | 0.463 | 0.203 | 0.377 | 0.62 |
| PC ae C30:2 | 0.335 | 0.319 | 0.396 | 0.98 |
| PC ae C32:1 | 0.826 | 0.756 | 0.0723 | 0.26 |
| PC ae C32:2 | 1.36 | 1.30 | 0.551 | 0.93 |
| PC ae C34:0 | 0.596 | 0.543 | 0.8320 | 0.96 |
| PC ae C34:1 | 2.69 | 2.94 | 0.447 | 0.48 |
| PC ae C34:2 | 3.47 | 3.71 | 0.398 | 0.67 |
| PC ae C34:3 | 4.73 | 5.01 | 0.613 | 0.74 |
| PC ae C36:0 | 0.583 | 0.568 | 0.0711 | 0.88 |
| PC ae C36:1 | 3.77 | 4.31 | 0.908 | 0.66 |
| PC ae C36:2 | 7.84 | 8.12 | 1.439 | 0.89 |
| PC ae C36:3 | 2.61 | 2.61 | 0.477 | 1.00 |
| PC ae C36:4 | 2.25 | 2.26 | 0.198 | 0.96 |
| PC ae C36:5 | 2.09 | 2.21 | 0.166 | 0.60 |
| PC ae C38:0 | 0.515 | 0.564 | 0.0694 | 0.48 |
| PC ae C38:1 | 0.711 | 0.748 | 0.1410 | 0.85 |
| PC ae C38:2 | 0.995 | 1.03 | 0.1929 | 0.89 |
| PC ae C38:3 | 1.90 | 2.08 | 0.307 | 0.66 |
| PC ae C38:4 | 1.82 | 2.09 | 0.318 | 0.56 |
| PC ae C38:5 | 1.51 | 1.65 | 0.146 | 0.51 |
| PC ae C38:6 | 1.88 | 1.52 | 0.139 | 0.44 |
| PC ae C40:1 | 0.154 | 0.144 | 0.0164 | 0.60 |
| PC ae C40:2 | 0.475 | 0.504 | 0.0553 | 0.69 |
| PC ae C40:3 | 0.509 | 0.534 | 0.0661 | 0.76 |
| PC ae C40:4 | 0.606 | 0.706 | 0.1113 | 0.51 |
| PC ae C40:5 | 1.36 | 1.58 | 0.213 | 0.47 |
| PC ae C40:6 | 0.579 | 0.614 | 0.0444 | 0.52 |
| PC ae C42:0 | 0.371 | 0.400 | 0.0280 | 0.44 |
| PC ae C42:1 | 0.108 | 0.0988 | 0.00902 | 0.46 |
| PC ae C42:2 | 0.0813 | 0.0800 | 0.00676 | 0.89 |
| PC ae C42:3 | 0.0675 | 0.0688 | 0.0088 | 0.92 |
| PC ae C42:4 | 0.0250 | 0.0313 | 0.3318 | 0.99 |
| PC ae C42:5 | 0.793 | 0.781 | 0.3152 | 0.98 |
| PC ae C44:3 | 0.0263 | 0.0263 | 0.4721 | 1.00 |
| PC ae C44:4 | 0.0488 | 0.0500 | 0.2761 | 1.00 |
| PC ae C44:5 | 0.0563 | 0.0638 | 0.06279 | 0.93 |
| PC ae C44:6 | 0.0352 | 0.0375 | 2.5281 | 1.00 |
|  |  |  |  |  |
| Sphingomyelins, µM |  |  |  |  |
| SM (OH) C14:1 | 3.59 | 3.69 | 0.329 | 0.78 |
| SM (OH) C16:1 | 4.27 | 4.04 | 0.405 | 0.66 |
| SM (OH) C22:1 | 5.78 | 6.48 | 0.542 | 0.34 |
| SM (OH) C22:2 | 3.30 | 3.44 | 0.396 | 0.80 |
| SM (OH) C24:1 | 0.958 | 1.03 | 0.1029 | 0.64 |
| SM C16:0 | 33.1 | 35.3 | 3.295 | 0.56 |
| SM C16:1 | 4.41 | 4.44 | 0.418 | 0.93 |
| SM C18:0 | 3.87 | 3.77 | 0.394 | 0.84 |
| SM C18:1 | 1.73 | 1.85 | 0.168 | 0.57 |
| SM C20:2 | 0.035 | 0.038 | 0.0023 | 1.00 |
| SM C22:3 | 0.0163 | 0.0200 | 0.0077 | 0.72 |
| SM C24:0 | 9.27 | 9.28 | 0.895 | 0.99 |
| SM C24:1 | 6.17 | 6.65 | 0.621 | 0.33 |
| SM C26:0 | 0.280 | 0.256 | 0.0226 | 0.36 |
| SM C26:1 | 0.283 | 0.306 | 0.0395 | 0.50 |
|  |  |  |  |  |
| Sum of hexoses, µM | 4744 | 5051 | 180 | 0.14 |

1C = carnitine; lysoPC = lysophosphatidylcholine; PC = phosphatidylcholine; aa = diacyl; ae = acyl-alkyl; SM = sphingomyelin.

**2**CON = control.