Supplementary Material

# Supplementary Figures and Tables

## Supplementary Figures



**Supplementary Figure 1.** Heatmap for the expression levels of important AAs, FAs, metabolites and bacteria in SG, BG and CG.

## Supplementary Tables

**Supplementary Table 1.** Evaluation parameters of OPLS-DA models in the positive ion mode.

|  |  |  |  |
| --- | --- | --- | --- |
|  | R2X (cum) | R2Y (cum) | Q2 (cum) |
| SG vs BG | 0.655 | 0.989 | 0.898 |
| SG vs CG | 0.424 | 0.994 | 0.906 |
| BG vs CG | 0.746 | 0.998 | 0.709 |

**Supplementary Table 2.** Effect of different feeding redimes on alpha diversity of rumen bacteria of Black Tibetan sheep.

|  |  |  |  |
| --- | --- | --- | --- |
| Items | Groups | SEM | *P*-value |
|  | SG | BG | CG |  |  |
| shannon | 6.49b | 7.53a | 6.03b | 0.26 | < 0.01 |
| simpson | 0.95b | 0.98a | 0.92c | 0.01 | < 0.01 |
| chao1 | 1513.78a | 1583.17a | 1321.34b | 57.93 | < 0.01 |
| ace | 1565.03a | 1658.75a | 1381.06b | 60.61 | < 0.01 |

a, b, c different superscripts within a row indicate significantly different values for *P*-value < 0.05.

**Supplementary Table 3.** DMs in the key metabolic pathways between the three comparisons (SG vs BG; SG vs CG; BG vs CG) in the *longissimus lumborum* of Black Tibetan sheep.

|  |  |
| --- | --- |
| metabolic pathways | metabolites  |
| SG vs BG |
| upregulation in the SG group | upregulation/downregulation in the SG group |
| Pentose phosphate pathway | glyceric acid, pyruvate, D-erythrose 4-phosphate / D-glucosaminic acid |
| Phenylalanine metabolism | phenaceturic acid, 3-hydroxyphenylacetic acid, pyruvate / hippuric acid |
| Arginine and proline metabolism | pyruvate, urea, L-hydroxyarginine, creatinine / |
| Taurine and hypotaurine metabolism | pyruvate, taurine / |
| Valine, leucine, and isoleucine biosynthesis | pyruvate, L-isoleucine / |
| Carbohydrate digestion and absorption | maltotriose, D-lactose / |
| D-Alanine metabolism | Pyruvate / |
| Ascorbate and aldarate metabolism | pyruvate, L-ascorbic acid / |
| Glycine, serine, and threonine metabolism | glyceric acid, pyruvate / |
| Phosphotransferase system (PTS) | D-lactose, pyruvate, L-ascorbic acid / D-glucosaminic acid |
| HIF-1 signaling pathway | pyruvate, L-ascorbic acid / |
| SG vs CG |
| upregulation in the SG group | upregulation/downregulation in the SG group |
| Carbohydrate digestion and absorption | maltotriose, D-lactose, D-glucose 6-phosphate / |
| Arginine and proline metabolism | L-hydroxyarginine, urea, creatinine / |
| Starch and sucrose metabolism | isomaltose, D-glucose 6-phosphate / |
| Phosphotransferase system (PTS) | D-mannose 6-phosphate, D-lactose, D-glucose 6-phosphate / D-glucosaminic acid |
| Thyroid hormone synthesis | glutathione, D-glucose 6-phosphate / |
| downregulation in the SG group | upregulation/downregulation in the SG group |
| Bile secretion | glutathione / chenodeoxycholate, choline, L-carnitine |
| BG vs CG |
| upregulation in the CG group | upregulation/downregulation in the CG group |
| Taurine and hypotaurine metabolism | taurocholate, pyruvate, taurine / |
| Valine, leucine, and isoleucine biosynthesis | pyruvate, L-isoleucine / |
| Pentose phosphate pathway | pyruvate, glyceric acid / |
| Valine, leucine, and isoleucine degradation | methylmalonic acid, L-isoleucine / |
| D-Alanine metabolism | pyruvate / |
| Ascorbate and aldarate metabolism | L-ascorbic acid, pyruvate / |
| Sphingolipid metabolism | ceramide (d18:1/18:1(9Z)), sphingomyelin (d18:1/18:0) / |
| Primary bile acid biosynthesis | taurocholate, glycocholic acid, taurine / |
| Secondary bile acid biosynthesis | taurocholate, glycocholic acid / |
| Cholesterol metabolism | taurocholate, glycocholic acid / |
| HIF-1 signaling pathway | L-ascorbic acid, pyruvate / |
| downregulation in the CG group | upregulation/downregulation in the CG group |
| beta-Alanine metabolism | / pantothenate, aspartic acid, anserine |
| Histidine metabolism | / aspartic acid, anserine |

**Supplementary Table 4.** Effect of different feeding modes on the AA composition in the *longissimus lumborum* of Black Tibetan sheep (μg/g tissue).

|  |  |  |  |
| --- | --- | --- | --- |
| Items | Groups | SEM | *P*-value |
|  | SG | BG | CG |  |  |
| glutamate | 47.35  | 36.38  | 79.45  | 31.88  | 0.43  |
| glycine | 323.20  | 362.28  | 280.03  | 53.15  | 0.37  |
| lysine | 44.70  | 44.18  | 35.36  | 7.16  | 0.40  |
| aspartate | 8.22  | 71.68  | 17.84  | 26.31  | 0.10  |
| arginine | 108.46  | 66.26  | 90.77  | 14.61  | 0.07  |
| serine | 55.42  | 49.75  | 41.19  | 6.28  | 0.15  |
| methionine | 5.87a  | 4.07b  | 5.71a  | 0.35  | < 0.01  |
| phenylalanine | 44.25a  | 26.90b  | 39.47a  | 3.22  | < 0.01  |
| tyrosine | 47.02a  | 27.64b  | 38.63ab  | 5.86  | 0.04  |
| leucine | 74.33a  | 47.42b  | 66.43a  | 5.74  | < 0.01  |
| isoleucine | 23.84a  | 17.04b  | 22.02a  | 1.32  | < 0.01  |
| histidine | 68.88  | 97.71  | 95.37  | 23.46  | 0.44  |
| proline | 77.08a  | 40.81b  | 35.98b  | 10.40  | 0.01  |
| valine | 54.07a  | 37.86b  | 43.56b  | 4.26  | 0.02  |
| threonine | 57.63  | 22.92  | 45.43  | 12.53  | 0.08  |
| alanine | 542.20  | 276.66  | 496.99  | 120.95  | 0.14  |
| asparagine | 43.93a  | 20.48b  | 34.95ab  | 6.26  | 0.03  |
| creatine | 1051.36  | 1138.96  | 1138.05  | 45.21  | 0.16  |
| citrulline | 43.14  | 60.94  | 28.53  | 14.95  | 0.18  |
| glutamine | 1212.64  | 686.30  | 1705.48  | 514.41  | 0.22  |
| cysteine | 0.30  | 1.66  | 1.66  | 0.85  | 0.26  |
| creatinine | 13.26  | 12.00  | 12.20  | 0.58  | 0.15  |
| tryptophan | 39.51b  | 54.88a  | 42.05b  | 4.91  | 0.04  |
| hydroxyproline | 36.43a  | 10.80b  | 36.59a  | 7.94  | 0.03  |
| ornithine | 39.99  | 8.54  | 13.27  | 13.51  | 0.12  |
| taurine | 1014.74a  | 365.16b  | 678.34ab  | 188.75  | 0.04  |
| choline | 22.14  | 190.63  | 50.89  | 64.24  | 0.08  |
| aminoadipic acid | 26.24a  | 9.19b  | 16.09ab  | 5.31  | 0.05  |
| EAAs 1 | 344.21  | 255.26  | 300.03  | 33.19  | 0.09  |
| NEAAs | 4781.98  | 3533.80  | 4892.30  | 798.59  | 0.25  |
| TAAs | 5126.19  | 3789.06  | 5192.33  | 830.41  | 0.24  |

a, b different superscripts within a row indicate significantly different values for *P*-value < 0.05. EAAs: essential amino acids, NEAAs: non-essential amino acids, TAAs: total amino acids.

1 EAAs = (leucine + methionine + valine + isoleucine + threonine + phenylalanine + lysine + tryptophan)

**Supplementary Table 5.** Effect of different feeding modes on the FA composition in the *longissimus lumborum* of Black Tibetan sheep (μg/g tissue).

|  |  |  |  |
| --- | --- | --- | --- |
| Items | Groups | SEM | *P*-value |
|  | SG | BG | CG |  |  |
| C6:0 | 0.04  | 0.04  | 0.06  | 0.02  | 0.57  |
| C10:0 | 1.01  | 1.65  | 1.16  | 0.75  | 0.70  |
| C11:0 | 0.02  | 0.09  | 0.08  | 0.06  | 0.45  |
| C12:0 | 3.87  | 12.75  | 12.60  | 7.85  | 0.48  |
| C13:0 | 0.23  | 0.77  | 1.03  | 0.52  | 0.35  |
| C14:0 | 130.07  | 211.77  | 185.81  | 93.63  | 0.69  |
| C14:1N5 | 4.38  | 5.61  | 3.52  | 2.26  | 0.67  |
| C15:0 | 12.22  | 17.28  | 32.52  | 11.57  | 0.27  |
| C15:1N5 | 3.49  | 4.68  | 7.74  | 2.54  | 0.30  |
| C16:0 | 1560.05  | 1441.80  | 1635.60  | 607.10  | 0.95  |
| C16:1N7 | 130.02  | 75.24  | 101.33  | 55.32  | 0.64  |
| C17:0 | 49.03  | 45.84  | 88.80  | 29.66  | 0.34  |
| C17:1N7 | 40.22  | 29.20  | 49.95  | 16.11  | 0.48  |
| C18:0 | 1237.42  | 1000.61  | 1456.91  | 526.62  | 0.70  |
| C18:1N9 | 2630.31  | 1790.71  | 2098.61  | 858.51  | 0.64  |
| C18:2N6 | 520.10  | 515.18  | 706.96  | 76.09  | 0.08  |
| C18:3N6 | 6.20a  | 3.19b  | 7.01a  | 1.09  | 0.03  |
| C18:3N3 | 26.54  | 30.90  | 52.72  | 9.59  | 0.07  |
| C20:0 | 8.67  | 7.14  | 10.81  | 3.68  | 0.63  |
| C20:1N9 | 22.48a  | 10.94b  | 13.22b  | 2.09  | < 0.01  |
| C21:0 | 3.61  | 3.40  | 3.44  | 0.37  | 0.84  |
| C20:3N6 | 18.68  | 28.17  | 25.19  | 3.65  | 0.10  |
| C20:4N6 | 2.81  | 2.07  | 2.56  | 0.24  | 0.06  |
| C20:3N3 | 187.83  | 310.29  | 190.08  | 47.26  | 0.07  |
| C22:0 | 1.51  | 1.53  | 2.24  | 0.47  | 0.28  |
| C20:5N3 | 14.40b  | 44.02a  | 21.83ab  | 9.45  | 0.05  |
| C22:1N9 | 8.26  | 4.63  | 5.42  | 1.93  | 0.22  |
| C22:2N6 | 0.38  | 0.30  | 0.30  | 0.15  | 0.81  |
| C23:0 | 0.88  | 0.72  | 1.17  | 0.17  | 0.10  |
| C22:4N6 | 27.53  | 18.07  | 26.48  | 3.91  | 0.10  |
| C22:5N6 | 8.02  | 6.27  | 6.71  | 1.03  | 0.29  |
| C24:0 | 1.60  | 1.73  | 2.31  | 0.36  | 0.19  |
| C22:5N3 | 39.09b  | 88.59a  | 69.11ab  | 12.97  | 0.02  |
| C22:6N3 | 6.77b  | 22.67a  | 8.90b  | 4.48  | 0.02  |
| TFAs | 6707.74 | 5737.86 | 6832.17  | 2164.09  | 0.86  |
| SFAs | 3010.23  | 2747.13  | 3434.54  | 1250.24  | 0.86  |
| MUFAs | 2839.16  | 1921.01  | 2279.78  | 931.81  | 0.63  |
| PUFAs | 858.35  | 1069.72  | 1117.85  | 137.12  | 0.21  |
| n-3 PUFAs | 274.63  | 496.47  | 342.64  | 71.72  | 0.05  |
| n-6 PUFAs | 583.73  | 573.25  | 775.21  | 78.04  | 0.07  |
| PUFAs/SFAs | 0.34  | 0.45  | 0.39  | 0.16  | 0.80  |
| n-6 PUFAs/n-3 PUFAs | 2.12a  | 1.19b  | 2.26a  | 0.17  | < 0.01  |
| C16:0/C18:1 | 0.58b  | 0.87a  | 0.77ab  | 0.09  | 0.05  |

a, b different superscripts within a row indicate significantly different values for *P*-value < 0.05. TFAs: total fatty acids, SFAs: saturated fatty acids, MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids, n-3 PUFAs: omega-3 polyunsaturated fatty acids, n-6 PUFAs: omega-6 polyunsaturated fatty acids.