**Supplementary Table S1**. Individualized lactation performance response to supplemental rumen-protected methionine in mid-lactating dairy cows based on data of our previous researches1.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Response2 | Mean | Maximum | Minimum | SD | % of RPM supplementation cows |
| Trial 1 |  |  |  |  |  |
| ∆ Milk yield, kg/d | 1.58 | 6.05 | -9.00 | 4.33 | 100 |
| ∆ > 0, n = 9 | 3.57 | 6.05 | 1.05 | 1.96 | 75 |
| ∆ < 0, n = 3 | -4.38 | -1.20 | -9.00 | 4.09 | 25 |
| ∆ ECM yield, kg/d | 2.54 | 6.77 | -8.23 | 4.69 | 100 |
| ∆ > 0, n = 9 | 4.93 | 6.77 | 2.23 | 1.44 | 75 |
| ∆ < 0, n = 3 | -4.62 | -2.37 | -8.23 | 3.16 | 25 |
| Trial 2 |  |  |  |  |  |
| ∆ Milk yield, kg/d | 1.02 | 9.93 | -7.46 | 3.89 | 100 |
| ∆ > 0, n = 19 | 3.21 | 9.93 | 0.35 | 2.61 | 65.5 |
| ∆ < 0, n = 10 | -3.14 | -0.28 | -7.46 | 2.08 | 34.5 |
| ∆ ECM yield, kg/d | 1.57 |  | -6.08 | 5.02 | 100 |
| ∆ > 0, n = 18 | 4.55 | 13.4 | 0.21 | 3.88 | 62.1 |
| ∆ < 0, n = 11 | -3.31 | -1.09 | -5.86 | 1.69 | 37.9 |

1All the experiments were randomized block design with mid-lactation cows, cows fed the same diets with or without RPM supplementation were taken as RPM group and Control group, RPM were supplemented for 8 weeks for trial 1, 12 weeks for trial 2.

2∆ Milk yield = ∆RPM Milk yield - ∆Control Milk yield. ∆RPM Milk yield = The mean milk yield of the last four week after RPM supplementation for the RPM supplementation cow from certain block minus its milk yield before RPM supplementation; ∆Control Milk yield = The mean milk yield of the last four week for Control cow from the same block minus its milk yield before RPM supplementation. The calculation of ECM (energy-corrected milk) was the same as the milk yield.

**Supplementary Table S2**. Rumen-protected methionine (RPM) degradation constants based on the model P = a + b(1-exp(-ct)).

|  |  |  |
| --- | --- | --- |
| Item | RPM Degradation | SEM |
| a, % | -1.51 | 1.15 |
| b, % | 47.6 | 2.21 |
| c, %/h | 0.06 | 0.01 |
| dg1 | 30.2 | 0.93 |

1dg (effective degradability) = *a* + *bc* / (*c*+*kp*)1, assuming a passage rate (*kp*) of 3.3%/h according the prediction equation of Krizsan et al.2. *Kp =* 0.83 + 1.54 + 0.0866 × NDF intake (g/kg of body weight).

**Supplementary Table S3**. Differences in lactation performance, body weight, days in milk, and parity between positive response (PR) and limited response (LR) cows at wk 0.

| Item | PR, n = 10 | LR, n = 10 | SEM | *P*-value |
| --- | --- | --- | --- | --- |
| DMI, kg/d | 24.3 | 25.1 | 0.98 | 0.59 |
| Yield1, kg/d |  |  |  |  |
| Milk | 37.4 | 37.5 | 1.22 | 0.97 |
| ECM | 40.9 | 41.7 | 1.19 | 0.63 |
| FCM | 39.1 | 40.2 | 1.27 | 0.58 |
| Milk composition2, % | | | | |
| Protein | 3.48 | 3.45 | 0.07 | 0.78 |
| Fat | 3.83 | 3.95 | 0.16 | 0.59 |
| Lactose | 5.15 | 5.03 | 0.05 | 0.18 |
| total solid | 12.7 | 12.5 | 0.18 | 0.44 |
| MUN, mg/dl | 15.5 | 16.9 | 0.92 | 0.31 |
| SCC, ×103/ml | 46.5 | 83.5 | 22.3 | 0.34 |
| Feed efficiency3 | 1.70 | 1.69 | 0.07 | 0.88 |
| BW, kg | 647 | 689 | 20.7 | 0.19 |
| Day in milk, d | 141 | 139 | 3.10 | 0.80 |
| Parity | 1.30 | 1.70 | 0.18 | 0.14 |

1ECM: energy-corrected milk yield, ECM = 0.3246 × milk yield + 13.86 × milk fat yield + 7.04 × milk protein yield; FCM: fat-corrected milk yield, FCM = 0.432 × milk yield + 16.216 × milk fat yield.

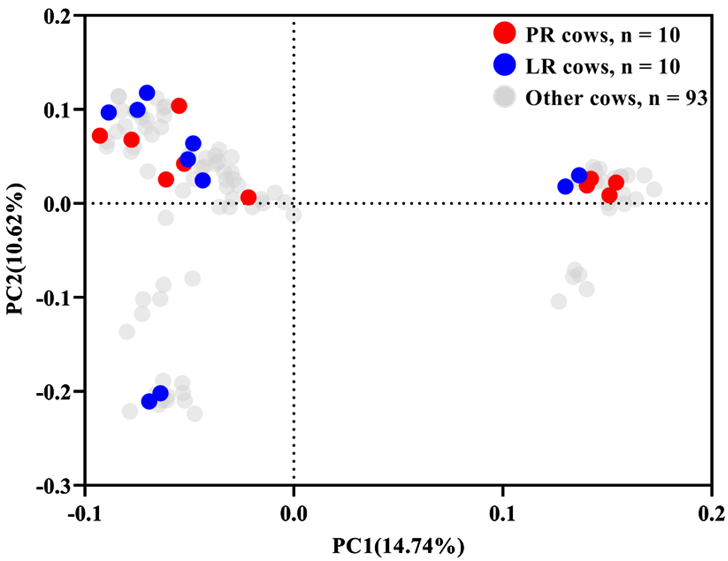
2SCC: somatic cell count; MUN: milk urea nitrogen.

3Feed efficiency calculated as ECM yield (kg/d)/DMI(kg/d), Nitrogen efficiency calculated as milk protein yield (kg/d)/total CP intake (kg/d).

**Supplementary Table S4**. Individualized lactation performance changes after rumen-protected methionine supplementation in mid-lactating dairy cows

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Response1 | Mean | Maximum | Minimum | SD | CV，% |
| ∆ Milk yield, kg/d | 0.17 | 3.41 | -4.98 | 1.45 | 840 |
| ∆ > 0, n = 68 | 1.11 | 3.41 | 0.02 | 0.82 | 73.8 |
| ∆ < 0, n = 45 | -1.24 | -0.05 | -4.98 | 0.98 | 79.1 |
| ∆ ECM yield, kg/d | 1.11 | 9.08 | -6.08 | 2.55 | 231 |
| ∆ > 0, n = 79 | 2.33 | 9.08 | 0.01 | 1.85 | 79.5 |
| ∆ < 0, n = 34 | -1.74 | -0.01 | -6.08 | 1.45 | 83.1 |
| ∆ FCM yield, kg/d | 1.25 | 9.91 | -8.05 | 2.84 | 227 |
| ∆ > 0, n = 79 | 2.59 | 9.91 | 0.05 | 2.04 | 78.5 |
| ∆ < 0, n = 34 | -1.88 | -0.02 | -8.05 | 1.76 | 94.1 |
| ∆ Milk protein content, % | 0.00 | 0.62 | -0.46 | 0.13 | 3343 |
| ∆ > 0, n = 53 | 0.11 | 0.62 | 0.00 | 0.12 | 120 |
| ∆ < 0, n = 60 | -0.08 | -0.00 | -0.46 | 0.08 | 92.2 |
| ∆ Milk fat content, % | 0.21 | 1.45 | -1.06 | 0.46 | 218 |
| ∆ > 0, n = 80 | 0.43 | 1.45 | 0.01 | 0.33 | 78.2 |
| ∆ < 0, n = 33 | -0.31 | -0.02 | -1.06 | 0.26 | 84.4 |
| ∆ Milk lactose content, % | -0.01 | 0.49 | -0.34 | 0.10 | 1036 |
| ∆ > 0, n = 43 | 0.08 | 0.49 | 0.00 | 0.10 | 129 |
| ∆ < 0, n = 70 | -0.06 | -0.00 | -0.34 | 0.06 | 89.6 |

1∆ Milk yield: change in milk yield = Mean of milk yield (wk 1–8) minus Mean of milk yield (wk 0). The calculation of other parameters was the same as the milk yield. ECM: energy-corrected milk, FCM: fat-corrected milk.



**Supplementary** **Figure S1**. Genotyping information of dairy cows. PCA showed no obvious clustering in genotyping between PR cows and LR cows.

**Supplementary Table S5.** Difference of free amino acid concentration in abdominal subcutaneous venous between at wk 8 and wk 0 of dairy cows.

| Item, mg/L | PR cows | | SEM | *P*-value | LR cows | | SEM | *P*-value |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| wk 0 | wk 8 | wk 0 | wk 8 |
| Arg | 12.8 | 10.1 | 1.07 | 0.06 | 12.9 | 11.8 | 1.06 | 0.30 |
| His | 7.87 | 7.05 | 0.43 | 0.14 | 8.04 | 7.38 | 0.49 | 0.05 |
| Ile | 14.5 | 13.0 | 0.80 | 0.14 | 13.7 | 13.9 | 1.04 | 0.81 |
| Leu | 19.7 | 16.8 | 1.08 | 0.06 | 19.5 | 19.3 | 1.52 | 0.88 |
| Lys | 9.77 | 8.60 | 0.75 | 0.28 | 10.6 | 10.4 | 0.77 | 0.72 |
| Met | 1.90 | 1.82 | 0.21 | 0.80 | 2.00 | 2.07 | 0.19 | 0.71 |
| Phe | 10.7 | 7.62 | 0.56 | < 0.01 | 9.99 | 7.85 | 0.41 | 0.01 |
| Thr | 26.8 | 22.0 | 1.67 | < 0.01 | 26.5 | 23.0 | 1.24 | 0.02 |
| Val | 38.3 | 33.9 | 1.43 | 0.01 | 35.8 | 35.3 | 2.51 | 0.82 |
| Ala | 20.7 | 20.8 | 1.35 | 0.88 | 20.3 | 21.8 | 0.96 | 0.09 |
| Asp | 1.65 | 1.69 | 0.27 | 0.90 | 1.36 | 1.70 | 0.15 | 0.04 |
| Glu | 17.9 | 18.5 | 0.82 | 0.38 | 17.5 | 18.9 | 0.97 | 0.09 |
| Gly | 18.1 | 18.1 | 1.33 | 0.99 | 16.5 | 17.1 | 0.87 | 0.30 |
| Pro | 12.1 | 10.6 | 0.91 | 0.11 | 12.2 | 11.7 | 0.90 | 0.51 |
| Ser | 8.59 | 5.73 | 0.66 | < 0.01 | 7.68 | 6.06 | 0.55 | < 0.01 |
| Tyr | 11.9 | 8.32 | 0.74 | < 0.01 | 11.6 | 9.25 | 0.66 | 0.02 |
| Cys | 10.8 | 9.47 | 0.87 | 0.13 | 8.36 | 8.13 | 0.72 | 0.66 |
| BCAA1 | 72.4 | 63.7 | 3.18 | 0.04 | 68.9 | 68.5 | 5.01 | 0.92 |
| TEAA2 | 142 | 121 | 6.51 | 0.02 | 139 | 131 | 7.91 | 0.30 |
| TNEAA3 | 102 | 93.2 | 5.22 | 0.11 | 95.5 | 94.5 | 3.63 | 0.77 |
| TAA4 | 244 | 214 | 10.7 | 0.04 | 234 | 225 | 11.2 | 0.40 |

1BCAA = branched-chain amino acids (Val + Ile + Leu).

2TEAA = total essential amino acids (Arg+ His + Ile + Leu + Lys + Met + Phe + Thr + Val).

3TNEAA = total non-essential amino acids (Ala + Asp + Glu + Gly + Pro + Ser + Tyr + Cys).

4TAA = total amino acids (TEAA + TNEAA).

**Supplementary Table S6.** Difference of the arterial-venous difference (A-V difference) between at wk 8 and wk 0 of dairy cows.

| Item, mg/L | PR cows | | SEM | *P*-value | LR cows | | SEM | *P*-value |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| wk 0 | wk 8 | wk 0 | wk 8 |
| Arg | 6.02 | 6.34 | 0.67 | 0.75 | 5.95 | 5.53 | 1.01 | 0.78 |
| His | 2.09 | 2.75 | 0.25 | 0.06 | 1.75 | 2.29 | 0.26 | 0.24 |
| Ile | 6.06 | 6.57 | 0.56 | 0.53 | 7.33 | 6.04 | 0.72 | 0.18 |
| Leu | 9.22 | 10.3 | 0.83 | 0.34 | 11.0 | 9.95 | 0.97 | 0.38 |
| Lys | 7.47 | 7.88 | 0.59 | 0.62 | 7.62 | 7.41 | 0.79 | 0.85 |
| Met | 1.93 | 2.13 | 0.18 | 0.43 | 1.94 | 2.00 | 0.18 | 0.78 |
| Phe | 7.94 | 2.51 | 1.23 | 0.02 | 5.55 | 2.66 | 0.77 | 0.07 |
| Thr | 6.33 | 12.8 | 1.12 | < 0.01 | 4.79 | 11.9 | 0.83 | < 0.01 |
| Val | 5.60 | 6.27 | 0.98 | 0.67 | 8.49 | 5.31 | 0.93 | 0.03 |
| Ala | 3.62 | 3.01 | 0.69 | 0.58 | 3.08 | 1.81 | 0.81 | 0.36 |
| Asp | 1.37 | 0.95 | 0.20 | 0.25 | 1.45 | 0.69 | 0.21 | 0.01 |
| Glu | 7.31 | 4.31 | 0.64 | 0.03 | 7.46 | 3.64 | 0.76 | < 0.01 |
| Gly | 1.00 | 1.89 | 0.77 | 0.52 | 1.39 | 1.00 | 0.44 | 0.56 |
| Pro | 1.08 | 0.44 | 0.36 | 0.31 | 1.53 | 0.31 | 0.40 | 0.04 |
| Ser | 2.60 | 3.30 | 0.46 | 0.32 | 2.95 | 2.85 | 0.32 | 0.78 |
| Tyr | 1.23 | 3.53 | 0.49 | < 0.01 | 1.92 | 3.18 | 0.43 | 0.08 |
| Cys | -1.41 | -1.05 | 0.66 | 0.76 | 0.88 | -0.69 | 0.40 | 0.04 |
| BCAA1 | 20.9 | 23.2 | 2.31 | 0.51 | 26.9 | 21.3 | 2.52 | 0.12 |
| TEAA2 | 52.7 | 57.6 | 4.28 | 0.34 | 54.5 | 53.1 | 4.86 | 0.83 |
| TNEAA3 | 16.8 | 16.4 | 3.02 | 0.94 | 20.7 | 12.8 | 2.47 | 0.07 |
| TAA4 | 69.5 | 74.0 | 6.63 | 0.64 | 75.1 | 65.9 | 6.76 | 0.35 |

1BCAA = branched-chain amino acids (Val + Ile + Leu).

2TEAA = total essential amino acids (Arg+ His + Ile + Leu + Lys + Met + Phe + Thr + Val).

3TNEAA = total non-essential amino acids (Ala + Asp + Glu + Gly + Pro + Ser + Tyr + Cys).

4TAA = total amino acids (TEAA + TNEAA).

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