**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).

**REFERENCES**

1. Ørskov ER, Debhovell FD, Mould F. The use of the nylon bag technique for the evaluation of feedstuffs. *Trop Anim Prod* **5:**195-213 (1980).

2. Krizsan SJ, Ahvenjarvi S, Huhtanen P. A meta-analysis of passage rate estimated by rumen evacuation with cattle and evaluation of passage rate prediction models. *J Dairy Sci* **93(12):**5890-5901 (2010).