**Supplemental Information**

**Supplemental Table S1** The basal diet formulation

|  |  |
| --- | --- |
| Items (%, DM basis) |  |
| Corn silage | 40.0 |
| Corn | 35.0 |
| Wheat bran | 8.0 |
| Soybean meal | 5.0 |
| Sunflower seeds | 8.0 |
| NaCl | 1.0 |
| Premix\* | 1.8 |
| NaHCO3 | 1.2 |
| Total | 100.0 |
|  |  |
| Nutrient composition (% of DM) |  |
| NEL (MJ/kg) | 6.7 |
| CP | 15.2 |
| NDF | 33.5 |
| ADF | 17.2 |
| NFC | 40.4 |
| Ca | 0.7 |
| P | 0.5 |

\* The premix provided these nutrients per kg of PREMIX: Vitamin A 200,000 IU, Vitamin D 70,000 IU, Vitamin E 1,000 IU, Fe 2,000 mg, Cu 600 mg, Zn 2,400 mg, Mn 1,300 mg, I 6 mg, Co 7 mg.

DM, Dry Matter; NEL, Net energy for lactation; CP, Crude protein; NDF, Neutral detergent fibre; ADF, Acid detergent fibre; NFC, Non-fibre carbohydrate.

**Supplemental Table S2.** Basic description of nonHYK and HYK cows1, 2

|  |  |  |  |
| --- | --- | --- | --- |
| Item2 | nonHYK (n = 10) | HYK (n = 10) | *P*-value |
| BW (kg) | 629.53 ± 7.82 | 651.28 ± 6.94 | 0.0782 |
| BCS | 3.36 ± 0.06 | 3.51 ± 0.08 | <0.0001 |
| Milk yield (kg) | 31.02 ± 0.57 | 28.12 ± 0.39 | <0.0001 |
| DMI (kg) | 20.10 ± 0.43 | 18.78 ± 0.42 | <0.0001 |
| DIM at the start of blood sampling and milk yield measurement period (d) | 5.9 ± 0.62 | 6.6 ± 0.5 | 0.3919 |
| DIM of the adipose tissue sampling (d) | 8.9 ± 0.62 | 9.6 ± 0.5 | 0.3919 |
| BHB (m*M*) | 0.35 ± 0.08 | 2.23 ± 0.18 | <0.0001 |
| Fatty acids (m*M*) | 0.43 ± 0.06 | 0.86 ± 0.13 | <0.0001 |
| Glucose (m*M*) | 3.76 ± 0.07 | 2.89 ± 0.05 | <0.0001 |

1 Data are expressed as mean ± standard error of the mean.

2 nonHYK = non-hyperketonemic; HYK = hyperketonemic; BCS = body condition score; DMI = dry matter intake; DIM = days in milk; BHB = β-hydroxybutyrate.

**Supplemental Table S3.** The primers sequences and efficiencies.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *Genes* | Sequence number | Primer sequences (5’-3’) | Length (bp) | Annealing temperature (°C) | Efficiency (E) (%) | Correlation coefficient (R2) |
| *MAP1LC3B* | NM\_001001169.1 | For TAAGGAAACCGTGCTGCTGT | 124 | 60 | 95 | 0.9968 |
| Rev GCAGTGGTGTTTTTCCGTGT |
| *ATG5* | NM\_001034579.2 | For CCAACTTGCTTCACCCTGTA | 188 | 60 | 97 | 0.9957 |
| Rev CATTTCAGTGGTGTGCCTTC |
| *ATG7* | XM\_024983114.1 | For GCGGTTGCCGGAAGTTG | 125 | 60 | 96 | 0.9978 |
| Rev ACTCATGCCAGAAGCCAACA |
| *ACTB* | NM\_173979.3 | For GCCCTGAGGCTCTCTTCCA | 101 | 60 | 98 | 0.9913 |
| Rev GCGGATGTCGACGTCACA |
| *GAPDH* | NM\_001034034.2 | For GGCGTGAACCACGAGAAGTATAARev CCTCCACGATGCCAAAGTG | 118 | 60 | 97 | 0.9952 |

*ACTB* = β-actin; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase; *MAP1LC3B* = microtubule-associated protein 1 light chain 3 beta; *ATG5* = autophagy-related gene 5; *ATG7* = autophagy-related gene 7.



**Supplemental Figure S1.** The expression of housekeeping genes in different groups. (A, B) Ct values of β-actin (*ACTB*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) in adipose tissue of non-hyperketonemic (nonHYK; n = 10) and hyperketonemic (HYK; n = 10) cows. (C-D) Ct values of *ACTB* and *GAPDH* in adipocytes. Calf adipocytes were treated with 250 n*M* Torin1 or 10 µ*M* isoproterenol (ISO) for 3 h, or pretreated with 10 μg/mL leupeptin for 4 h, followed by 10 µ*M* ISO for 3 h. A and B were analyzed using unpaired *t*-tests. C and D were analyzed using a two-way mixed-ANOVA with subsequent Bonferroni correction. The data presented are the mean ± SEM.