**Supplementary material**

# The Effects of Artificial Diets on the Expression of Molecular Marker Genes Related to Honey Bee Health

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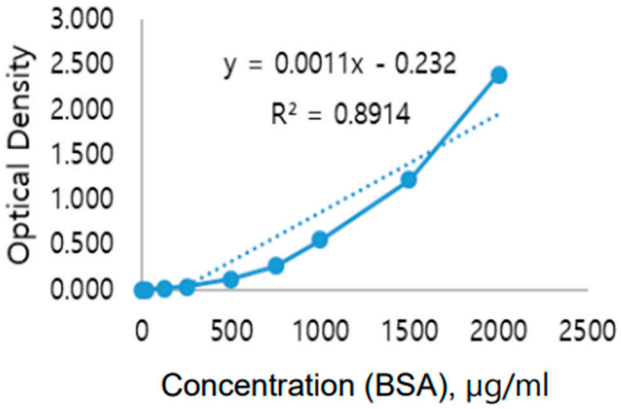


**Figure S1.** Calibration curve for the colorimetric method using the Pierce™ BCA Protein Assay Kit. BSA stands for Bovine Serum Albumin, and BCA stands for BiCinchoninic Acid.

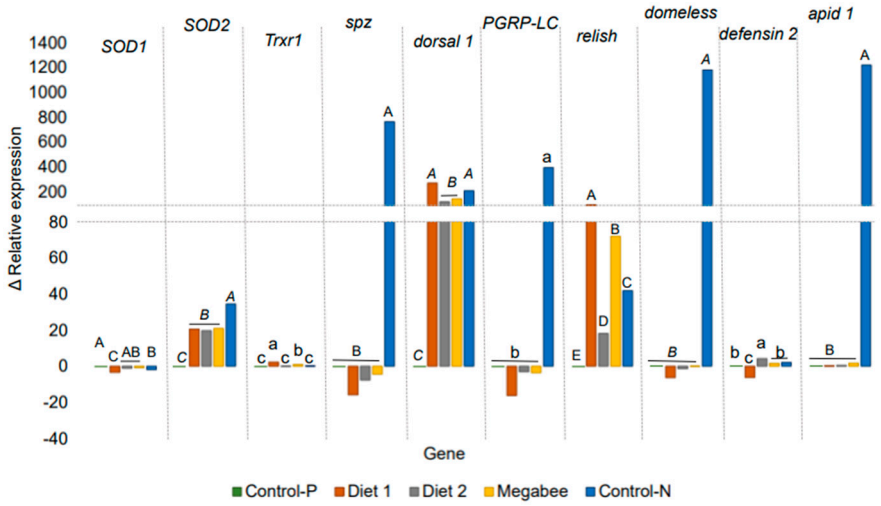


**Figure S2.** Differences in relative gene expression of defense system genes in honey bees under dietary conditions. Difference values were calculated by subtracting the relative gene expression in honey bees after dieting from that before dieting. One-way ANOVA and Duncan post hoc tests ($p < 0.05$) were conducted and marked in the chart with upper or lowercase letters.

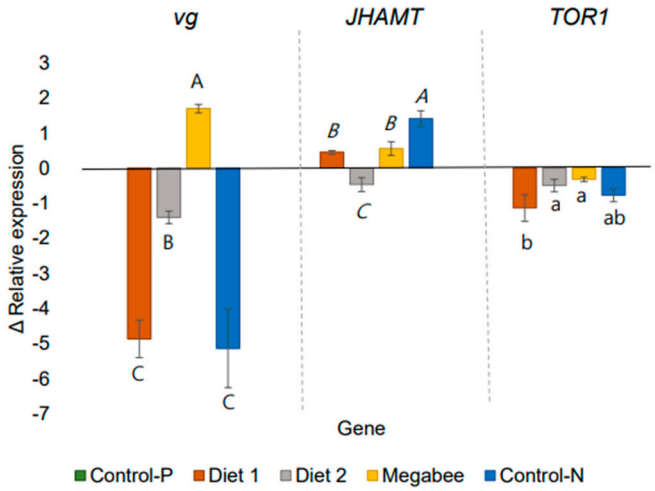


**Figure S3.** Differences in relative gene expression of nutrition markers in honey bees under dietary conditions. Difference values were calculated by subtracting the relative gene expression in honey bees after dieting from that before dieting. One-way ANOVA and Duncan post hoc tests ($p < 0.05$) were conducted and marked in the chart with upper or lowercase letters.

**Table S1** PCR primer sequences.

| | Locus | 5`-3` | Primers | Amplicon length, bp | NCBI Reference Sequence | Efficiency (%) |
|---|---|---|---|---|---|---|
| 1 | *defensin-2* | F | ACCGCTGCTACCACTACGACA | 139 | NM_001011638.1 | 110. |
| 2 | | R | GCCATTTCTGCAACTACCGCCT | | | |
| 3 | *relish* | F | TCCATTGCATGCAGCACTTCG | 264 | XM_026444175.1 | 97 |
| 4 | | R | ACACATGCACCAGCTTCAGGA | | | |
| 5 | *dorsal-1* | F | TGCAGCAAGTGGAACAACCAGT | 114 | XM_006566999.3 | 92 |
| 6 | | R | CAGGCCTACCTGCACCGAGA | | | |
| 7 | *domeless* | F | GCCGCTGCTCTTTGGCATCT | 238 | XM_006567690.3 | 108 |
| 8 | | R | GCCAAATTGTTGTTCCAACAGCCC | | | |
| 9 | *apid-1* | F | TTGTTGTTACCTTTGTAGTCGCGGT | 70 | NM_001011642.1 | 110 |
| 10 | | R | AGGCGCGTAGGTCGAGTAGG | | | |
| 11 | *PGRP-LC* | F | TGCAATGCGATGGCGACACA | 105 | XM_026441962.1 | 110 |
| 12 | | R | AGCGACTTGAGCACACCACAC | | | |
| 13 | *spz (spaetzel)* | F | TGGACGACAGCCCTCTTTGTCA | 371 | XM_006565534.3 | 92 |
| 14 | | R | GCGCCTTCGACGTGACGATT | | | |
| 15 | *SBV* | F | GTGGAACCCGAGTGTTTTGTAACCC | 156 | KY273489.1 | 98 |
| 16 | | R | AAGCTAAAAGCGTCCACTCTGTACTCT | | | |
| 17 | *DWV* | F | TGT GAA GTG GCG GAC GTT ACA GA | 211 | KT215904.1 | 100 |
| 18 | | R | GTA TTC TGG ACC CCA TCC GAA TGC | | | |
| 19 | *β-actin* | F | GGATTCCTATGTTGGTGATGAAGCCC | 177 | NM_001185145.1 | 100 |
| 20 | | R | GGTGCCTCAGTAAGAAGTACCGGATG | | | |
| 21 | *SOD1* | F | GCAGTGTGCGTTCTTCAGGGT | 86 | NM_001178027.1 | 99 |
| 22 | | R | TGACCGGTGACCTTCACGGA | | | |
| 23 | *SOD2* | F | GGCGGTAAACCAGACGCTGC | 126 | NM_001178048.2 | 110 |
| 24 | | R | TCCAAGCCAACCCCAACCAGA | | | |
| 25 | *Trxr-1* | F | CCTGTTGCTATACATGCGGGTCG | 141 | XM_006563201.3 | 110 |
| 26 | | R | TGCTGCTTCTTCGCTAAGGCCA | | | |
| 27 | *vg* | F | GCA GAA TAC ATG GAC GGT GT | 146 | NM_001011578.1 | |
| 28 | *(vitellogenin)* | R | GAA CAG TCT TCG GAA GCT TG | | | 92.36 |
| 29 | *JHAMT* | F | TTG GAC ATA GGT TGC GGA CC | 302 | NM_001327967.1 | |
| 30 | | R | AAT CCT TTT CCT CCT GGC CG | | | 89.20 |
| 31 | *TOR1* | F | AAC AAC TGT TGC TGA CGG TG | 153 | XM_006566642.3 | |
| 32 | | R | GTT GCA GTC CAG GCT TTT TG | | | 90.54 |