Supplementary Material

**Effect of fermented heat-treated rice bran on performance and possible role of intestinal microbiota in laying hens**

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# Supplementary Tables

Table S1 Analysis of L18（35）orthogonal test results of fermented rice bran

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Factors / levels /No. | Inoculation quantity (‰)-A | *B. subtilis*: *L. plantarum-B* | Solid-liquid ratio (m: v)-C | Fermentation temperature (℃)-D | Fermentation time (h)-E | DPPH free radical scavenging activity (%) |
| 1 | 0.4 | 2: 8 | 1: 0.5 | 30 | 24 |
| 2 | 0.6 | 3: 7 | 1: 0.6 | 35 | 36 |
| 3 | 0.8 | 4: 6 | 1: 0.7 | 37 | 48 |
| 1 | 0.4 | 2: 8 | 1: 0.5 | 30 | 24 | 85.54±2.11 |
| 2 | 0.4 | 2: 8 | 1: 0.6 | 37 | 36 | 86.75±0.13 |
| 3 | 0.4 | 3: 7 | 1: 0.5 | 35 | 36 | 82.08±1.62 |
| 4 | 0.4 | 3: 7 | 1: 0.7 | 30 | 48 | 89.11±2.79 |
| 5 | 0.4 | 4: 6 | 1: 0.6 | 35 | 24 | 91.43±1.63 |
| 6 | 0.4 | 4: 6 | 1: 0.7 | 37 | 48 | 78.19±0.15 |
| 7 | 0.6 | 2: 8 | 1: 0.5 | 35 | 48 | 83.25±1.23 |
| 8 | 0.6 | 2: 8 | 1: 0.7 | 30 | 24 | 89.19±2.57 |
| 9 | 0.6 | 3: 7 | 1: 0.6 | 37 | 24 | 78.60±2.33 |
| 10 | 0.6 | 3: 7 | 1: 0.7 | 35 | 36 | 86.47±1.65 |
| 11 | 0.6 | 4: 6 | 1: 0.5 | 37 | 48 | 81.41±0.95 |
| 12 | 0.6 | 4: 6 | 1: 0.6 | 30 | 36 | 81.59±3.28 |
| 13 | 0.8 | 2: 8 | 1: 0.6 | 35 | 48 | 82.75±0.53 |
| 14 | 0.8 | 2: 8 | 1: 0.7 | 37 | 36 | 86.78±2.82 |
| 15 | 0.8 | 3: 7 | 1: 0.5 | 37 | 24 | 90.65±2.50 |
| 16 | 0.8 | 3: 7 | 1: 0.6 | 30 | 48 | 82.50±0.44 |
| 17 | 0.8 | 4: 6 | 1: 0.5 | 30 | 36 | 77.59±1.58 |
| 18 | 0.8 | 4: 6 | 1: 0.7 | 35 | 24 | 88.93±1.88 |
| K1 | 85.52 | 85.71 | 83.42 | 84.25 | 86.07 |  |
| K2 | 85.14 | 85.13 | 83.62 | 83.87 | 87.39 |  |
| K3 | 85.54 | 85.53 | 83.54 | 84.62 | 84.80 |  |
| R | 0.41 | 0.58 | 0.20 | 0.75 | 2.59 |  |
| Ranking of factors affecting DPPH radical scavenging activity of FHRB: E>D>B>A>C. Theoretical optimal combination of FHRB: A3B1C2D2E2. Inoculation quantity was 0.8‰, *B. subtilis*: *L. plantarum* = 2: 8, solid-liquid ratio was 1: 0.6, fermentation temperature was 35℃, fermentation time was 36 hours. |

**Table** S**2 Statistics of sequencing quantity for each sample**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample ID | Input | Filtered | Denoised | Merged | Non-chimeric | Non-singleton |
| 2.5%HRB1 | 116348 | 102095 | 97846 | 79423 | 56731 | 54720 |
| 2.5%HRB2 | 143088 | 128406 | 124176 | 101595 | 65642 | 62812 |
| 2.5%HRB3 | 118927 | 106157 | 102386 | 84818 | 56282 | 54261 |
| 2.5%HRB4 | 116666 | 103184 | 99439 | 81514 | 53823 | 51201 |
| 2.5%HRB5 | 110138 | 98463 | 94813 | 77872 | 51458 | 49506 |
| 2.5%HRB6 | 136714 | 120102 | 115912 | 95984 | 67926 | 65597 |
| 2.5%HRB7 | 116388 | 104461 | 100710 | 82479 | 56765 | 54796 |
| 2.5%HRB8 | 124420 | 110119 | 106229 | 84783 | 58917 | 57080 |
| 5.0%HRB1 | 132449 | 117207 | 113175 | 90404 | 65285 | 62533 |
| 5.0%HRB2 | 147658 | 131317 | 126904 | 105931 | 66997 | 64180 |
| 5.0%HRB3 | 145243 | 130237 | 125970 | 103111 | 67451 | 65212 |
| 5.0%HRB4 | 134510 | 117813 | 113536 | 88191 | 58982 | 56187 |
| 5.0%HRB5 | 142198 | 124031 | 118869 | 91228 | 57539 | 53550 |
| 5.0%HRB6 | 146513 | 129460 | 125936 | 107808 | 70268 | 67853 |
| 5.0%HRB7 | 117636 | 105434 | 101464 | 80705 | 52677 | 50460 |
| 5.0%HRB8 | 133284 | 117752 | 114042 | 93224 | 64805 | 62678 |
| 2.5%FHRB1 | 87511 | 76091 | 72644 | 54920 | 39161 | 37248 |
| 2.5%FHRB2 | 109720 | 96610 | 92890 | 73237 | 50491 | 48145 |
| 2.5%FHRB3 | 110620 | 97868 | 93944 | 74694 | 51721 | 49243 |
| 2.5%FHRB4 | 117185 | 102948 | 99550 | 80683 | 57021 | 55488 |
| 2.5%FHRB5 | 139309 | 122514 | 117889 | 93089 | 62311 | 59540 |
| 2.5%FHRB6 | 136958 | 121058 | 116559 | 92313 | 63815 | 61496 |
| 2.5%FHRB7 | 142078 | 127081 | 122447 | 96370 | 62915 | 59606 |
| 2.5%FHRB8 | 140630 | 123033 | 118894 | 96432 | 61925 | 59612 |
| 5.0%FHRB1 | 138026 | 122997 | 118271 | 94521 | 66640 | 63889 |
| 5.0%FHRB2 | 134759 | 118633 | 115223 | 96259 | 66418 | 64277 |
| 5.0%FHRB3 | 135951 | 121421 | 117632 | 97542 | 73087 | 71145 |
| 5.0%FHRB4 | 111261 | 97259 | 93318 | 72506 | 50715 | 48898 |
| 5.0%FHRB5 | 125456 | 110134 | 105771 | 81454 | 53128 | 49930 |
| 5.0%FHRB6 | 146843 | 129701 | 125383 | 103169 | 65627 | 62506 |
| 5.0%FHRB7 | 87149 | 76967 | 73886 | 60069 | 44680 | 43306 |
| 5.0%FHRB8 | 93634 | 81417 | 78547 | 63228 | 45450 | 44067 |

**Table S3 Relative abundances of taxon at the phylum and genus level**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Taxon at the phylum level (%) | 2.5% HRB | 2.5% FHRB | 5.0% HRB | 5.0% FHRB |
| Firmicutes | 50.22 | 55.89 | 56.83 | 53.37 |
| Bacteroidetes | 38.47 | 34.07 | 36.09 | 37.25 |
| Proteobacteria | 3.19 | 3.32 | 2.62 | 3.08 |
| Actinobacteria | 1.64 | 1.49 | 1.34 | 1.47 |
| Verrucomicrobia | 0.19 | 0.97 | 0.68 | 0.51 |
| Synergistetes | 0.49 | 0.60 | 0.34 | 0.37 |
| Tenericutes | 0.11 | 0.24 | 0.10 | 0.13 |
| Deferribacteres | 0.08 | 0.22 | 0.09 | 0.15 |
| Elusimicrobia | 0.08 | 0.15 | 0.11 | 0.13 |
| Fusobacteria | 0.02 | 0.12 | 0.09 | 0.10 |
| Taxon at the genus level (%) | 2.5% HRB | 2.5% FHRB | 5.0% HRB | 5.0% FHRB |
| Bacteroides | 23.58 | 21.91 | 20.32 | 22.42 |
| Ruminococcus | 13.81 | 11.08 | 15.60 | 15.20 |
| Lachnospira | 8.52 | 6.92 | 8.57 | 7.53 |
| Lactobacillus | 6.41 | 6.64 | 6.44 | 7.68 |
| Clostridiales | 6.36 | 5.45 | 5.25 | 4.02 |
| Faecalibacterium | 3.92 | 5.62 | 5.24 | 4.95 |
| Peptococcus | 3.24 | 1.43 | 1.42 | 2.73 |
| Prevotella | 2.14 | 2.84 | 1.43 | 1.30 |
| Paraprevotella | 1.69 | 2.37 | 1.52 | 1.61 |
| Oscillospira | 1.73 | 1.45 | 1.97 | 1.93 |
| Megamonas | 1.97 | 1.89 | 0.59 | 1.23 |
| Coprococcus | 1.69 | 1.16 | 2.23 | 1.56 |
| Blautia | 1.40 | 1.23 | 1.07 | 1.56 |
| Desulfovibrio | 1.35 | 1.05 | 1.16 | 1.06 |
| Phascolarctobacterium | 1.04 | 0.64 | 1.12 | 1.07 |
| S24-7 | 1.33 | 1.24 | 0.73 | 0.95 |
| Paraprevotellaceae | 2.37 | 1.52 | 1.61 | 1.69 |
| Peptostreptococcaceae | 0.35 | 0.46 | 1.38 | 1.80 |
| Phascolarctobacterium | 0.64 | 1.12 | 1.07 | 1.04 |
| Ruminococcus | 0.69 | 0.90 | 0.76 | 0.91 |
| Parabacteroides | 0.50 | 0.87 | 0.64 | 0.86 |
| Coriobacteriaceae | 0.70 | 0.77 | 0.55 | 0.45 |
| Paraprevotella | 0.24 | 0.44 | 0.92 | 0.73 |
| Turicibacter | 0.20 | 0.35 | 0.80 | 0.98 |
| Akkermansia | 0.19 | 0.96 | 0.68 | 0.49 |
| Subdoligranulum | 0.53 | 0.35 | 0.83 | 0.44 |
| Sutterella | 0.46 | 0.56 | 0.32 | 0.46 |
| Synergistaceae | 0.48 | 0.60 | 0.34 | 0.36 |
| Mogibacteriaceae | 0.32 | 0.43 | 0.39 | 0.27 |
| Butyricicoccus | 0.18 | 0.51 | 0.33 | 0.34 |

**Table** S**4 Differential metabolites identified between the two groups**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Metabolites | *P*-value | VIP | FC **(2.5%FHRB /2.5%HRB)** | Metabolites | *P*-value | VIP | FC **(5.0%HRB /2.5%HRB)** |
| Triethylamine | 0.028  | 1.70  | 0.33  | p-Cresol | 0.028  | 1.68  | 2.03  |
| 1-Aminocyclopropanecarboxylic acid | 0.010  | 1.87  | 1.94  | 1,2,3-Trihydroxybenzene | 0.049  | 1.57  | 1.44  |
| Choline | 0.007  | 1.39  | 4.81  | Isochavicol | 0.021  | 2.19  | 0.57  |
| 2-Phenylethanol | 0.028  | 1.60  | 0.70  | Indole-3-carboxylic acid | 0.015  | 2.48  | 0.34  |
| m-Cresol | 0.021  | 2.02  | 0.27  | Safrole | 0.015  | 2.47  | 0.30  |
| Glutarate semialdehyde | 0.015  | 1.47  | 0.72  | 5-Hydroxylysine | 0.021  | 2.13  | 1.11  |
| Indole | 0.048  | 1.02  | 1.14  | Pterin | 0.049  | 1.88  | 0.39  |
| 2,6-Dimethylaniline | 0.038  | 1.23  | 0.74  | S-Carboxymethyl-L-cysteine | 0.038  | 1.86  | 0.37  |
| N-Acetylputrescine | 0.049  | 1.41  | 1.91  | Thiabendazole | 0.007  | 2.41  | 1.32  |
| L-Leucine | 0.002  | 1.84  | 2.26  | Spermine | 0.003  | 2.18  | 1.21  |
| Creatine | 0.015  | 1.48  | 1.80  | 5-Methoxyindoleacetate | 0.010  | 2.19  | 0.43  |
| Ureidopropionic acid | 0.049  | 1.58  | 1.97  | Prephenate | 0.049  | 1.38  | 0.67  |
| Cinnamaldehyde | 0.010  | 1.81  | 0.56  | N-Acetyl-L-2-amino-6-oxopimelate | 0.007  | 1.77  | 1.68  |
| 1,3-Dihydro-(2H)-indol-2-one | 0.028  | 1.55  | 0.59  | Fenfluramine | 0.021  | 2.30  | 0.24  |
| Isochavicol | 0.007  | 1.86  | 0.52  | N(omega)-Nitro-L-arginine methyl ester | 0.049  | 2.31  | 1.89  |
| Pulegone | 0.038  | 1.54  | 0.66  | Confertifolin | 0.015  | 2.11  | 2.55  |
| Phenylacetic acid | 0.021  | 1.76  | 1.27  | Linoleic acid | 0.038  | 2.20  | 2.39  |
| Acetylphosphate | 0.001  | 2.22  | 1.23  | 15-Deoxy-d-12,14-PGJ2 | 0.010  | 1.93  | 0.77  |
| 2-Naphthylamine | 0.021  | 1.42  | 0.51  | (R)-10-Hydroxystearate | 0.010  | 2.10  | 2.03  |
| 2-Dehydropantoate | 0.049  | 1.61  | 0.36  | Allopregnanolone | 0.021  | 2.19  | 2.08  |
| L-Glutamine | 0.028  | 1.69  | 0.52  | N, N-Dimethylsphing-4-enine | 0.028  | 1.54  | 2.24  |
| L-Methionine | 0.038  | 1.53  | 1.37  | all-trans-5,6-Epoxyretinoic acid | 0.049  | 1.89  | 0.68  |
| Metaraminol | 0.003  | 1.80  | 0.54  | Labetalol | 0.049  | 2.01  | 0.64  |
| D-Cathinone | 0.005  | 1.90  | 0.50  | Carnosol | 0.038  | 2.27  | 0.47  |
| D-Arabitol | 0.038  | 1.47  | 0.72  | 12-Keto-tetrahydro-leukotriene B4 | 0.010  | 2.47  | 0.49  |
| 3D-3,5\_4-Trihydroxycyclohexane-1,2-dione | 0.021  | 1.67  | 0.47  | 6-Keto-prostaglandin F1a | 0.001  | 2.57  | 2.79  |
| 4-Hydroxy-2-quinolone | 0.049  | 1.55  | 0.73  | Maslinic acid | 0.028  | 2.22  | 0.28  |
| 5-Hydroxylysine | 0.002  | 2.18  | 1.17  | Soyasaponin I | 0.021  | 1.83  | 4.11  |
| Normetanephrine | 0.021  | 1.49  | 1.78  | Phenylacetaldehyde | 0.015  | 2.09  | 1.66  |
| 2,4,6\_3,5-Pentahydroxycyclohexanone | 0.049  | 1.66  | 0.59  | Purine | 0.003  | 2.78  | 3.30  |
| S-Carboxymethyl-L-cysteine | 0.007  | 1.94  | 0.41  | N-Acetylornithine | 0.049  | 1.77  | 0.62  |
| 4-Acetamidobenzoic acid | 0.015  | 1.82  | 0.55  | Shikimic acid | 0.010  | 2.26  | 0.60  |
| Beta-Tyrosine | 0.015  | 1.87  | 0.65  | Hippuric acid | 0.028  | 2.69  | 1.41  |
| Phosphorylcholine | 0.038  | 1.78  | 0.58  | 4-Hydroxyphenylpyruvic acid | 0.049  | 2.11  | 1.78  |
| N-Alpha-acetyllysine | 0.049  | 1.58  | 0.70  | L-Tryptophan | 0.038  | 1.96  | 0.50  |
| 2-Keto-6-acetamidocaproate | 0.049  | 1.62  | 0.49  | Adenosine diphosphate ribose | 0.021  | 2.02  | 3.19  |
| Homocitrulline | 0.010  | 1.85  | 0.59  | Metabolites | *P*-value | VIP | FC **(5.0%FHRB /2.5%FHRB)** |
| Glycylleucine | 0.001  | 2.47  | 3.23  | Cytosine | 0.049  | 1.03  | 1.67  |
| meso-2,6-Diaminoheptanedioate | 0.038  | 1.40  | 0.61  | L-Proline | 0.015  | 1.51  | 1.69  |
| (-)-Jasmonic acid | 0.028  | 1.61  | 0.51  | 2,6-Dimethylaniline | 0.021  | 1.69  | 1.45  |
| 2-Amino-2-deoxy-D-gluconate | 0.028  | 1.74  | 0.69  | Agmatine | 0.015  | 1.72  | 0.40  |
| Thiabendazole | 0.005  | 1.81  | 1.26  | Pulegone | 0.021  | 1.69  | 1.61  |
| O-Acetylcarnitine | 0.005  | 1.88  | 0.40  | 4-Hydroxyphenylacetaldehyde | 0.038  | 1.75  | 0.58  |
| 5-Methoxyindoleacetate | 0.007  | 1.86  | 0.41  | Methylimidazoleacetic acid | 0.010  | 1.66  | 2.37  |
| N-Acetyl-D-phenylalanine | 0.028  | 1.40  | 0.35  | 2,3-Dihydroxy-3-methylvalerate | 0.002  | 1.66  | 1.66  |
| Neostigmine | 0.021  | 1.20  | 0.54  | N-Methyltyramine | 0.049  | 1.33  | 1.58  |
| Hydroxykynurenine | 0.021  | 1.94  | 0.58  | p-Octopamine | 0.028  | 1.12  | 1.06  |
| Prephenate | 0.003  | 1.93  | 0.67  | N-Acetylhistamine | 0.049  | 1.33  | 1.27  |
| N-Acetyl-L-2-amino-6-oxopimelate | 0.007  | 1.62  | 1.47  | L-Histidine | 0.049  | 1.31  | 1.40  |
| Fenfluramine | 0.049  | 1.52  | 0.44  | 4-Acetamido-2-aminobutanoic acid | 0.038  | 1.51  | 0.65  |
| N2-Succinyl-L-ornithine | 0.021  | 1.78  | 1.65  | 3D-3,5\_4-Trihydroxycyclohexane-1,2-dione | 0.038  | 1.41  | 1.93  |
| Costunolide | 0.015  | 1.88  | 0.43  | 4-Hydroxy-2-quinolone | 0.002  | 2.03  | 1.63  |
| D-Octopine | 0.038  | 1.51  | 1.59  | L-Homophenylalanine | 0.049  | 1.53  | 1.35  |
| Pyridoxal 5'-phosphate | 0.002  | 2.06  | 0.63  | 2-Deoxystreptamine | 0.015  | 1.71  | 1.38  |
| Juvenile hormone III | 0.049  | 1.54  | 1.68  | L-Methionine S-oxide | 0.049  | 1.48  | 0.40  |
| Rivastigmine | 0.007  | 2.25  | 0.21  | Nalpha-Methylhistidine | 0.015  | 1.32  | 1.64  |
| Estrone | 0.028  | 1.51  | 0.66  | Indole-3-acetate | 0.049  | 1.29  | 0.50  |
| Genistein | 0.028  | 1.67  | 0.71  | 4-Acetamidobenzoic acid | 0.010  | 1.66  | 1.81  |
| Retinal | <0.001  | 2.45  | 0.09  | 4-Pyridoxic acid | 0.002  | 1.95  | 1.51  |
| 6beta-Hydroxytestosterone | 0.015  | 1.53  | 5.80  | Phosphoserine | 0.028  | 1.68  | 1.29  |
| Phenyllactic acid | <0.001  | 2.23  | 0.32  | 5-Guanidino-3-methyl-2-oxopentanoate | 0.028  | 1.27  | 0.71  |
| Adrenosterone | 0.049  | 1.62  | 0.53  | (2S,4S)-4-Hydroxy-2,3,4,5-tetrahydrodipicolinate | 0.038  | 1.49  | 1.56  |
| Allopregnanolone | 0.010  | 1.82  | 2.42  | N-Alpha-acetyllysine | 0.010  | 1.85  | 1.55  |
| Sphinganine | 0.028  | 1.61  | 0.47  | Homocitrulline | 0.005  | 1.73  | 1.50  |
| Dihydrocapsaicin | 0.049  | 1.46  | 0.60  | meso-2,6-Diaminoheptanedioate | 0.049  | 1.37  | 1.57  |
| Praziquantel | 0.049  | 1.28  | 3.19  | Neocnidilide | 0.007  | 1.51  | 1.94  |
| all-trans-5,6-Epoxyretinoic acid | 0.003  | 2.12  | 0.55  | 2-Amino-2-deoxy-D-gluconate | 0.001  | 2.08  | 1.62  |
| Ubiquinone-1 | 0.038  | 1.46  | 0.73  | L-Dopa | 0.010  | 1.83  | 1.84  |
| 5,6-DHET | 0.049  | 1.61  | 0.49  | N-Acetylhistidine | 0.005  | 1.76  | 1.80  |
| Citalopram | 0.049  | 1.54  | 0.67  | gamma-Glutamyl-beta-aminopropiononitrile | 0.038  | 1.53  | 1.77  |
| 2,3-Dinor-8-iso prostaglandin F2alpha | 0.021  | 1.35  | 0.39  | Dodecanoic acid | 0.038  | 1.62  | 1.60  |
| 17beta-Acetamidoandrost-4-en-3-one | 0.038  | 1.58  | 6.12  | N-Acetyl-D-galactosamine | 0.010  | 1.81  | 1.61  |
| Labetalol | 0.038  | 1.56  | 0.62  | N6-Acetyl-N6-hydroxy-L-lysine | 0.049  | 1.40  | 0.38  |
| Kyotorphin | 0.015  | 1.65  | 0.59  | Isoelemicin | <0.001  | 2.10  | 1.62  |
| 17alpha,21-Dihydroxypregnenolone | 0.021  | 1.57  | 0.69  | Pilocarpine | 0.049  | 1.55  | 1.53  |
| Misoprostol | 0.007  | 1.68  | 0.42  | Harmine | 0.015  | 1.40  | 1.63  |
| Resolvin D2 | 0.005  | 1.99  | 0.28  | N-a-Acetylcitrulline | 0.010  | 1.44  | 1.70  |
| N-Acetyllactosamine | 0.021  | 1.69  | 0.38  | N-Acetylserotonin | <0.001  | 1.99  | 2.12  |
| 6-Keto-prostaglandin F1a | 0.007  | 1.96  | 2.42  | Prephenate | 0.015  | 1.79  | 1.31  |
| Lovastatin | 0.028  | 1.55  | 0.49  | Deoxyuridine | 0.015  | 1.66  | 1.57  |
| Allocholic acid | 0.038  | 1.35  | 0.66  | Trioxsalen | 0.038  | 1.47  | 1.40  |
| LysoPA(16\_0\_0\_0) | 0.015  | 1.69  | 0.43  | Traumatic Acid | 0.015  | 1.53  | 1.69  |
| Licoricidin | 0.007  | 2.01  | 3.41  | Propazine | 0.038  | 1.39  | 1.93  |
| alpha-Tocopherol | <0.001  | 2.65  | 0.16  | Costunolide | 0.010  | 1.55  | 1.90  |
| Hecogenin | 0.021  | 1.80  | 0.19  | N-[(2S)-2-Amino-2-carboxyethyl]-L-glutamate | 0.003  | 1.91  | 1.53  |
| 16-Feruloyloxypalmitate | 0.021  | 1.68  | 0.46  | Equol | 0.028  | 1.55  | 1.66  |
| N1,N8-Bis(4-coumaroyl)spermidine | 0.041  | 1.25  | 0.18  | Ribavirin | 0.038  | 1.44  | 1.45  |
| Maslinic acid | 0.038  | 1.76  | 0.46  | beta-Alanyl-L-arginine | 0.002  | 1.95  | 1.57  |
| alpha-D-Galactosyl-1,3-beta-D-galactosyl-1,4-N-acetyl-D-glucosamine | 0.003  | 1.93  | 0.20  | Pyridoxamine 5'-phosphate | 0.007  | 1.74  | 1.52  |
| NAD | 0.001  | 2.32  | 0.19  | 6-Hydroxymelatonin | 0.038  | 1.59  | 1.56  |
| Succinic acid semialdehyde | 0.021  | 1.63  | 2.88  | Deoxyadenosine | 0.015  | 1.57  | 1.34  |
| alpha-Ketoisovaleric acid | 0.049  | 1.66  | 2.15  | 16-Hydroxy hexadecanoic acid | 0.010  | 1.71  | 1.55  |
| 5-Aminopentanoic acid | 0.028  | 1.77  | 1.48  | Imidacloprid | <0.001  | 1.69  | 3.13  |
| Purine | 0.028  | 1.61  | 1.96  | Parthenin | 0.038  | 1.39  | 1.59  |
| L-Cysteine | 0.001  | 2.49  | 0.44  | Linoleic acid | 0.038  | 1.59  | 1.81  |
| 2-Dehydro-3-deoxy-D-xylonate | 0.021  | 1.70  | 2.68  | Tramadol | 0.005  | 1.84  | 1.80  |
| D-Ribose | 0.015  | 1.86  | 1.35  | Thiamine | 0.015  | 1.23  | 2.20  |
| 3,4-Dihydroxybenzoate | 0.049  | 1.83  | 3.46  | Nevirapine | 0.015  | 1.50  | 1.89  |
| Citrulline | 0.021  | 1.72  | 0.56  | Androstenedione | 0.010  | 1.59  | 1.50  |
| L-Tryptophan | 0.038  | 1.68  | 0.46  | Estrone | 0.015  | 1.55  | 1.57  |
| Indolelactic acid | 0.007  | 2.11  | 0.26  | Nordiazepam | 0.005  | 1.72  | 2.17  |
| N-Acetyl-L-phenylalanine | 0.015  | 1.77  | 0.38  | Genistein | 0.001  | 2.17  | 1.83  |
| Citrinin | 0.007  | 1.91  | 0.60  | Sotalol | 0.028  | 1.19  | 1.79  |
| N2-gamma-Glutamylglutamine | 0.028  | 1.41  | 3.00  | N2-Succinyl-L-arginine | 0.028  | 1.38  | 1.44  |
| 9,10-DHOME | 0.005  | 1.95  | 1.94  | Saccharopine | 0.038  | 1.45  | 1.77  |
| 6-Ketoprostaglandin E1 | 0.038  | 1.69  | 0.58  | 2-Cyano-3,3-diphenylacrylic acid ethyl ester | 0.049  | 1.17  | 1.41  |
| FMN | 0.028  | 1.83  | 0.21  | 1-Methyladenosine | 0.003  | 1.99  | 1.51  |
| Adenosine diphosphate ribose | 0.049  | 1.39  | 2.66  | Oleamide | 0.015  | 1.34  | 1.80  |
| Metabolites | *P*-value | VIP | FC **(5.0%FHRB /5.0%HRB)** | Norcodeine | 0.005  | 1.63  | 1.76  |
| L-Leucine | 0.028  | 1.53  | 0.71  | Piperine | 0.028  | 1.59  | 1.50  |
| Hypoxanthine | 0.038  | 1.73  | 0.67  | N1,N12-Diacetylspermine | 0.007  | 1.77  | 1.86  |
| Methylimidazoleacetic acid | 0.015  | 2.20  | 0.33  | Anastrozole | 0.049  | 1.36  | 1.47  |
| Pimelate | 0.049  | 1.94  | 1.89  | Trimeprazine | 0.049  | 1.47  | 1.42  |
| Phosphorylcholine | 0.002  | 2.40  | 1.59  | Sphingosine | 0.021  | 1.51  | 1.79  |
| Acetylphosphate | <0.001  | 2.01  | 0.80  | Adrenosterone | 0.021  | 1.37  | 1.66  |
| O-Acetylcarnitine | 0.038  | 1.83  | 1.60  | Sphinganine | 0.049  | 1.37  | 1.75  |
| Cytosine | 0.049  | 1.54  | 0.48  | Nicotianamine | 0.028  | 1.36  | 1.40  |
| Biocytin | 0.038  | 1.71  | 0.61  | 8,9-EET | 0.007  | 1.65  | 1.76  |
| Thiamine | 0.015  | 1.93  | 0.43  | 4'-Oxolividamine | 0.038  | 1.55  | 1.79  |
| N-Acetylleucine | 0.021  | 2.11  | 0.34  | Clomipramine | 0.021  | 1.52  | 2.24  |
| Thymine | 0.038  | 1.41  | 0.52  | Ubiquinone-1 | 0.002  | 1.96  | 1.62  |
| Saccharopine | 0.003  | 1.92  | 0.35  | Citalopram | <0.001  | 2.10  | 1.90  |
| Nalpha-Methylhistidine | 0.010  | 2.04  | 0.57  | 2alpha-Methylpregn-4-ene-3,20-dione | 0.028  | 1.10  | 1.67  |
| Agmatine | 0.038  | 1.81  | 2.07  | Hydroxychloroquine | 0.015  | 1.24  | 1.66  |
| Pyrophosphate | 0.049  | 1.90  | 0.63  | 12-Keto-tetrahydro-leukotriene B4 | 0.049  | 1.48  | 0.72  |
| Alpha-Tocotrienol | 0.028  | 1.76  | 0.55  | Kyotorphin | 0.003  | 1.84  | 1.66  |
| N1,N8-Bis(4-coumaroyl)spermidine | 0.001  | 2.63  | 4.96  | Erucic acid | 0.028  | 1.51  | 0.81  |
| 3-Keto-4-methylzymosterol | 0.015  | 1.76  | 2.83  | Bioresmethrin | 0.049  | 1.41  | 1.49  |
| Hecogenin | <0.001  | 2.54  | 5.67  | Naltrexone | 0.007  | 1.68  | 1.63  |
| p-Octopamine | 0.010  | 2.28  | 0.94  | Coniferin | 0.007  | 1.79  | 2.46  |
| Hydroquinone | 0.028  | 1.85  | 0.66  | L-Alanyl-gamma-D-glutamyl-L-lysine | 0.010  | 1.96  | 3.20  |
| alpha-Tocopherol | 0.005  | 2.40  | 4.79  | N-Acetylmuramoyl-Ala | 0.038  | 1.13  | 1.33  |
| Phenylacetaldehyde | 0.038  | 1.88  | 1.64  | 17alpha,21-Dihydroxypregnenolone | 0.021  | 1.67  | 1.60  |
| Retinal | <0.001  | 3.18  | 48.27  | Prostaglandin I2 | 0.005  | 1.85  | 2.28  |
| Phenyllactic acid | <0.001  | 2.53  | 2.43  | Vincamine | 0.021  | 1.45  | 2.39  |
| 19-Hydroxytestosterone | 0.021  | 1.82  | 0.51  | Yohimbine | 0.028  | 1.60  | 1.50  |
| 5-Nitro-2-benzoic acid | 0.038  | 1.71  | 0.55  | Nitrendipine | 0.005  | 1.80  | 1.51  |
| 2-Dehydro-3-deoxy-L-rhamnonate | 0.049  | 1.72  | 0.69  | 1-Arachidonoylglycerol | 0.021  | 1.58  | 0.63  |
| Metaraminol | 0.038  | 1.87  | 1.29  | Chelirubine | 0.038  | 1.38  | 0.49  |
| Caprylic acid | 0.038  | 1.97  | 1.96  | Enalapril | 0.028  | 1.44  | 1.41  |
| Coniferin | 0.049  | 1.82  | 0.40  | S-Inosyl-L-homocysteine | 0.001  | 2.25  | 2.08  |
| Cinnamaldehyde | 0.038  | 1.64  | 1.44  | beta-Sitosterol | 0.010  | 1.78  | 0.47  |
| 4-Methoxy-2,2'-bipyrrole-5-carbaldehyde | 0.015  | 1.83  | 0.33  | Licoricidin | 0.038  | 1.51  | 0.38  |
| Neocnidilide | 0.001  | 2.47  | 0.44  | Copal-8-ol diphosphate | 0.015  | 1.55  | 1.70  |
| Purine | 0.015  | 2.37  | 2.58  | Protoporphyrin IX | 0.021  | 1.16  | 0.42  |
| alpha-D-Galactosyl-1,3-beta-D-galactosyl-1,4-N-acetyl-D-glucosamine | <0.001  | 2.87  | 9.44  | (R)-2,3-Dihydroxy-isovalerate | 0.038  | 1.22  | 1.82  |
| 6-Methoxymellein | 0.038  | 1.86  | 0.31  | L-Cysteine | 0.038  | 1.59  | 1.48  |
| Xanthosine | 0.015  | 2.09  | 2.00  | L-Aspartic acid | 0.010  | 1.96  | 0.29  |
| Prostaglandin I2 | 0.038  | 1.98  | 0.45  | L-Ribulose | 0.028  | 1.62  | 1.33  |
| Hippuric acid | 0.021  | 2.11  | 1.37  | Uric acid | 0.010  | 1.91  | 0.58  |
| Resolvin D2 | <0.001  | 2.85  | 6.21  | myo-Inositol | 0.010  | 1.82  | 2.34  |
| Pseudouridine | 0.049  | 1.61  | 0.57  | D-Mannose | 0.007  | 2.28  | 1.67  |
| delta-Tocotrienol | 0.002  | 2.46  | 0.18  | Cysteine-S-sulfate | 0.049[  | 1.73  | 1.58  |
| Dethiobiotin | 0.015  | 2.11  | 0.42  | Pantothenic acid | 0.021  | 2.02  | 2.01  |
| 5'-S-Methyl-5'-thioinosine | 0.005  | 2.44  | 0.52  | Thymidine | <0.001  | 2.76  | 1.80  |
| o-Cresol | <0.001  | 2.04  | 0.53  | Pentadecanoic acid | 0.049  | 1.58  | 1.85  |
|  |  |  |  | Arbutin | 0.015  | 1.84  | 1.74  |
|  |  |  |  | N2-gamma-Glutamylglutamine | 0.049  | 1.47  | 0.38  |
|  |  |  |  | 5'-S-Methyl-5'-thioinosine | 0.002  | 2.28  | 2.10  |
|  |  |  |  | Chenodeoxycholic acid | 0.021  | 1.92  | 1.66  |

**Table** S**5 Enrichment analysis of differentially expressed metabolites in cecal chyme**

|  |
| --- |
| Metabolic pathways between 2.5% HRB and 2.5% FHRB groups |
| Pathway name | Hits | *P-*value | Impact |
| Oxidative phosphorylation | 3 | 0.016  | 0.15  |
| Pantothenate and CoA biosynthesis | 4 | 0.019  | 0.11  |
| Aminoacyl-tRNA biosynthesis | 5 | 0.032  | 0.10  |
| Phenylalanine metabolism | 5 | 0.055  | 0.08  |
| Vitamin B6 metabolism | 3 | 0.077  | 0.13  |
| Thiamine metabolism | 3 | 0.090  | 0.11  |
| Lysine degradation | 4 | 0.093  | 0.03  |
| Glycine, serine and threonine metabolism | 4 | 0.093  | 0.05  |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 3 | 0.111  | 0.08  |
| Arginine and proline metabolism | 5 | 0.131  | 0.06  |
| mTOR signaling pathway | 1 | 0.131  | 0.25  |
| Taurine and hypotaurine metabolism | 2 | 0.175  | 0.09  |
| Valine, leucine and isoleucine biosynthesis | 2 | 0.188  | 0.13  |
| Arginine biosynthesis | 2 | 0.188  | 0.15  |
| Retinol metabolism | 2 | 0.214  | 0.13  |
| Phototransduction | 1 | 0.246  | 0.10  |
| Alanine, aspartate and glutamate metabolism | 2 | 0.252  | 0.05  |
| Arachidonic acid metabolism | 4 | 0.260  | 0.03  |
| Ferroptosis | 2 | 0.265  | 0.09  |
| Sulfur relay system | 1 | 0.322  | 0.13  |
| Pentose phosphate pathway | 2 | 0.343  | 0.01  |
| D-Glutamine and D-glutamate metabolism | 1 | 0.368  | 0.10  |
| Cysteine and methionine metabolism | 3 | 0.373  | 0.13  |
| Valine, leucine and isoleucine degradation | 2 | 0.430  | 0.06  |
| Steroid hormone biosynthesis | 4 | 0.450  | 0.04  |
| Inositol phosphate metabolism | 2 | 0.489  | 0.00  |
| Nitrogen metabolism | 1 | 0.489  | 0.03  |
| Riboflavin metabolism | 1 | 0.507  | 0.18  |
| Tyrosine metabolism | 3 | 0.511  | 0.02  |
| ABC transporters | 5 | 0.525  | 0.04  |
| Glycerophospholipid metabolism | 2 | 0.543  | 0.08  |
| Tryptophan metabolism | 3 | 0.554  | 0.04  |
| Nicotinate and nicotinamide metabolism | 2 | 0.574  | 0.07  |
| Pentose and glucuronate interconversions | 2 | 0.584  | 0.02  |
| Sphingolipid metabolism | 1 | 0.587  | 0.05  |
| Linoleic acid metabolism | 1 | 0.629  | 0.03  |
| Pyrimidine metabolism | 2 | 0.666  | 0.03  |
| Pyruvate metabolism | 1 | 0.666  | 0.03  |
| beta-Alanine metabolism | 1 | 0.678  | 0.04  |
| Sulfur metabolism | 1 | 0.689  | 0.02  |
| Glutathione metabolism | 1 | 0.740  | 0.02  |
| Butanoate metabolism | 1 | 0.774  | 0.05  |
| alpha-Linolenic acid metabolism | 1 | 0.790  | 0.02  |
| Primary bile acid biosynthesis | 1 | 0.811  | 0.03  |
| Drug metabolism - cytochrome P450 | 2 | 0.812  | 0.03  |
| Ubiquinone and other terpenoid-quinone biosynthesis | 2 | 0.836  | 0.02  |
| Drug metabolism - other enzymes | 1 | 0.842  | 0.02  |
| Purine metabolism | 2 | 0.849  | 0.02  |
| Glyoxylate and dicarboxylate metabolism | 1 | 0.890  | 0.00  |
| Metabolic pathways between 5.0% HRB and 5.0% FHRB groups |
| Pathway name | Hits | *P-*value | Impact |
| Biotin metabolism | 3 | 0.008  | 0.08  |
| mTOR signaling pathway | 1 | 0.059  | 0.25  |
| Pyrimidine metabolism | 3 | 0.073  | 0.01  |
| VEGF signaling pathway | 1 | 0.087  | 0.17  |
| Phototransduction | 1 | 0.114  | 0.10  |
| Sulfur relay system | 1 | 0.154  | 0.07  |
| Ubiquinone and other terpenoid-quinone biosynthesis | 3 | 0.160  | 0.02  |
| Lipoic acid metabolism | 1 | 0.179  | 0.08  |
| Neuroactive ligand-receptor interaction | 2 | 0.184  | 0.04  |
| Vascular smooth muscle contraction | 1 | 0.216  | 0.06  |
| Oxidative phosphorylation | 1 | 0.216  | 0.10  |
| Phenylalanine metabolism | 2 | 0.228  | 0.05  |
| Caffeine metabolism | 1 | 0.285  | 0.03  |
| Taurine and hypotaurine metabolism | 1 | 0.285  | 0.03  |
| Valine, leucine and isoleucine biosynthesis | 1 | 0.295  | 0.06  |
| Retinol metabolism | 1 | 0.317  | 0.12  |
| ABC transporters | 3 | 0.345  | 0.02  |
| Ferroptosis | 1 | 0.357  | 0.02  |
| Pyruvate metabolism | 1 | 0.377  | 0.03  |
| Thiamine metabolism | 1 | 0.377  | 0.07  |
| Purine metabolism | 2 | 0.422  | 0.04  |
| Valine, leucine and isoleucine degradation | 1 | 0.474  | 0.02  |
| Histidine metabolism | 1 | 0.513  | 0.04  |
| Lysine degradation | 1 | 0.535  | 0.05  |
| Glycerophospholipid metabolism | 1 | 0.549  | 0.01  |
| Aminoacyl-tRNA biosynthesis | 1 | 0.549  | 0.02  |
| Fructose and mannose metabolism | 1 | 0.562  | 0.01  |
| Fatty acid biosynthesis | 1 | 0.589  | 0.01  |
| Steroid biosynthesis | 1 | 0.589  | 0.02  |
| Cysteine and methionine metabolism | 1 | 0.619  | 0.00  |
| Arachidonic acid metabolism | 1 | 0.684  | 0.01  |
| Tyrosine metabolism | 1 | 0.698  | 0.00  |
| Arginine and proline metabolism | 1 | 0.698  | 0.01  |
| Steroid hormone biosynthesis | 1 | 0.783  | 0.00  |
| Metabolic pathways between 2.5% HRB and 5.0% HRB groups |
| Pathway name | Hits | *P-*value | Impact |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 4 | 0.001  | 0.13  |
| Arachidonic acid metabolism | 3 | 0.054  | 0.04  |
| PPAR signaling pathway | 1 | 0.056  | 0.20  |
| Phenylalanine metabolism | 2 | 0.151  | 0.05  |
| Arginine biosynthesis | 1 | 0.235  | 0.02  |
| Tryptophan metabolism | 2 | 0.248  | 0.04  |
| Retinol metabolism | 1 | 0.253  | 0.01  |
| Linoleic acid metabolism | 1 | 0.278  | 0.06  |
| Pantothenate and CoA biosynthesis | 1 | 0.295  | 0.01  |
| beta-Alanine metabolism | 1 | 0.311  | 0.03  |
| Glutathione metabolism | 1 | 0.358  | 0.01  |
| Glycine, serine and threonine metabolism | 1 | 0.443  | 0.00  |
| Lysine degradation | 1 | 0.443  | 0.01  |
| Aminoacyl-tRNA biosynthesis | 1 | 0.456  | 0.02  |
| Neuroactive ligand-receptor interaction | 1 | 0.456  | 0.02  |
| Biosynthesis of unsaturated fatty acids | 1 | 0.581  | 0.02  |
| Arginine and proline metabolism | 1 | 0.600  | 0.02  |
| Tyrosine metabolism | 1 | 0.600  | 0.03  |
| Ubiquinone and other terpenoid-quinone biosynthesis | 1 | 0.662  | 0.02  |
| Purine metabolism | 1 | 0.673  | 0.00  |
| Steroid hormone biosynthesis | 1 | 0.689  | 0.00  |
| Metabolic pathways between 2.5% FHRB and 5.0% FHRB groups |
| Pathway name | Hits | *P-*value | Impact |
| Pantothenate and CoA biosynthesis | 4 | 0.028  | 0.14  |
| beta-Alanine metabolism | 4 | 0.035  | 0.16  |
| Cysteine and methionine metabolism | 6 | 0.036  | 0.11  |
| Aminoacyl-tRNA biosynthesis | 5 | 0.052  | 0.08  |
| Sulfur relay system | 2 | 0.067  | 0.20  |
| Histidine metabolism | 4 | 0.112  | 0.14  |
| Vascular smooth muscle contraction | 2 | 0.129  | 0.13  |
| Glycine, serine and threonine metabolism | 4 | 0.132  | 0.08  |
| Neuroactive ligand-receptor interaction | 4 | 0.147  | 0.08  |
| Lysosome | 1 | 0.149  | 0.25  |
| Apoptosis | 1 | 0.149  | 0.25  |
| ABC transporters | 8 | 0.175  | 0.06  |
| PPAR signaling pathway | 1 | 0.182  | 0.20  |
| VEGF signaling pathway | 1 | 0.215  | 0.17  |
| Valine, leucine and isoleucine biosynthesis | 2 | 0.229  | 0.06  |
| Arginine biosynthesis | 2 | 0.229  | 0.11  |
| Sphingolipid metabolism | 2 | 0.259  | 0.12  |
| Galactose metabolism | 3 | 0.271  | 0.02  |
| Vitamin B6 metabolism | 2 | 0.318  | 0.10  |
| Necroptosis | 1 | 0.331  | 0.08  |
| Thiamine metabolism | 2 | 0.347  | 0.13  |
| C-type lectin receptor signaling pathway | 1 | 0.383  | 0.08  |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 2 | 0.390  | 0.05  |
| Oxidative phosphorylation | 1 | 0.475  | 0.05  |
| Pyrimidine metabolism | 3 | 0.476  | 0.04  |
| Steroid hormone biosynthesis | 4 | 0.554  | 0.08  |
| Inositol phosphate metabolism | 2 | 0.559  | 0.04  |
| Arachidonic acid metabolism | 3 | 0.574  | 0.04  |
| Taurine and hypotaurine metabolism | 1 | 0.588  | 0.06  |
| Arginine and proline metabolism | 3 | 0.601  | 0.03  |
| Tyrosine metabolism | 3 | 0.601  | 0.07  |
| Tryptophan metabolism | 3 | 0.643  | 0.07  |
| Drug metabolism - cytochrome P450 | 3 | 0.675  | 0.03  |
| Linoleic acid metabolism | 1 | 0.677  | 0.06  |
| Alanine, aspartate and glutamate metabolism | 1 | 0.677  | 0.15  |
| Phosphatidylinositol signaling system | 1 | 0.690  | 0.02  |
| Ferroptosis | 1 | 0.690  | 0.07  |
| Glycolysis / Gluconeogenesis | 1 | 0.714  | 0.00  |
| Sulfur metabolism | 1 | 0.737  | 0.02  |
| Pentose phosphate pathway | 1 | 0.757  | 0.01  |
| Glutathione metabolism | 1 | 0.785  | 0.02  |
| Biosynthesis of unsaturated fatty acids | 2 | 0.798  | 0.03  |
| alpha-Linolenic acid metabolism | 1 | 0.832  | 0.02  |
| Neomycin, kanamycin and gentamicin biosynthesis | 2 | 0.837  | 0.02  |
| Primary bile acid biosynthesis | 1 | 0.851  | 0.03  |
| Ascorbate and aldarate metabolism | 1 | 0.868  | 0.03  |
| Lysine degradation | 1 | 0.868  | 0.05  |
| Fructose and mannose metabolism | 1 | 0.888  | 0.04  |
| Nicotinate and nicotinamide metabolism | 1 | 0.893  | 0.02  |
| Purine metabolism | 2 | 0.896  | 0.02  |
| Pentose and glucuronate interconversions | 1 | 0.897  | 0.07  |
| Fatty acid biosynthesis | 1 | 0.905  | 0.01  |
| Steroid biosynthesis | 1 | 0.905  | 0.02  |
| Ubiquinone and other terpenoid-quinone biosynthesis | 1 | 0.977  | 0.01  |
| Amino sugar and nucleotide sugar metabolism | 1 | 0.988  | 0.01  |
| Porphyrin and chlorophyll metabolism | 1 | 0.997  | 0.02  |

Notes: Hits, number of overall differential metabolites in target metabolic pathways; *P*-value, of hypergeometric distribution test, smaller represents more significant impact of detected differential metabolites on the pathway; impact, metabolic pathway impact value, the larger the effect of detected differential metabolites on the target pathway.