**Calorie restriction improves serum lipid metabolism, colon metabolites and microbiota in pigs**

**JL Liu\_ Online Supplementary Material**

**Supplemental Table 1**. Effects of calorie restriction on serum free amino acid of pigs

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Items** | **Con** | **CR** | **SEM** | ***P*-value** |
| Aspartic acid (Asp)  | 1.34±0.03 | 1.35±0.05 | 0.03 | 0.82 |
| Threonine (Thr)  | 15.8±0.14 | 15.3±0.23 | 0.15 | 0.06 |
| Serine (Ser) | 8.35±0.12 | 8.31±0.11 | 0.08 | 0.84 |
| Glutamic acid (Glu) | 22.7±0.24 | 22.8±0.17 | 0.14 | 0.69 |
| Glycine (Gly) | 73.5±0.62 | 72.2±0.76 | 0.50 | 0.21 |
| Alanine (Ala) | 26.1±0.10 | 25.8±0.16 | 0.10 | 0.27 |
| Cystine (Cys) | 1.82±0.02 | 1.79±0.02 | 0.02 | 0.39 |
| Valine (Val) | 24.5±0.31 | 24.7±0.15 | 0.17 | 0.69 |
| Methionine (Met) | 5.35±0.15 | 5.18±0.06 | 0.08 | 0.29 |
| Isoleucine (Ile) | 12.6±0.08 | 12.6±0.18 | 0.09 | 0.88 |
| Leucine (Leu) | 22.9±0.13 | 23.3±0.21 | 0.13 | 0.19 |
| Tyrosine (Tyr) | 1.46±0.03 | 1.47±0.05 | 0.03 | 0.92 |
| Phenylalanine (Phe) | 12.7±0.16 | 11.5±1.37 | 0.69 | 0.39 |
| Lysine (Lys) | 14.8±0.15 | 14.8±0.11 | 0.09 | 0.92 |
| Histidine (His) | 11.8±0.13 | 11.8±0.16 | 0.10 | 0.97 |
| Arginine (Arg) | 21.3±0.32 | 21.7±0.26 | 0.20 | 0.33 |
| Proline (Pro)  | 23.1±0.76 | 22.6±0.63 | 0.48 | 0.61 |
| Total | 300±1.06 | 297±2.28 | 1.28 | 0.25 |

Con, control; CR, calorie restriction. The amount of feed in the CR group was 70% of that in the Con group. Values are means ± SEMs, n= 8/group. In the same row, values with no letter superscripts mean no significant difference (*P* > 0.05), while with different small letter superscripts mean significant difference (*P* ≤ 0.05).

**Supplemental Table 2**. Effects of calorie restriction on visceral organ weight and indices of pigs.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Items** | **Con** | **CR** | **SEM** | ***P*-value** |
| Final BW (kg) | 122±1.52a | 113±1.86b | 1.66 | < 0.01 |
| Heart weight (kg) | 0.428±0.018a | 0.372±0.013b | 0.01 | 0.02 |
| Liver weight (kg) | 1.81±0.07 | 1.78±0.04 | 0.04 | 0.66 |
| Spleen weight (kg) | 0.209±0.02 | 0.174±0.009 | 0.01 | 0.14 |
| Heart index (%) | 0.350±0.01 | 0.351±0.01 | 0.008 | 0.26 |
| Liver index (%) | 1.49±0.05 | 1.58±0.06 | 0.039 | 0.22 |
| Spleen index (%) | 0.172±0.02 | 0.156±0.01 | 0.010 | 0.42 |

Con, control; CR, calorie restriction. BW, body weight. The amount of feed in the CR group was 70% of that in the Con group. Values are means ± SEMs, n= 8/group. In the same row , values with no letter superscripts mean no significant difference (*P* ＞ 0.05), while with different small letter superscripts mean significant difference (*P* ≤ 0.05).

**Supplemental Figure 1**. Rarefaction curves of observed species (A), Chao 1 index (B) and Venn of colon OTUs number (C) for gut microbiota diversity of pigs in the Con group and CR group. The rarefaction curve of observed species and Chao 1 index of gut microbiota plateaued with the increase of reads. The venn diagram showed that the OTUs increased in the CR group than in the Con group. Con, control; CR, calorie restriction. The Con pigs were provided with *ad libitum* access to chow diet, and the amount of feed in the CR group was 70% of that in the Con group.



**Supplemental Figure 2**. Correlations between the differences of bacteria (genus) and the metabolites or the SCFAs in the colon. (A) Correlations between the differences of bacteria (genus) and metabolites. (B) Correlations between bacteria (genus) and SCFAs in the colon. The Spearman correlation between differential bacteria (genus) and metabolites was explored in 16 individuals in two groups. The concentrations of L-Glutamic acid and gamma-aminobutyric acid were significantly negatively correlated with *Lachnospiraceae\_XPB1014\_group*. The concentrations of xanthine and 9,10-epoxyoctadecanoic acid were significantly negatively correlated with *Romboutsia*. We also analyzed the Spearman correlations between differential bacteria (genus) and SCFAs. Bacteria from genera *Escherichia-Shigella,* *Romboutsia* and *Candidatus\_Saccharimonas* were positively correlated with the body weight, whereas were negatively correlated with the valeric acid. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high density lipoproteins cholesterol; LDL-C, low density lipoproteins cholesterol; SCFAs, short-chain fatty acid; TC, total cholesterol; TG, total triglyceride.