**Animal: An International Journal of Animal Bioscience**

**Protein restriction and succedent realimentation affecting ileal morphology, ileal microbial composition and metabolites in weaned piglets**

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**Supplementary material**

**Supplementary Table S1** *The ingredient and nutrient composition of diets (as fed basis) in weaned piglets.*

|  |  |  |
| --- | --- | --- |
| Ingredient（kg/100kg） | NPD1 | LPD2 |
| corn | 41.62 | 60.91 |
| cornstarch | 18.30 | 16.22 |
| soybean meal | 25.97 | 11.20 |
| fishmeal | 5.00 | 1.41 |
| soybean oil | 4.00 | 4.00 |
| L-lysine | 0.70 | 1.30 |
| DL-methionine | 0.23 | 0.34 |
| L-threonine | 0.18 | 0.50 |
| L-tryptophan | 0.01 | 0.12 |
| premix3 | 4.00 | 4.00 |
| total | 100.00 | 100.00 |
| Calculated nutritional values |  |  |
| Digestible energy, Mcal/kg | 3.49 | 3.54 |
| Crude protein, % | 18.83 | 13.05 |
| Lysine, % | 1.52 | 1.51 |
| Methionine+Cysteine, % | 0.83 | 0.74 |
| Threonine, % | 0.91 | 0.92 |
| Methionine, % | 0.55 | 0.54 |
| Dry matter, % | 90.26 | 89.83 |
| Tryptophan, % | 0.25 | 0.25 |

1NPD = normal protein diet.

2LPD = low protein diet.

3The premix per kg contains vitamin A (KIU) 100-400, vitamin D3 (KIU) 25-125, vitamin E (mg) ≥ 300, vitamin K3 (mg) ≥ 30, vitamin B1 (mg) ≥ 25, vitamin B2 (mg) ≥ 75, vitamin B6 (mg) ≥ 35, vitamin B12 (mg) ≥ 0.25, niacin (mg) ≥ 400, pantothenic acid (mg) ≥ 300, folic acid (mg) ≥ 14, D-biotin (mg) ≥ 1.0, choline chloride (mg) ≥ 5000, Cu (mg) 500-5000, Fe (mg) 500-7500, Zn (mg) 500-3750, Mn (mg) 500-3750, I (mg) ≥ 3, Se (mg) 2.5-12.5, Ca (%) 10-20, total P (%) ≥ 1.5, sodium chloride (%) 5-14, arginine (%)≥ 3.7, phytase (U) ≥ 20000.

**Supplementary Table S2** *The primer sequences of ileal mucosal genes of weaned piglets.*

|  |  |
| --- | --- |
| Gene | Primer pairs sequence |
| *β-actin* | F- ATGCTTCTAGACGGACTGCG |
| R- GTTTCAGGAGGCTGGCATGA |
| *ZO-1* | F- GAGGATGGTCACACCGTGGT |
| R- GGAGGATGCTGTTGTCTCGG |
| *Occludin* | F- ATGCTTTCTCAGCCAGCGTA |
| R- AAGGTTCCATAGCCTCGGTC | |
| *MUC-2* | F-CTGCTCCGGGTCCTGTGGGA | |
| R-CCCGCTGGCTGGTGCGATAC | |
| *Claudin-1* | F-GCCACAGCAAGGTATGGTAAC | |
| R-AGTAGGGCACCTCCCAGAAG | |
| *Claudin-2* | F-CTCGTTGGCCTGTATCATCACC | |
| R-CAGGGGGGAGTAGAAGTCCC | |
| *TGF-β* | F-GGTGCTAATGGTGGAAAGCG | |
| R-GGAGCTCCGACGTGTTGAA | |
| *IL-1β* | F- AGTGGAGAAGCCGATGAAGA | |
| R- CATTGCACGTTTCAAGGATG | |
| *APA* | F- GTCTCTACCACCTGACGAT | |
| R- CTCTGTAAGTGATGAGTCC | |
| *APN* | F- ACATCACTCTCATCCACCCT | |
| R- GCAATCACAGTGACAACTCG | |
| *PEPT-1* | F- GATGAAATGTGAGCGTATGGG | |
| R-AAAGAGGGAGGATCTGGAAAA | |
| *SGLT-1* | F- CCACTTTCCCTATAAAACCTCAC | |
| R-CTCCATCAAACTTCCATCCTCAG | |

*ZO-1* = zonula occludens-1; *MUC-2* = mucin-2; *TGF-β* = transforming growth factor-β; *IL-1β* = interleukin-1β; *APA* = amino-peptidase A; *APN* = amino-peptidase N; *PEPT-1* = peptide transporter-1; *SGLT-1* = sodium-glucose transporter-1.

**Supplementary Table S3** *Effect of protein restriction and realimentation on bacterial alpha diversity in the* *ileal content of weaned piglets.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Item | CG | TG | SEM | *P* value |
| day 14 |  |  |  |  |
| Shannon | 1.82 | 2.12 | 0.29 | 0.322 |
| Simpson | 0.39 | 0.27 | 0.09 | 0.216 |
| Chao | 158.33 | 161.83 | 16.44 | 0.836 |
| OTU | 134.41 | 144.58 | 13.12 | 0.456 |
| day 28 |  |  |  |  |
| Shannon | 2.42 | 2.07 | 0.33 | 0.301 |
| Simpson | 0.19 | 0.23 | 0.06 | 0.543 |
| Chao | 200.11 | 162.61 | 20.92 | 0.103 |
| OTU | 188.67 | 155.67 | 19.83 | 0.127 |

CG = control group; TG = treatment group.

OTU = Operational Taxonomic Units.

**Supplementary Table S4** *The altered relative abundance of microbiota at the phylum level in the* *ileal content of weaned piglets.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Item | CG | TG | SEM | *P* value |
| day 14 |  |  |  |  |
| Firmicutes | 79.95 | 78.20 | 5.17 | 0.742 |
| Proteobacteria | 19.55 | 17.30 | 4.67 | 0.641 |
| Actinobacteria | 0.39 | 2.52 | 0.60 | 0.005 |
| day 28 |  |  |  |  |
| Firmicutes | 75.38 | 92.95 | 6.74 | 0.029 |
| Proteobacteria | 21.00 | 5.01 | 4.64 | 0.026 |
| Actinobacteria | 1.32 | 0.69 | 0.41 | 0.153 |

CG = control group; TG = treatment group.

**Supplementary Table S5** *The altered relative abundance of microbiota at the family level in the ileal content of weaned piglets.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Item | CG | TG | SEM | *P* value |
| day 14 |  |  |  |  |
| Lactobacillaceae | 69.62 | 27.22 | 4.35 | <0.001 |
| Clostridiaceae 1 | 0.49 | 19.81 | 1.27 | <0.001 |
| Pseudomonadaceae | 2.99 | 3.16 | 2.13 | 0.936 |
| Enterobacteriaceae | 1.91 | 1.78 | 1.26 | 0.921 |
| Moraxellaceae | 0.11 | 0.76 | 0.12 | 0.358 |
| Halomonadaceae | 2.33 | 9.76 | 1.80 | 0.002 |
| day 28 |  |  |  |  |
| Lactobacillaceae | 59.13 | 71.23 | 5.32 | 0.046 |
| Clostridiaceae 1 | 0.28 | 2.04 | 0.38 | 0.001 |
| Pseudomonadaceae | 6.90 | 0.29 | 0.62 | <0.001 |
| Enterobacteriaceae | 3.62 | 0.23 | 1.06 | 0.017 |
| Moraxellaceae | 1.09 | 0.06 | 0.36 | 0.029 |
| Halomonadaceae | 7.01 | 2.11 | 0.80 | <0.001 |

CG = control group; TG = treatment group.

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**Supplementary Figure S1** Effect of protein restriction and realimentation on bacterial community composition in the ileal content of piglets. (a) Effect of protein restriction and realimentation on ileal bacterial community composition at phylum level in piglets. (b) at family level in piglets. (c) at genus level in piglets. Phyla, families and genera with proportion less than 1% are not listed. CG = control group; TG = treatment group.