Intrauterine growth restriction affects colonic barrier function via regulating the Nrf2/Keap1 and TLR4-NFκB/ERK pathways and altering colonic microbiota and metabolome homeostasis in growing-finishing pig model

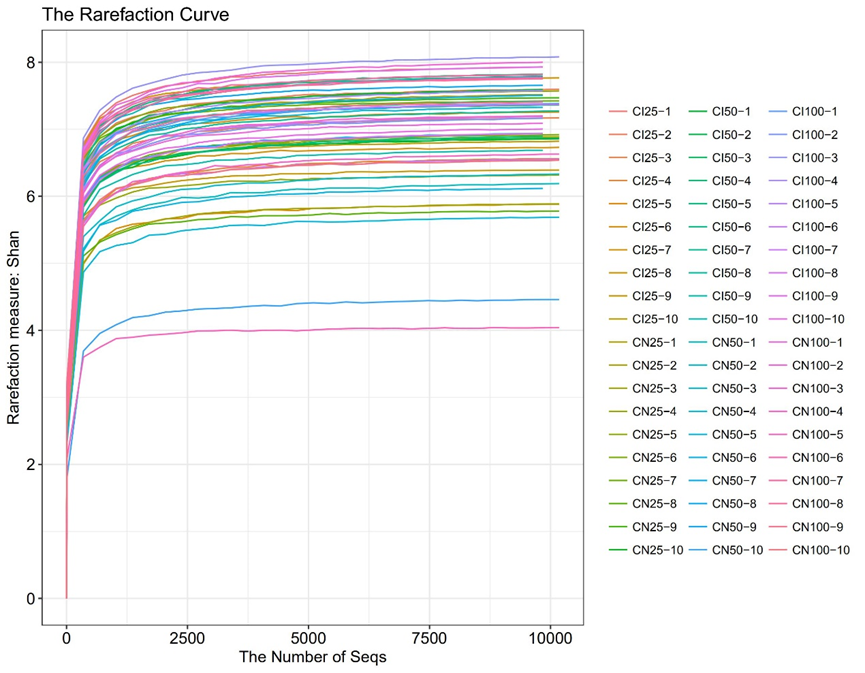
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**Figure S1.** Rarefaction curve analysis was used to evaluate whether further sequencing would likely detect additional taxa. CI and CN represent samples obtained from the colon contents of intrauterine growth restriction (IUGR) pigs and normal birth weight (NBW) pigs, respectively; 25, 50, and 100 represent 25, 50, and 100 kg body weight stages of NBW pigs, respectively.

**Table S1** Ingredients and chemical composition of the experimental diets (as-fed basis).

|  |  |  |  |
| --- | --- | --- | --- |
| Items | Nursery pig feed  (28-69 day-old) | Growing pig feed  (70-103 day-old) | Finishing pig feed  (104-165 day-old) |
| Ingredients (%) |  |  |  |
| Corn | 60.00 | 61.00 | 61.17 |
| Soybean meal | 27.50 | 25.00 | 25.50 |
| Barley | 6.00 | 8.00 | 8.00 |
| Soybean oil | 2.00 | 1.50 | 1.00 |
| Lysine | 0.16 | 0.18 | 0.13 |
| CaHPO4 | 0.10 | 0.10 | 0.00 |
| Threonine | 0.10 | 0.07 | 0.08 |
| Methionine | 0.02 | 0.03 | 0.00 |
| Anti-mildew agent | 0.10 | 0.10 | 0.10 |
| Anti-oxidant | 0.02 | 0.02 | 0.02 |
| Nursery pigs premix1) | 4.00 | 0.00 | 0.00 |
| Growing-finishing pigs premix2) | 0.00 | 4.00 | 4.00 |
| Total | 100.00 | 100.00 | 100.00 |
| Nutrient levels3) |  |  |  |
| Digestible energy (MJ/kg) | 13.91 | 13.77 | 13.64 |
| Crude protein | 17.20 | 16.40 | 16.50 |
| Crude fat | 4.70 | 4.30 | 3.80 |
| Crude fiber | 2.70 | 2.70 | 2.80 |
| Digestible lysine | 1.17 | 1.08 | 1.05 |
| Digestible methionine | 0.33 | 0.30 | 0.28 |
| Digestible threonine | 0.77 | 0.71 | 0.73 |
| Total calcium | 0.77 | 0.74 | 0.66 |
| Total phosphorus | 0.56 | 0.52 | 0.45 |

1) The nursery pig premix supplied for per kg diet: vitamin A 8 000 IU, vitamin D3 228 IU, vitamin E 15 IU; vitamin K3 3.00 mg, vitamin B1 1.30 mg, vitamin B2 3.10 mg, vitamin B6 1.20 mg, vitamin B12 0.03 mg, calcium pantothenate 13.40 mg, choline chloride 500 mg, iron 120 mg, copper 10 mg, zinc 130 mg, manganese 100 mg, iodine 0.30 mg, and selenium 0.30 mg.

2) The growing-finishing pig premix supplied for per kg diet: vitamin A 15 000 IU, vitamin D3 200 IU, vitamin E 50 IU, vitamin K3 4.00 mg, vitamin B1 4.00 mg, vitamin B2 10 mg, vitamin B6 3.00 mg, vitamin B12 0.04 mg, calcium pantothenate 20.00 mg, choline chloride 800 mg, iron 120 mg, copper 20 mg, zinc 112 mg, manganese 124 mg, iodine 0.50 mg, and selenium 0.40 mg.

3) Nutrient levels were calculated values.

**Table S2** Primer sequences used in the RT-PCR.

|  |  |  |  |
| --- | --- | --- | --- |
| Target genes | Primers | Sequences (5′-3′) | Product size (bp) |
| *β-actin* | Forward | GATCTGGCACCACACCTTCTACAAC | 107 |
| Reverse | TCATCTTCTCACGGTTGGCTTTGG |
| *GPX1* | Forward | TGGGGAGATCCTGAATT | 184 |
| Reverse | GATAAACTTGGGGTCGG |
| *GPX4* | Forward | GATTCTGGCCTTCCCTTGC | 173 |
| Reverse | TCCCCTTGGGCTGGACTTT |
| *SOD1* | Forward | GAGACCTGGGCAATGTGACT | 189 |
| Reverse | CCAAACGACTTCCAGCATTT |
| *SOD2* | Forward | TGTATCCGTCGGCGTCCAAGG | 93 |
| Reverse | TCCTGGTTAGAACAAGCGGCAATC |
| *IL-1β* | Forward  Reverse | ACCTGGACCTTGGTTCTC  GGATTCTTCATCGGCTTC | 124 |
| *IL-10* | Forward  Reverse | CACTGCTCTATTGCCTGATCTTCC  AAACTCTTCACTGGGCCGAAG | 136 |
| *TNF-α* | Forward  Reverse | ACGCTCTTCTGCCTACTGC | 162 |
| TCCCTCGGCTTTGACATT |

*GPX*, glutathione peroxidase; *SOD*, superoxide dismutase; *IL*, interleukin; *TNF-α*, tumor necrosis factor α.