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**Title: Effects of early protein restriction on the growth performance and gut development of pigs fed diets with or without antibiotic**

X. Zhao1, a, H. Y. Fu1, a, S. N. Qiu1, a, T. Teng1, a, G. D. Bai1, a, D. X. Ju1, a, Y. C. Sun1, a and B. M. Shi1, a

1*Institute of Animal Nutrition, Northeast Agricultural University, Harbin, 150030, P. R. China*

a *Present address:* *Institute of Animal Nutrition, Northeast Agricultural University, Harbin, 150030, P. R. China*

Corresponding author: B. M. Shi. Email: shibaoming1974@163.com

Table S1 *Ingredient and nutrient composition of experimental diets for pigs (%)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Phase/Trial days (d) | Restriction phase | | Re-alimentation phase | |
| d 0 to d 30 | | d 31 to d 65 | d 66 to d 104 |
| Items | NP(AI/AF) | PR(AI/AF) | NP | NP |
| Ingredients, % |  |  |  |  |
| Corn | 60.20 | 76.50 | 71.70 | 78.70 |
| Soybean meal (46.2%CP) | 19.00 | 11.00 | 25.00 | 18.00 |
| Corn gluten meal | 2.00 | — | — | — |
| Extruded full-fat soybean | 9.00 | — | — | — |
| Whey powder | 3.00 | 3.00 | — | — |
| Fish meal (64%CP) | 3.00 | 3.00 | — | — |
| Soybean oil | 0.50 | 2.00 | — | — |
| L-Lysine HCl (98%) | 0.17 | 0.71 | — | 0.10 |
| DL-Methionine | — | 0.11 | — | — |
| L-Threonine | — | 0.28 | — | — |
| L-Tryptophan | — | 0.09 | — | — |
| Dicalcium phosphate | 0.84 | 0.96 | 1.10 | 1.00 |
| Limestone | 0.89 | 0.95 | 0.80 | 0.80 |
| Salt | 0.40 | 0.40 | 0.40 | 0.40 |
| Vitamin- mineral premix | 1.001 | 1.001 | 1.002 | 1.002 |
| Analyzed nutrient levels (%) |  |  |  |  |
| Crude protein | 20.28 | 13.90 | 17.35 | 15.16 |
| Lysine | 1.12 | 1.15 | 1.02 | 0.80 |
| Methionine | 0.56 | 0.61 | 0.61 | 0.50 |
| Threonine | 0.70 | 0.73 | 0.87 | 0.53 |
| Tryptophan | 0.23 | 0.23 | 0.21 | 0.16 |
| Calcium | 0.74 | 0.74 | 0.62 | 0.57 |
| Phosphorus | 0.57 | 0.54 | 0.53 | 0.49 |
| Calculated nutrient levels |  |  |  |  |
| Metabolizable energy, Mcal/kg | 3.14 | 3.14 | 3.08 | 3.09 |
| Available phosphorus (%) | 0.36 | 0.36 | 0.31 | 0.30 |

NP=normal protein; PR= protein restriction; AF=antibiotic free; AI=antibiotic intervention. Composition of antibiotic: 20 mg/kg colistin sulfate with 50 mg/kg kitasamycin. A dash (—) indicate not applicable

1 Supplied per kilogram of diet in the restriction phase: 10000 IU vitamin A, 1000 IU vitamin D3, 30 mg vitamin E, 5.00 mg vitamin B1, 5.00 mg vitamin B2, 5.00 mg vitamin B6, 0.02 mg vitamin B12, 24 mg pantothenic acid, 45 mg niacin, 0.05 mg biotin, 0.39 mg folic acid, 190 mg Cu, 140 mg Zn, 0.4 mg Se, 45 mg Mn, 190 mg Fe, 0.5 mg I.

2 Supplied per kilogram of diet in the re-alimentation phase: 8000 IU vitamin A, 2000 IU vitamin D3, 30 mg vitamin E, 1.60 mg vitamin B1, 1.60 mg vitamin B6, 12 μg vitamin B12, 20 mg pantothenic acid, 15 mg niacin, 0.05 mg biotin, 100 mg Cu, 80 mg Zn, 0.3 mg Se, 25 mg Mn, 100 mg Fe, 0.3 mg I.

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**Table S2** *The primers corresponding to the pigs used for quantitative real-time PCR*

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Primer sequence （5'to3'） | Product length（bp） | GenBank |
| IL-1β | F: GTTCTCTGAGAAATGGGAGC | 143 | NM\_214055.1 |
| R: CTGGTCATCATCACAGAAGG |
| IL-6 | F: AGCAAGGAGGTACTGGCAGA | 257 | NM\_001252429.1 |
| R: GTGGTGGCTTTGTCTGGATT |
| TNF-α | F: CATGAGCACTGAGAGCATGA | 170 | NM\_214022.1 |
| R: CGATAACTTCGAAGTGCAGT |
| TLR-4 | F: CAGATAAGCGAGGCCGTCATT | 113 | NM\_001293316.1 |
| R: TTGCAGCCCACAAAAAGCA |
| ZO-1 | F: TGGTTCAGTGCCGCCTCCTG | 163 | XM\_021098896.1 |
| R: CACGGTGTGACCATCCTCATCTTC |
| Occludin | F: GAGTACATGGCTGCTGCTGA | 173 | NM\_001163647.2 |
| R: AACAAGGTGGCCTCTGTCTC |
| β-actin | F: ATGCTTCTAGGCGGACTGT | 211 | AY550069 |
| R: CCATCCAACCG ACTGCT |

IL= interleukin; TNF-α= tumor necrosis factor-α; TLR= toll-like receptor; ZO-1= zonula occludens-1.

**Table S3** The alpha diversity in colonic microbiota of pigs fed two protein levels diets (20% CP vs.14% CP) supplemented with or without antibiotic in the restriction phase (Day 0 to 30) and pigs switched to the same diets in the re-alimentation phase (Day 31 to 104).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Items | Trial days | AF | | AI | | SEM | *P*-value | | |
| NP | PR | NP | PR | P | A | P×A |
| ACE1 |  |  |  |  |  |  |  |  |  |
|  | Day 30 | 636.04 | 532.09 | 477.86 | 412.90 | 21.786 | 0.086 | 0.013 | 0.666 |
| Day 104 | 738.14 | 748.03 | 758.91 | 748.52 | 15.297 | 0.994 | 0.753 | 0.747 |
| Chao12 |  |  |  |  |  |  |  |  |  |
|  | Day 30 | 650.28 | 561.94 | 491.23 | 401.80 | 23.742 | 0.093 | 0.010 | 0.964 |
| Day 104 | 749.99 | 768.04 | 773.64 | 760.80 | 16.359 | 0.938 | 0.807 | 0.647 |
| Simpson3 |  |  |  |  |  |  |  |  |  |
|  | Day 30 | 0.04 | 0.07 | 0.08 | 0.08 | 0.004 | 0.090 | 0.024 | 0.123 |
| Day 104 | 0.02 | 0.02 | 0.02 | 0.02 | 0.002 | 0.768 | 0.897 | 0.615 |
| Shannon4 |  |  |  |  |  |  |  |  |  |
|  | Day 30 | 4.19 | 3.89 | 3.71 | 3.57 | 0.069 | 0.136 | 0.026 | 0.600 |
| Day 104 | 4.79 | 4.76 | 4.80 | 4.81 | 0.055 | 0.909 | 0.784 | 0.838 |

AF = antibiotic free; AI = antibiotic intervention; NP = normal crude protein level; PR = protein restriction. P, main effect of protein; A, main effect of antibiotic; P×A, interaction effect between protein and antibiotic.

1,2 indicate the richness of species.

3,4 indicate diversity of the community.

All of the values are expressed as the means and pooled SEM.

**Figure. S1** Colonic microbiota composition ofpigs fed two protein levels diets (20% CP vs.14% CP) supplemented with or without antibiotic in the restriction phase (Day 0 to 30) and pigs switched to the same diets in the re-alimentation phase (Day 31 to 104). (A) The top 10 phyla of bacteria on d 30. (B) The top 10 phyla of bacteria on d 104*.* AFNP, antibiotic free-normal crude protein; AFPR, antibiotic free-protein restriction; AINP, antibiotic intervention-normal crude protein; AIPR, antibiotic intervention-protein restriction.

