Title: Epidermal growth factor promotes intestinal secretory cell differentiation in weaning piglets via Wnt/β-catenin signaling

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**Supplementary Table** **S1** *Ingredient composition of piglet diets*

|  |  |  |
| --- | --- | --- |
| Component | Content, % | |
| Corn | 37.66 | |
| Extruded corn | 20.00 | |
| Soybean meal, 43% CP1 | 8.00 | |
| Concentrated soy protein | 7.00 | |
| Whey | 10.00 | |
| Fish meal, 63% CP1 | 5.00 | |
| Plasma protein powder | 4.50 | |
| L-Lysine HCl, 98% | 0.33 | |
| DL-Methionine | 0.08 | |
| L-Threonine | 0.03 | |
| L-Tryptophane | 0.01 | |
| Glucose | 2.00 | |
| Soybean oil | 2.00 | |
| Limestone | 1.04 | |
| Monocalcium phosphate | 0.50 | |
| Choline chloride, 50% | 0.10 | |
| Antioxidants | 0.05 | |
| Zinc oxide | 0.30 | |
| Citric acid | 0.30 | |
| Vitamin-mineral premix2 | 1.00 | |
| EGF premix or carrier3 | 0.10 | |
| Total | 100 | |
| Calculated composition |  | |
| CP1, % | 18.0 | |
| ME4, MJ/kg | 14.2 | |
| Lysine5, % | | 1.35 |
| Methionine5, % | | 0.39 |
| Methionine + Cysteine5, % | | 0.74 |
| Threonine5, % | | 0.79 |
| Tryptophane5, % | | 0.22 |

1 CP = crude protein

2 Vitamin-mineral premix supplied per kilogram of feed: 10 000 IU of Vitamin A, 1 000 IU of Vitamin D3, 80 IU of Vitamin E, 2.0 mg of Vitamin K3, 0.03 mg of Vitamin B12, 12 mg of riboflavin, 40 mg of niacin, 25 mg of d-pantothenic acid, 0.25 mg of biotin, 1.6 mg of folic acid, 3.0 mg of thiamine, 2.25 mg of pyridoxine, 300 mg of choline chloride, 150 mg of Fe (FeSO4), 100 mg of Zn (ZnSO4), 30 mg of Mn (MnSO4), 25 mg of Cu (CuSO4), 0.5 mg of I (KIO3), 0.3 mg of Co (CoSO4), 0.3 mg of Se (Na2SeO3), and 4.0 mg of ethoxyquin.

3 EGF = epidermal growth factor; the spray dried egg yolk powder was used as carrier.

4 ME = metabolism energy

5 Standardized ileal-digestible.

**Supplementary Table S2** *Primers used in real-time PCR analysis of piglet intestinal epithelial cells*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene1 | Primer sequences, 5′-3′ | | Annealing temperature, °C | | Amplicon size, bp |
| *Hes1* | F: AAGCTGGAGAAGGCGGACAT | | 57.40 | | 152 |
|  | R: AAGCGGGTCACCTCGTTCAT | |  | |  |
| *Elf3* | F: CAAGTGGAGAAGCACAAATACG | | 57.40 | | 157 |
|  | R: TGGAAGTGAGGTCCCATAGC | |  | |  |
| *Atoh1* | F: GGTGGTAGACGAGCTGGTTTG | | 55.60 | | 170 |
|  | R: CGTTGTTGAAGGACGGGATAA | |  | |  |
| *Gfi1* | F: GCGTCCTCTAAAGTGTTGGTT | | 55.60 | | 246 |
|  | R: GGTAACTGTGAGCCTTCTTGC | |  | |  |
| *SPDEF* | F: GCCTCTTAGCATCTTAGCCC | | 55.40 | | 159 |
|  | R: GTGAGCCTGAATCTGTGGAA | |  | |  |
| *Klf4* | F:TTGCCCGCTCAGATGAA | | 52.20 | | 100 |
|  | R:TGGTCCGACCTTGAAAATG | |  | |  |
| *Ngn3* | F: ACCAGACCGAGCAGCCTTTC | | 57.30 | | 246 |
|  | R: GCATTCGATTGCGCTCACG | |  | |  |
| *Pdx1* | F: CACCACCTCCCGGCTCAG | | 57.60 | | 193 |
|  | R: GCTTGTTCTCCTCGGGCTCT | |  | |  |
| *Sox9* | F: GCCTCTACTCCACCTTCACCTA | | 55.20 | | 185 |
|  | R: ATCACGGGCCATCATCACT | |  | |  |
| *Mucin2* | F: ACGCCATCCTGGGTGAGCT | | 59.50 | | 121 |
|  | R: ACGCTGCCGTCCGACTTGA | |  | |  |
| *ITF3* | F: ATGTTCTGGCTGCTAGTGGTGCTCC | | 59.60 | | 231 |
|  | R: TCAGAAGGTGCATTCTGTTTCCTGC | |  | |  |
| *ChgA* | F:CCAGCACCCACCCCTTAGCC | | 59.60 | | 192 |
|  | R:CTTCTTCCTCCGGGACCGCC | |  | |  |
| *Lysozymee* | F: AATAGCCGCTACTGGTGTAATGATG | | 57.90 | | 148 |
|  | R: ATGCTTTAACGCCTAGTGGATCTCT | |  | |  |
| *PLA2* | F: AGTAGCTTTCGGTGTAGGGATTGTC | | 59.60 | | 225 |
|  | R: CAAGGGCATTATGGCAGTTTCGTAG | |  | |  |
| *EGFR* | F: GGAGATCAGCGACGGAGAC | | 57.30 | | 171 |
|  | R: GAGCACAGCGGGTTACAGA | |  | |  |
| *β-actin* | F:AGTTGAAGGTGGTCTCGTGG | | 57.40 | | 215 |
|  | R:TGCGGGACATCAAGGAGAAG |  | |  | |

1 *Hes1*, hairy and enhancer of split-1;*Elf3,* [E74 like ETS transcription factor 3](https://www.ncbi.nlm.nih.gov/nuccore/XM_021064529.1); *Atoh1*, atonal homologue 1 gene; *Gfi1,* growth factor-independent 1; *SPDEF,*[SAM pointed domain containing ETS transcription factor](https://www.ncbi.nlm.nih.gov/nuccore/NM_001190254.1); *Klf4*, [Kruppel like factor 4](https://www.ncbi.nlm.nih.gov/nuccore/NM_001031782.2); *Ngn3*, neurogenin3; *Pdx1*, pancreatic and duodenal homeobox 1; *Sox9*, SRY-box containing gene 9; ITF3, intestinal trefoil factor 3; ChgA, chromogranin A; PLA2, phospholipase A2.