**Pigs that are divergent in feed efficiency, differ in Intestinal enzyme and nutrient transporter gene expression, nutrient digestibility and microbial activity**

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Short title: Feed efficiency, nutrient digestion and absorption

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**Supplementary Table S1** *Composition and chemical analysis of experimental diets (as fed basis)*

|  |  |  |  |
| --- | --- | --- | --- |
| Ingredient | Weaning (g/kg) | Grower (g/kg) | Finisher (g/kg) |
| Whey permeate | 125 |  |  |
| Wheat | 444.2 | 400 | 382.6 |
| Barley |  | 273.5 | 250 |
| Soybean meal | 142.5 | 180 | 195 |
| Maize |  |  | 150 |
| Soya oil | 65 | 10 | 18 |
| Whey protein isolate | 130 |  |  |
| Full-fat soyabean | 80 | 100 |  |
| Vitamins and mineralsa | 5 | 3 | 2.5 |
| Limestone |  | 11 | 12.5 |
| Salt |  | 3 | 5 |
| Dicalcium phosphate  |  | 12.5 |  |
| Monocalcium phosphate |  |  | 6.6 |
| Lysine HCL | 4.5 | 4 | 2.3 |
| DL-methionine | 1.6 | 1.5 |  |
| L-threonine | 2.2 | 1.5 | 0.5 |
| Analysis (g/kg, unless otherwise stated) |
| DM  | 892.5 | 881.1 | 884.2 |
| CP (N X 6.25) | 224.2 | 195 | 193.8 |
| Gross Energy (MJ/kg) | 18.2 | 16.5 | 16.4 |
| Digestible Energy  | 16 | 14.5 | 14 |
| Ash | 43.7 | 50 | 49.8 |
| Neutral-detergent fibre | 110.3 | 139.2 | 130.5 |
| Lysine† | 16.5 | 13.04 | 11.1 |
| Methionine and cysteine† | 9.9 | 8.02 | 6.6 |
| Threonine† | 10.7 | 8.09 | 7.7 |
| Tryptophan† | 2.5 | 2.3 | 2 |
| Calcium† | 8 | 9.5 | 5.8 |
| Phosphorous† | 6 | 6.1 | 5.8 |

Weaner diet provided (mg/kg completed diet): Cu 175, Fe 140, Mn 47, Zn 120, I .6, Se 0.3, retinol 1.8, cholecalciferol 0.025, alpha-tocopherol 67, phytylmenaquinone 4, cyanocobalamin 0.01, riboflavin 2, nicotinic acid 12, pantothenic acid 10, choline chloride 250, thiamine 2, pyridoxine 0.015.

Grower diet provided (mg/kg completed diet) Cu 25, Zn 100, Se 0.3, Fe 100, Mn 25, I 0.2, retinol 3, cholecalciferol 0.05, alpha tocopherol 40.

Finisher diet provided (mg/kg completed diet) Cu 25, Zn 100, Se 0.3, Fe 100, Mn 25, I 0.2, retinol 4.2, cholcalciferolecalciferol 0.07, alpha-tocopherol 80.

† Calculated from tabulated nutritional composition (Sauvant et al., 2004).

**Supplementary Table S2** *Oligonucleotide sequences of forward and reverse primers used for qPCR of bacterial 16s rRNA*

|  |  |  |  |
| --- | --- | --- | --- |
| Target bacteria  | Primer Sequence (5’- 3’) | Amplicon size (bp) | Tm (oC) |
|  Firmicutes | GGAGTATGTGGTTTAATTCGAAGCA | 126 | 59 |
|  | AGCTGACGACAACCATGCAC |  |  |
|  Bifidobacteria | CGGGTGAGTAATGCGTGACC | 125 | 59 |
|  | TGATAGGACGCGACCCCA |  |  |
|  Lactobacillus | TGGATCACCTCCTTTCTAAGGAAT | 340 | 55 |
|  | TGTTCTCGGTTTCATTATGAAAAAATA |  |  |
|  Enterobacteria | CATTGACGTTACCCGCAGAAGAAGC | 190 | 58 |
|  | CTCTACGAGACTCAAGCTTGC |  |  |
|  Bacteroides | AACGCTAGCTACAGGCTT | 276 | 54 |
|  | CAAATGTGGGGGACCTTC |  |  |

Forward primer sequence (5’- 3’) and 2= reverse primer sequence (5’- 3’).

**Supplementary Table S3** *Porcine specific primers used for real-time PCR*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene | Accession Number | Primer Sequence (5’- 3’) | Melting Temp (oC) | Product Length (BP) |
| Reference Genes |
|  *YHAZ* | XM\_001927228.1 | GGACATCGGATACCCAAGGA | 59.4 | 71 |
|  |  | AAGTTGGAAGGCCGGTTAATTT | 56.6 |  |
|  *ACTB* | AY550069.1 | CAAATGCTTCTAGGCGGACTGT | 60.3 | 75 |
|  |  | TCTCATTTTCTGCGCAAGTTAGG | 58.9 |  |
| Carbohydrate Transporters  |
|  *SGLT1* | NM\_001164021.1 | GGCTGGACGAAGTATGGTGT | 59.4 | 153 |
|  |  | ACAACCACCCAAATCAGAGC | 57.3 |  |
|  *GLUT2* | AF054835.1 | CCAGGCCCCATCCCCTGGTT | 65.5 | 96 |
|  |  | GCGGGTCCAGTTGCTGAATGC | 63.7 |  |
|  *GLUT5* | EU\_012359 | CCCAGGAGCCGGTCAAG | 60 | 60 |
|  |  | TCAGCGTCGCCAAAGCA | 55.2 |  |
|  *GLUT7* | XM\_003127552.3 | ACATCGCCGGACATTCCATA | 57.3 | 75 |
|  |  | GCGAGGACTGCAGGAAGATC | 61.4 |  |
|  *GLUT8* | XM\_003480608.1 | AGCGCCTTTGGCACCTACTT | 59·4 | 62 |
|  |  | TGCACGTGCGAGGAGTTG | 58·2 |  |
| Fatty Acid Transporters  |
|  *FABP2* | NM\_001031780.1 | TCGGGATGAAATGGTCCAGACT | 62.4 | 102 |
|  |  | TGTGTTCTGGGCTGTGCTCCA | 61.8 |  |
|  *CD36* | NM\_001044622.1 | GGAGAAAAGATCACTACCATCATGAG | 61.6 | 78 |
|  |  | CTCCTGAAGTGCAATGTACTGACA | 61 |  |
| Protein Transporters  |
|  *PEPT1* | NM\_214347.1 | GGATAGCCTGTACCCCAAGCT | 61.8 | 73 |
|  |  | CATCCTCCACGTGCTTCTTGA | 59.8 |  |
|  *SLC6A19* | XM\_003359855 | GCCACCGTGGTCTACTCCAT | 61.4 | 129 |
|  |  | GAAGTTCTCCTGCGTCACGTT | 59.8 |  |
|  *SLC7A11* | XM\_003360551 | CGGCTCCTGGGAAATTTCTC | 59.4 | 72 |
|  |  | ACCATTCATGGAGCCAAAGC | 57.3 |  |
|  *SLC7A1* | NM\_001012613 | TCTCATCCTAACGGGACTTTTAACTC | 61.6 | 85 |
|  |  | AATGGGACGGCTGGAGTTC | 58.8 |  |
| Enzymes  |
|  *SI* | NM\_001041 | GAACTCACAAAAAGAACTGAAGGATTAC | 60.4 | 171 |
|  |  | GCAAAACAGATGATCCATCACTAAGA | 59.97 |  |
|  *AMY2*  | NM\_214195.1 | CGCTAGACTGTACAAAGTGGCAGTT | 63.0 | 124 |
|  |  | CCCAACGGTAGCTTGACATCA | 59.8 |  |
|  *PGA* | NM\_213873.2 | GTGGGTGCCCTCTGTCTACTG | 63.7 | 135 |
|  |  | GAGGAGTCATCAGGGTTGAACTG | 62.4 |  |
|  *PNLIP* | NM\_001177912.2 | TGCATCTGTGTGGACTGGAAA | 57.9 | 76 |
|  |  | ACGATGCGGATGTTCTGTGA | 57.3 |  |
|  *TRPGEN* | NM\_001162891.1 | TCATCAATGCCGCCAAGA | 53.7 | 221 |
|  |  | ATCAGCATGATGTCGTTATCTAAGGT | 60.1 |  |
|  *CNDP1* | KF800733.1 | CCAGGCCACCATCCAGAA | 58.2 | 234 |
|  |  | GAGAGTGCTCGCCATCATCA | 59.4 |  |

Forward primer sequence (5’- 3’) and 2= reverse primer sequence (5’- 3’).

*YWHAZ*: Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta; *ACTB*: Beta actin; *SGLT1*: sodium/ glucose co-transporter 1; *GLUT*: glucose transporter; *FABP2*: fatty acid binding protein 2; *CD36*: cluster of differentiation 36; *PEPT1*: peptide transporter 1; *SLC6A19*: sodium-dependent neutral amino acid transporter B(0)AT1; *SLC7A11*: anionic amino acid transporter light chain, xc- system; *SLC7A1*: Cationic Amino Acid Transporter, Y+ System; *SI*: sucrase-isomaltase; *AMY2*: pancreatic amylase 2B; *PGA*: pepsinogen A; *PNLIP*: pancreatic lipase; *TRPGEN*: trypsinogen; *CNDP1*: carnosine dipeptidase.

Supplementary Table S4 Characterization of the effect of residual feed intake on enzyme gene expression in the duodenum, ileum and pancreas (Least square means and SEM)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| RFIb | Higha | Low | SEM | P Value |
| Duodenum |  |  |  |  |
|  *SI* | 1.04 | 0.94 | 0.16 | 0.659 |
|  *AMY2*  | 1.17 | 1.30 | 0.15 | 0.567 |
|  *TRPGEN* | 2.58 | 2.45 | 1.02 | 0.931 |
|  *CNDP1* | 1.70 | 1.78 | 0.24 | 0.826 |
|  *PGA* | 3.95 | 3.03 | 1.15 | 0.584 |
|  *PNLIP* | 7.78 | 5.20 | 2.75 | 0.518 |
| Ileum |  |  |  |  |
|  *SI* | 1.00 | 1.16 | 0.30 | 0.704 |
|  *AMY2*  | 0.89 | 0.81 | 0.10 | 0.547 |
|  *TRPGEN* | 0.85 | 0.87 | 0.10 | 0.896 |
|  *CNDP1* | 0.79 | 0.38 | 0.21 | 0.194 |
|  *PGA* | 0.85 | 0.51 | 0.18 | 0.206 |
|  *PNLIP* | 0.86 | 0.45 | 0.20 | 0.165 |
| Pancreas |  |  |  |  |
|  *SI*  | 0.99 | 1.53 | 0.48 | 0.445 |
|  *AMY2*  | 1.19 | 0.93 | 0.12 | 0.144 |
|  *TRPGEN* | 1.05 | 1.06 | 0.12 | 0.971 |
|  *CNDP1* | 1.30 | 1.95 | 0.68 | 0.505 |
|  *PGA* | 1.04 | 1.54 | 0.45 | 0.450 |
|  *PNLIP* | 1.08 | 1.13 | 0.17 | 0.849 |

RFI: Residual feed intake; *SI*: sucrase-isomaltase; *AMY2*: pancreatic amylase 2B; *PGA*: pepsinogen A; *PNLIP*: pancreatic lipase; *TRPGEN*: trypsinogen; *CNDP1*: carnosine dipeptidase

a High = RFI was >0.5 SD above the mean; medium = RFI was ±0.5 SD above and below the mean; low = RFI was <−0.5 SD below the mean

b RFI evaluated when pigs were 105 days old with 8 pigs per RFI group.

Supplementary Table S5 Characterization of the effect of residual feed intake on the gene expression of intestinal nutrient transporters in the duodenum, ileum (Least square means and SEM)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| RFIb | Higha | Low | SEM | P Value |
| Duodenum |  |  |  |  |
|  *GLUT1*  | 1.23 | 1.22 | 0.29 | 0.981 |
|  *GLUT2* | 2.46 | 2.21 | 0.31 | 0.572 |
|  *PEPT1* | 1.77 | 1.62 | 0.30 | 0.723 |
|  *SGLT1* | 1.14 | 1.13 | 0.20 | 0.954 |
|  *FABP2* | 2.15 | 1.77 | 0.33 | 0.425 |
|  *SLC7A11* | 1.03 | 1.50 | 0.24 | 0.173 |
|  *SLC7A1* | 0.87 | 1.05 | 0.13 | 0.325 |
|  *SLC6A19* | 1.17 | 1.29 | 0.26 | 0.741 |
|  *SLC1A4* | 1.31 | 1.32 | 0.16 | 0.963 |
|  *GLUT7* | 2.63 | 2.69 | 0.50 | 0.442 |
|  *GLUT5* | 1.59 | 1.86 | 0.24 | 0.445 |
|  *CD36* | 2.75 | 2.04 | 0.27 | 0.216 |
|  *SLC1A2* | 1.71 | 1.64 | 0.15 | 0.746 |
| Ileum  |  |  |  |  |
|  *GLUT1* | 1.19 | 1.22 | 0.12 | 0.841 |
|  *GLUT2* | 0.63 | 0.37 | 0.31 | 0.562 |
|  *PEPT1* | 0.64 | 0.50 | 0.30 | 0.743 |
|  *SGLT1* | 0.73 | 0.78 | 0.20 | 0.867 |
|  *FABP2* | 0.48 | 0.25 | 0.33 | 0.629 |
|  *SLC7A11* | 1.50 | 1.23 | 0.24 | 0.435 |
|  *SLC7A1* | 1.40 | 1.34 | 0.13 | 0.714 |
|  *SLC6A19* | 0.89 | 0.82 | 0.26 | 0.855 |
|  *SLC1A4* | 1.08 | 0.89 | 0.16 | 0.432 |
|  *GLUT7* | 0.90 | 0.28 | 0.50 | 0.471 |
|  *GLUT5* | 0.74 | 0.49 | 0.24 | 0.476 |
|  *CD36* | 0.47 | 0.36 | 0.27 | 0.763 |
|  *SLC1A2* | 1.18 | 0.93 | 0.15 | 0.244 |

RFI: Residual feed intake; SGLT1: sodium/ glucose co-transporter 1; GLUT: glucose transporter; FABP2: fatty acid binding protein 3; CD36:cluster of differentiation 36; PEPT1: peptide transporter 1; SLC6A19: sodium-dependent neutral amino acid transporter B(0)AT1; SLC7A11: anionic amino acid transporter light chain, xc- system; SLC7A1: Cationic Amino Acid Transporter, Y+ System.

a High = RFI was >0.5 SD above the mean; medium = RFI was ±0.5 SD above and below the mean; low = RFI was <−0.5 SD below the mean.

b RFI evaluated when pigs were 105 days old with 8 pigs per RFI group.

Supplementary Table S6 Effect of residual feed intake on organ weights. (Least square means and SEM)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| RFIc | Higha | Low | SEM | P Value |
| Trait (g) |  |  |  |  |
|  BW | 77.70 | 76.68 | 1.36 | 0.833 |
|  Stomach | 690.9 | 655.9 | 36.1 | 0.504 |
|  Small Int. | 2900.0 | 2690.0 | 152.7 | 0.347 |
|  Large Int. | 2193.0 | 1954.0 | 137.4 | 0.240 |
|  Liver | 1978.0 | 1897.0 | 102.0 | 0.581 |
|  Kidneys | 389.0 | 372.0 | 17.1 | 0.490 |
|  Testes | 326.0 | 356.0 | 35.5 | 0.558 |
|  Heart | 439 | 360 | 23.4 | 0.033 |
|  Spleen | 241.0 | 275.0 | 68.8 | 0.506 |
|  Thyroid | 16.0 | 16.0 | 1.0 | 0.698 |
|  Total Tract | 5093.0 | 4644.0 | 170.5 | 0.084 |
|  Viscerab | 5920 | 5331 | 193.1 | 0.048 |

RFI: Residual feed intake, BW: Body Weight.

a High = RFI was >0.5 SD above the mean; medium = RFI was ±0.5 SD above and below the mean; low = RFI was <−0.5 SD below the mean.

b Viscera: Combined weight of small int., large int., heart, liver, kidneys

c RFI evaluated when pigs were 105 days old with 8 pigs per RFI group.