

Supplementary

Table S1. Oligonucleotide primers used for quantitative PCR.

Genes ¹	Accession number ²		Primer sequence (5' to 3')	Amplification efficiencies	Amplicon size (bp)	Reference ³
<i>SLC34A1</i>	NM_001044623.1	F:	TCAACTCTCTGCTCAAGGGC	94.0	183	newly designed
		R:	CACCTAGGCCAATGAGTGGG			
<i>SLC34A2</i>	NM_001256772.1	F:	CGTGTCTCTCGTCTGACTCTGA	90.0	280	newly designed
		R:	CCAGCGGTACTTGGATGAGAT			
<i>TRPV5</i>	XM_021078896.1,	F:	TCCCTGTAACCTGCCAGTGC	99.7	103	newly designed
	XR_002340352.1	R:	TGCTGATCCAGTCTTGCTG			
<i>TRPV6</i>	FJ268731.2	F:	GAATGCGGTTGCAATTGAGCA	89.8	112	newly designed
		R:	TTACACCCCTTCCACAGCCG			
<i>CALB1</i>	NM_001130226.1	F:	ATTTCGACGCTGACGGAAGT	94.1	224	newly designed
		R:	TTGCTGGCATCGGAATAGCA			
	X53456.1,	F:	GAAAATGGTTCCTGCTGCC			
<i>PMCA1b</i>	XR_002343820.1,			96.4	275	newly designed
	XM_021091182.1,	R:	GCAACCGAGTTGTTTGCCAT			
	NM_214352.3					
<i>VDR</i>	NM_001097414.1	F:	TGGTTGGAAGTGTCTGGGAG	99.8	117	newly designed
		R:	GGGGTCAGGTAAGGAAGTGC			
<i>CYP24A1</i>	NM_214075.2	F:	TGGGTTCGTTCTGACTCCG	90.6	103	newly designed
		R:	TCCACGGTTTGATCTCCAGC			
<i>CYP27B1</i>	NM_213995.1,	F:	TCATGGGTGGCTGAGGAAGAA	97.2	86	newly designed
	XM_021091124.1	R:	ATAACTGTTTAGCCAGCAGCAC			
<i>FGF23</i>	XM_001926525.4	F:	CGCAGGCTTCGTGGTCATAA	98.9	146	newly designed
		R:	GGTACACGTCGTAGCCGTC			
	AY150038.1,	F:	ACGAGCTGGCTGATCACATC			
<i>OCN</i>	XM_013996978.2,	R:	CTGCGAGGTCTAGGCTATGC	94.2	71	newly designed
	NM_001164004.1					
<i>OPG</i>	XM_003481346.4	F:	GACCTCTGTGAAAACAGTGTGC	111.3	276	newly designed
		R:	CTTCAGACTCTGTGTGACGGT			
<i>RANKL</i>	AY606802.1,	F:	CCAAGACGCCACGTACTTT		71	newly designed
	XM_001925694.6	R:	AAACAACCTCCACAAAATGGGGC	93.4		
<i>CDH1</i>	NM_001163060.1	F:	TACCTGAACGAGTGGGGCAA	107.0	118	[1]
		R:	CCCATCACATGAGCGTAGGG			
<i>CLDN1</i>	NM_001244539.1	F:	TGATGAGGTGCAGAAGATGC	91.4	88	[1]
		R:	CCATGCTGTGGCAACTAAGA			
<i>CLDN4</i>	NM_001161637.1	F:	CAACTGCGTGGATGATGAGA	90.1	140	[1]
		R:	CCAGGGGATTGTAGAAGTCG			
<i>OCN</i>	NM_001163647.1	F:	TTGTGGGACAAGGAACGTATTTA	92.0	76	[1]
		R:	TGCCTGCCGACACGTTT			
<i>ZO1</i>	XM_003353439.2	F:	AAGCCCTAAGTTCAATCACAACTCT	103.1	130	[1]
		R:	ATCAAACCTCAGGAGGCGGC			
<i>MUC2</i>	XM_013989745.1	F:	TGCCATCTACACCAAGGTCTATTC	90.6	137	[1]
		R:	TCTGCAGGCCGTTGTAGTCTC			
<i>MUC4</i>	XM_001926442.1	F:	GATGCCCTGGCCACAGAA	97.9	89	[1]
		R:	TGATTCAAGGTAGCATTCAATTGC			
<i>ACTG</i>	XM_003357928.4	F:	GGGCATCCTGACCCTCAAG	96.7	89	[1]
		R:	TGTAGAAGGTGTGATGCCAGATCT			
<i>GAPDH</i>	NM_001206359.1	F:	GGCGTGAACCATGAGAAGTATG	97.0	60	[2]
		R:	GGTGCAGGAGGCATTGCT			
<i>B2M</i>	NM_213978.1	F:	CCCCCGAAGGTTTCAGGTT	100.6	66	[2]
		R:	GCAGTTCAGGTAATTTGGCTTTC			
<i>HPRT</i>	NM_001032376.2	F:	AGAAAAGTAAGCAGTCAGTTTCATATCAGT	92.3	131	[2]
		R:	ATCTGAACAAGAGAGAAAATACAGTCAATAG			
<i>OAZ1</i>	NM_001122994.1	F:	TCGGCTGAATGTAACAGAGGAA	96.1	70	[2]
		R:	GAGCCTGGATTGGACGTTTAA			

¹ *SLC34A1*, Na⁺-Pi cotransporter 1; *SLC34A2*, Na⁺-Pi cotransporter 2; *TRPV5*, transient receptor potential vanilloid 5; *TRPV6*, transient receptor potential vanilloid 6; *CALB1*, calbindin; *PMCA1b*, plasma membrane Ca²⁺-adenosintriphosphatase; *VDR*, vitamin D receptor; ; *CYP24A1*, cytochrome P450, family 24, subfamily A,

polypeptide 1; *CYP27B1*, cytochrome P450, family 24, subfamily B, polypeptide 1; *FGF23*, fibroblast growth factor 23; *OCN*, osteocalcin; *OPG*, osteo-protegrin; *RANKL*, receptor activator of NF- κ B ligand; *CDH1*, cadherin-1; *CLDN1*, claudin-1; *CLDN4*, claudin-4; *OCLN*, occludin; *ZO1*, zona occludens-1; *MUC2*, mucin 2; *MUC4*, mucin 4; *ACTB*, β -actin; *GAPDH*, glyceraldehyde-3-phosphate-dehydrogenase; *B2M*, β 2-microglobulin; *HPRT*, hypoxanthin-guanine phosphoribosyl transferase; *OAZ1*, ornithine decarboxylase antizyme.

² National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>).

³ [1] Klinsoda, J.; Vötterl, J.; Zebeli, Q.; Metzler-zebeli, B.U. Alterations of the viable ileal microbiota of gut-mucosa-lymph node axis in pigs fed phytase and lactic acid-treated cereals. *Appl. Environ. Microbiol.* 2020, 86, e02128.

[2] Metzler-Zebeli, B.U.; Ertl, R.; Grüll, D.; Molnar, T.; Zebeli, Q. Enzymatically modified starch up-regulates expression of incretins and sodium-coupled monocarboxylate transporter in jejunum of growing pigs. *Animal* 2017, 11, 1180–1188.

Table S2. Effect of dietary phytase and lactic acid-treated cereals on performance parameters.

Parameter ²	Dietary treatment ¹				SEM	<i>p</i> - Value		
	Con	LA	Con-Phytase	LA-Phytase		Phytase	LA	Phytase × LA
BW start (kg)	13.3	13.1	13.1	13.1	0.64	0.832	0.862	0.832
BW final (kg)	23.6	24.3	23.7	24.3	0.95	0.948	0.510	0.938
ADG (g/d)	555	609	578	607	35.1	0.772	0.251	0.726
ADFI (g/d)	779	771	814	790	20.2	0.192	0.430	0.675
FCR	1.40	1.33	1.41	1.30	0.051	0.856	0.089	0.727

¹ Experimental period of 19 days; Con, control; LA, lactic acid treated cereals, Phytase 500 FTU/ kg diet (VM Phytase XP 897420).

² Values are presented as least square means ± SEM; *n* = 8/diet; Performance data were calculated for the time period experimental day 0 to experimental day 18/ 19; BW, body weight; BWG, body weight gain; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio (kg feed/kg body weight gain)

Table S3. Effect of dietary phytase and lactic acid-treated cereals on phosphorus, calcium and nitrogen balance and ratio of calcium to phosphorus balance.

Parameter ²	Dietary treatment ¹				SEM	<i>p</i> - Value		
	Con	LA	Con-Phytase	LA-Phytase		Phytase	LA	Phytase LA
P balance (g/d)								
P intake	4.54	4.34	4.66	4.39	0.058	0.168	<0.001	0.498
Total P excretion	1.94	1.64	1.37	1.08	0.079	<0.001	0.001	0.950
Faecal P excretion	1.89	1.60	1.16	0.92	0.066	<0.001	<0.001	0.731
Urinary P excretion	0.06	0.05	0.20	0.16	0.028	<0.001	0.366	0.642
P absorption	2.65	2.75	3.50	3.47	0.079	<0.001	0.697	0.429
P retention	2.60	2.70	3.30	3.31	0.087	<0.001	0.515	0.580
P balance (% of daily intake)								
P total excretion	42.76	37.91	29.21	24.38	1.631	<0.001	0.007	0.994
P faecal excretion	41.53	36.85	24.91	20.70	1.398	<0.001	0.004	0.868
P urine excretion	1.23	1.06	4.30	3.68	0.575	<0.001	0.501	0.702
P absorption	58.47	63.15	75.09	79.30	1.398	<0.001	0.004	0.868
P retention	57.24	62.09	70.79	75.62	1.631	<0.001	0.007	0.994
Ca balance (g/d)								
Ca intake	5.49	5.45	5.57	5.46	0.069	0.460	0.292	0.623
Total Ca excretion	3.37	2.85	2.15	1.84	0.135	<0.001	0.005	0.443
Faecal Ca excretion	1.48	1.26	0.99	0.93	0.071	<0.001	0.050	0.263
Urinary Ca excretion	0.85	1.42	0.12	0.16	0.077	<0.001	0.001	0.002
Ca absorption	4.00	4.19	4.58	4.54	0.096	<0.001	0.469	0.250
Ca retention	3.15	2.77	4.46	4.38	0.142	<0.001	0.119	0.300
Ca balance (% of daily intake)								
Ca total excretion	61.28	52.55	38.53	33.34	2.327	<0.001	0.006	0.455
Ca faecal excretion	26.87	23.06	17.67	16.70	1.231	<0.001	0.063	0.258
Ca urine excretion	15.64	26.27	2.20	2.91	1.379	<0.001	<0.001	0.001
Ca absorption	73.13	76.94	82.33	83.30	1.231	<0.001	0.063	0.258
Ca retention	57.49	50.68	80.14	80.39	2.258	<0.001	0.159	0.130
N balance (g/d)								
N intake	28.19	28.38	29.08	29.05	0.405	0.064	0.848	0.787
Total N excretion	8.30	8.22	8.57	7.66	0.356	0.681	0.180	0.251
Faecal N excretion	3.21	3.16	3.30	3.03	0.124	0.881	0.211	0.399
Urinary N excretion	5.09	5.07	5.27	4.63	0.383	0.741	0.394	0.421
N absorption	24.98	25.22	25.79	26.02	0.394	0.053	0.554	0.990
N retention	19.89	20.15	20.52	21.39	0.436	0.043	0.205	0.490
N balance (% of daily intake)								
N total excretion	29.42	28.69	29.38	26.26	1.152	0.294	0.108	0.310
N faecal excretion	11.44	11.06	11.29	10.43	0.432	0.376	0.164	0.581
N urine excretion	17.98	17.63	18.09	15.83	1.268	0.511	0.315	0.459
N absorption	88.56	88.94	88.71	89.57	0.432	0.376	0.164	0.581
N retention	70.58	71.31	70.62	73.74	1.152	0.294	0.108	0.310
Ratio of calcium to phosphorus balance								
Ratio of ingested Ca/P	1.21	1.26	1.20	1.25	0.005	0.062	<0.001	0.630
Ratio of totally excreted Ca/P ³	1.43	1.39	1.32	1.37	0.029	0.032	0.915	0.109
Ratio of excreted Ca/P in faeces ³	0.65	0.63	0.70	0.81	0.022	<0.001	0.057	0.010
Ratio of excreted Ca/P in urine ³	16.98	26.87	0.93	0.83	2.198	<0.001	0.035	0.032
Ratio of absorbed Ca/P ³	1.25	1.23	1.10	1.05	0.013	<0.001	0.010	0.510
Ratio of retained Ca/P ³	1.01	0.81	1.13	1.06	0.025	<0.001	<0.001	0.018

¹ Experimental period of 19 days; Con, control; LA, lactic acid treated cereals.² Values are presented as least square means ± SEM; *n* = 8/diet. The nutrient intake, nutrient excretion in faeces and urine as well as absorption and retention were calculated as the mean of the three days of sampling (experimental day 15 to day 17).³ Ratio calculated with data stated as a percentage per daily intake.

Table S4. Effect of dietary phytase and lactic acid-treated cereals on relative expression of genes (fold change) related to phosphorus and calcium absorption along the intestinal tract.

Parameter ²	Gut Site	Dietary treatment ¹				SEM	<i>p</i> - Value		
		Con	LA	Con-Phytase	LA-Phytase		Phytase	LA	Phytase × LA
<i>CDH1</i>	Duodenum	0.05	0.05	0.05	0.05	0.004	0.774	0.960	0.168
	Jejunum	0.40	0.43	0.40	0.52	0.052	0.371	0.159	0.435
	Ileum ³	0.03	0.03	0.01	0.02	0.005	0.012	0.336	0.505
	Caecum	0.09	0.10	0.08	0.08	0.005	0.037	0.089	0.685
	Colon	0.11	0.11	0.11	0.09	0.006	0.276	0.082	0.068
<i>CLDN1</i>	Duodenum	0.04	0.02	0.04	0.04	0.008	0.496	0.511	0.198
	Jejunum	0.25	0.24	0.39	0.31	0.071	0.171	0.541	0.640
	Ileum ³	0.08	0.09	0.10	0.05	0.017	0.535	0.231	0.152
	Caecum	0.03	0.02	0.02	0.02	0.005	0.682	0.334	0.440
	Colon	0.04	0.05	0.06	0.06	0.013	0.222	0.692	0.617
<i>CLDN4</i>	Duodenum	0.04	0.03	0.05	0.05	0.006	0.080	0.941	0.586
	Jejunum	0.43	0.33	0.50	0.39	0.052	0.229	0.053	0.961
	Ileum ³	0.02	0.02	0.02	0.01	0.004	0.841	0.408	0.163
	Caecum	0.02	0.02	0.02	0.02	0.003	0.157	0.578	0.867
	Colon	0.03	0.02	0.03	0.02	0.004	0.850	0.088	0.736
<i>OCLN</i>	Duodenum	0.02	0.02	0.02	0.02	0.002	0.392	1.000	0.073
	Jejunum	0.43	0.46	0.46	0.57	0.056	0.198	0.213	0.449
	Ileum ³	0.02	0.02	0.02	0.01	0.003	0.109	0.742	0.202
	Caecum	0.02	0.02	0.02	0.02	0.002	0.879	0.260	0.939
	Colon	0.04	0.04	0.03	0.03	0.002	0.041	0.257	0.257
<i>ZO1</i>	Duodenum	0.05	0.03	0.04	0.04	0.006	0.686	0.364	0.302
	Jejunum	0.41	0.36	0.29	0.52	0.070	0.754	0.207	0.060
	Ileum ³	0.03	0.03	0.04	0.03	0.011	0.940	0.805	0.922
	Caecum	0.07	0.09	0.07	0.08	0.004	0.172	0.001	0.473
	Colon	0.09	0.10	0.09	0.07	0.005	0.043	0.372	0.037
<i>MUC2</i>	Duodenum	0.09	0.10	0.07	0.08	0.013	0.075	0.731	0.880
	Jejunum	0.37	0.42	0.42	0.54	0.066	0.221	0.203	0.526
	Ileum ³	0.05	0.09	0.06	0.04	0.012	0.195	0.615	0.049
	Caecum	0.08	0.09	0.07	0.07	0.006	0.019	0.582	0.617
	Colon	0.17	0.19	0.14	0.13	0.017	0.015	0.850	0.385
<i>MUC4</i>	Duodenum	0.003	0.005	0.001	0.006	0.0027	0.733	0.243	0.689
	Jejunum	0.018	0.020	0.006	0.019	0.0059	0.267	0.213	0.336
	Ileum ³	0.01	0.03	0.01	0.01	0.007	0.423	0.287	0.387
	Caecum	0.51	0.63	0.36	0.38	0.064	0.005	0.262	0.416
	Colon	0.44	0.63	0.66	0.46	0.062	0.673	0.936	0.005

¹ Experimental period of 19 days; Con, control; LA, lactic acid treated cereals, Phytase 500 FTU/ kg diet (VM Phytase XP 897420).

² Values are presented as least square means ± SEM; *n* = 8/diet; *CDH1*, cadherin-1; *CLDN1*, claudin-1; *CLDN4*, claudin-4; *OCLN*, occludin; *ZO1*, zona occludens-1; *MUC2*, mucin 2; *MUC4*, mucin 4.

³ Relative gene expression data for innate immune genes from the ileum were already published in Klinsoda et al. 2020 (Klinsoda, J.; Vötterl, J.; Zebeli, Q.; Metzler-zebeli, B.U. Alterations of the viable ileal microbiota of gut-mucosa-lymph node axis in pigs fed phytase and lactic acid-treated cereals. Appl. Environ. Microbiol. 2020, 86, e02128.)

Table S6. Pearson's correlations coefficients between diet, bone parameters, and relative expression of genes related to phosphorus and calcium metabolism in the metacarpal bone and kidney (* $p < 0.05$; $|r| > 0.035$).

	Diet	Metric parameters in metacarpal bones										Relative gene expression in metacarpal bone										Relative gene expression in the kidney				
		Ca/P ratio	right metacarpal length (mm)	right metacarpal weight (g)	cortical wall thickness (mm)	cross sectional area (mm ²)	cortical index	density (g/cm ³)	DM (%)	Ash (%)	Calcium oxide (% of ash)	Phosphorus pentoxide (% of ash)	CYP24A1	CYP27B1	VDR	FGF23	OCN	OPG	RANKL	SLC34A1	TRPV5	TRPV6	CALB1	PMCA1b	VDR	CYP24A1
Diet	Ca/P ratio	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Metric parameters in metacarpal bones	right metacarpal length (mm)	0.16	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	right metacarpal weight (g)	-0.15	0.01	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	cortical wall thickness (mm)	-0.05	0.20	0.14	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	cross sectional area (mm ²)	-0.06	0.20	0.13	0.96*	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	cortical index	-0.05	0.13	0.12	0.91*	0.78*	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	density (g/cm ³)	0.11	0.03	0.03	-0.13	-0.17	-0.04	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DM (%)	-0.32	0.21	-0.04	-0.12	-0.10	-0.09	-0.31	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ash (%)	-0.22	0.00	-0.03	-0.12	-0.10	-0.15	-0.09	0.57*	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Calcium oxide (% of ash)	-0.50*	-0.35*	0.16	-0.04	-0.01	-0.07	-0.40*	0.25	0.07	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Phosphorus pentoxide (% of ash)	-0.46*	-0.28	0.03	-0.07	-0.04	-0.06	-0.17	0.30	0.05	0.70*	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Relative gene expression in metacarpal bone	CYP24A1	-0.17	0.12	-0.05	0.01	-0.07	0.12	0.08	-0.06	-0.31	-0.03	0.09	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
	CYP27B1	-0.18	0.25	-0.08	-0.04	-0.11	0.08	-0.05	0.23	-0.04	0.17	0.05	0.66*	1.00	-	-	-	-	-	-	-	-	-	-	-	-
	VDR	-0.34	0.02	-0.11	0.07	0.05	0.06	-0.13	0.43*	0.34	0.25	0.11	-0.01	0.18	1.00	-	-	-	-	-	-	-	-	-	-	-
	FGF23	-0.34	0.28	0.16	-0.25	-0.28	-0.16	0.15	0.28	0.30	-0.09	0	0.39*	0.48*	0.13	1.00	-	-	-	-	-	-	-	-	-	-
	OCN	-0.16	0.08	0.01	0.24	0.15	0.33	-0.15	0.21	-0.02	0.23	-0.02	0.40*	0.67*	0.46*	0.12	1.00	-	-	-	-	-	-	-	-	-
	OPG	-0.23	0.13	-0.04	0.14	0.05	0.26	-0.06	0.21	0.05	0.23	0.06	0.62*	0.83*	0.41*	0.35*	0.89*	1.00	-	-	-	-	-	-	-	-
	RANKL	-0.24	0.21	-0.07	-0.02	-0.10	0.08	-0.05	0.20	0.01	0.12	0.05	0.71*	0.92*	0.29	0.49*	0.69*	0.87*	1.00	-	-	-	-	-	-	-
Relative gene expression in kidney	SLC34A1	0.33	0.02	-0.03	-0.10	-0.10	-0.08	0.28	-0.05	0.23	-0.40*	-0.37*	-0.13	-0.15	-0.05	0.02	-0.10	-0.20	-0.18	1.00	-	-	-	-	-	-
	TRPV5	-0.08	0.09	-0.07	-0.28	-0.23	-0.31	0.21	0.31	0.62*	0	0.12	-0.30	-0.08	0.01	0.19	-0.39*	-0.21	-0.08	0.03	1.00	-	-	-	-	-
	TRPV6	0.28	0.01	0.29	0.04	0.04	0.04	0	-0.18	-0.05	-0.26	-0.47*	-0.02	-0.06	-0.17	0.04	0.12	-0.04	-0.14	0.25	-0.17	1.00	-	-	-	-
	CALB1	-0.08	-0.05	-0.06	-0.01	0.02	-0.04	0.28	0.21	0.65	0	0.14	-0.29	-0.20	-0.02	0.09	-0.36*	-0.22	-0.17	0.33	0.83*	-0.17	1.00	-	-	-
	PMCA1b	0.23	-0.13	-0.05	-0.12	-0.07	-0.18	0.18	-0.09	0.16	-0.18	-0.21	-0.25	-0.22	0	-0.26	0	-0.19	-0.18	0.77*	0.01	0.25	0.21	1.00	-	-
	VDR	-0.08	0.14	-0.13	-0.17	-0.12	-0.22	0.23	0.27	0.61*	-0.08	0.19	-0.32	-0.21	-0.05	0.22	-0.37*	-0.25	-0.20	0.19	0.67*	-0.31	0.70*	0.08	1.00	-
	CYP24A1	0.64*	-0.11	-0.03	0.09	0.07	0.12	0.20	-0.25	-0.18	-0.35*	-0.24	-0.28	-0.39*	-0.32	-0.41*	-0.23	-0.41*	-0.44*	0.14	-0.02	0.26	-0.001	0.07	0.07	1.00

