Phytase supplementation effects on amino acid digestibility in broiler chickens are influenced by dietary calcium concentrations but not by acid-binding capacity

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Online Supplementary Material

Table of contents

Supplemental Table 1. Ingredient composition of the experimental diets

Acid-binding capacity	$CaCO_3$				$CaCO_3$+formic acid				Calcium formate			
Calcium concentration	low		high		low		high		low		high	
Phytase	-	+	-	+	-	+	-	+	-	+	-	+
$CaCO_3$	7.32		13.83		7.32		13.83		0		0	
Formic acid	0		0		6		6		0		0	
Ca-formate	0		0		0		0		9.51		18.2	
Diamol	12.51		6		6.51		0		10.32		1.63	
Phytase (FTU/kg)[1]	0	1,500	0	1,500	0	1,500	0	1,500	0	1,500	0	1,500
Corn	440.67											
Soybean meal	300											
Rapeseed meal	100											
Sunflower meal	100											
Soybean oil	25											
Premix[2]	5											
Titanium dioxide	5											
NaCl	4											
DL-Methionine	0.5											

[1] Added on top of the diets.

[2] Supplied per kg of diet: 12,000 IU vitamin A (retinyl acetate), 2,500 IU vitamin D3 (cholecalciferol), 50 mg vitamin E (DL-α-tocopherol), 1.5 mg vitamin K3 (menadione), 2.0 mg vitamin B1 (thiamine), 7.5 mg vitamin B2 (riboflavin), 3.5 mg vitamin B6 (pyridoxine), 20 µg vitamin B12 (cyanocobalamin), 30 mg niacin, 12 mg pantothenic acid, 460 mg choline chloride, 1.0 mg folic acid, 0.2 mg biotin, 80 mg iron, 12 mg copper, 85 mg manganese, 60 mg zinc, 0.8 mg iodine, 0.15 mg selenium, 125 mg anti-oxidant.

Supplemental Table 2. Analyzed nutrient composition of the experimental diets (g/kg dry matter, unless otherwise stated).[1,2]

Acid-binding capacity	$CaCO_3$				$CaCO_3$+formic acid				Ca-formate			
Ca concentration	low		high		low		high		low		high	
Phytase[1]	-	+	-	+	-	+	-	+	-	+	-	+
Calcium	5.6	5.6	8.0	8.1	5.5	5.5	8.2	8.1	5.7	5.6	8.3	8.2
Phosphorus	5.3	5.3	5.3	5.2	5.3	5.3	5.4	5.3	5.2	5.4	5.3	5.3
Phytate-P	3.6	3.5	3.5	3.5	3.5	3.5	3.6	3.6	3.5	3.5	3.3	3.6
Phytase (FTU/kg)	< 60	1,450	< 60	1,580	< 60	1,460	< 60	1,450	< 60	1,470	< 60	1,480
Crude protein	266	260	263	262	264	262	262	262	259	263	259	262
Amino acids												
Alanine	13.0	13.0	12.7	12.6	12.8	13.0	12.8	12.8	12.7	12.8	12.5	12.6
Arginine	18.1	18.1	17.8	17.5	17.8	18.2	18.0	17.9	17.6	17.9	17.4	17.7
Aspartic acid/asparagine	27.2	27.2	26.8	26.5	26.8	27.6	27.0	27.1	26.6	27.2	26.3	26.7
Cysteine	4.4	4.3	4.3	4.3	4.3	4.3	4.3	4.2	4.2	4.2	4.1	4.2
Glutamic acid/glutamine	48.6	48.6	48.0	47.5	48.2	49.2	48.4	48.5	47.8	48.5	47.3	47.8
Glycine	12.2	12.3	12.0	11.9	12.0	12.4	12.1	12.0	12.0	12.1	11.8	11.8
Histidine	7.9	7.8	7.7	7.6	7.7	7.7	7.8	7.6	7.5	7.7	7.4	7.5
Isoleucine	11.3	11.4	11.2	11.0	11.1	11.7	11.1	11.5	11.3	11.3	11.1	11.1
Leucine	21.8	21.7	21.4	21.1	21.4	22.0	21.4	21.5	21.3	21.5	21.1	21.2
Lysine	14.1	14.2	14.0	13.8	13.9	14.4	14.0	14.1	13.8	14.1	13.7	13.8
Methionine	4.9	5.0	4.9	4.8	4.9	5.0	4.9	4.9	4.8	4.9	4.8	4.8
Phenylalanine	13.1	13.1	12.9	12.7	12.8	13.2	12.9	13.0	12.8	12.9	12.7	12.7
Proline	14.9	14.9	14.8	14.4	14.8	14.9	14.7	14.5	14.5	14.8	14.4	14.4
Serine	13.5	13.4	13.3	13.1	13.4	13.5	13.5	13.2	13.1	13.4	13.0	13.2
Threonine	10.9	10.9	10.7	10.6	10.7	10.9	10.8	10.7	10.6	10.8	10.5	10.6
Tyrosine	8.8	8.8	8.7	8.5	8.7	8.8	8.7	8.7	8.6	8.6	8.5	8.6
Valine	12.6	12.7	12.5	12.3	12.4	13.0	12.4	12.8	12.6	12.5	12.4	12.3

[1] - without phytase supplementation; + with supplementation of 1,500 FTU phytase/kg.

[2] Further analyzed compounds are shown in the companion paper (1).

Supplemental Table 3. Pearson correlation coefficients between prececal amino acid digestibility and relative abundance of OTUs in the crop and ileum content of broiler chickens.[1]

	Crop microbiota[2]		Ileum microbiota[3]		
	OTU2	OTU13	OTU1	OTU7	OTU10
Ala				0.32	-0.43
Arg			0.31		-0.33
Asx	-0.26	-0.26	0.31		-0.33
Cys			0.29		
Glx	-0.25	0.24	0.32		-0.34
Gly			0.32		-0.35
His					-0.40
Ile			0.31		-0.37
Leu			0.29	0.28	-0.41
Lys			0.28	0.27	-0.38
Met				0.31	-0.46
Phe			0.30		-0.37
Pro			0.30		-0.38
Ser			0.31		-0.35
Thr			0.28	0.29	-0.39
Tyr			0.29		-0.37
Val			0.30	0.27	-0.38

[1] n = 12 diets; only significant ($P \leq 0.05$) correlations are presented; values for relative abundance of OTUs published in the corresponding communication (1); Ala, alanine; Arg, arginine; Asx, aspartic acid/asparagine; Ca, calcium; Cys, cysteine; Glx, glutamic acid/glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

[2] OTU2 and OTU13 assigned to *Lactobacillus gallinarum* and *Lactobacillus reuteri*, respectively.

[3] OTU1, OTU7, and OTU10 assigned to *Lactobacillus johnsonii*, Uncultured Firmicutes, and *Salmonella alactolyticus*, respectively.

Supplemental Table 4. Percent of genes assigned to enzymes related to protein digestion and absorption as well as amino acid metabolism listed in the KEGG database in crop content of broiler chickens fed with differently acidified diets with low and high Ca concentrations without (-) and with (+) supplementation of 1,500 FTU phytase/kg.[1]

ABC	Ca con-centration	Phytase	01230	00220	00250	00260	00270	00280	00290	00300	00310	00330	00340	00350	00360	00380	00400	04974
Treatments																		
$CaCO_3$	low	-	0.040	0.296	0.710	1.006	0.878	0.166	0.064	0.675	0.116	0.226	0.063	0.225	0.038	0.096	0.094	2.426
		+	0.039	0.267	0.709	0.958	0.811	0.165	0.071	0.676	0.130	0.246	0.059	0.262	0.058	0.115	0.103	2.344
	high	-	0.033	0.324	0.739	0.989	0.902	0.151	0.070	0.711	0.114	0.246	0.040	0.259	0.063	0.093	0.102	2.448
		+	0.032	0.308	0.740	0.959	0.853	0.179	0.070	0.710	0.121	0.255	0.063	0.276	0.067	0.103	0.098	2.392
$CaCO_3$+	low	-	0.031	0.334	0.759	0.995	0.927	0.148	0.067	0.726	0.107	0.250	0.037	0.259	0.057	0.084	0.095	2.474
formic acid		+	0.030	0.333	0.756	0.963	0.900	0.182	0.080	0.713	0.117	0.270	0.060	0.274	0.078	0.097	0.115	2.467
	high	-	0.026	0.335	0.763	0.958	0.886	0.142	0.083	0.728	0.114	0.266	0.028	0.288	0.082	0.096	0.115	2.463
		+	0.028	0.331	0.764	0.947	0.874	0.135	0.081	0.720	0.116	0.263	0.030	0.286	0.075	0.098	0.109	2.436
Ca-formate	low	-	0.035	0.324	0.741	1.027	0.935	0.173	0.056	0.726	0.103	0.225	0.053	0.238	0.043	0.079	0.079	2.458
		+	0.030	0.320	0.753	1.001	0.899	0.178	0.056	0.736	0.104	0.234	0.059	0.262	0.049	0.084	0.074	2.425
	high	-	0.033	0.316	0.747	0.999	0.900	0.155	0.059	0.730	0.108	0.236	0.045	0.258	0.053	0.087	0.081	2.425
		+	0.033	0.306	0.738	0.960	0.853	0.195	0.070	0.711	0.121	0.254	0.070	0.278	0.070	0.103	0.099	2.387
	SEM		0.0023	0.0078	0.0082	0.0163	0.0172	0.0213	0.0085	0.0134	0.0064	0.0098	0.0133	0.0092	0.0114	0.0073	0.0145	0.0190
Significant two-way interactions[2]																		
ABC × Ca concentration														.			.	
$CaCO_3$	low		0.027^{c}	0.333^{a}	0.763^{a}	.	0.880^{bc}	.	.	0.724^{a}	.	.	.	.	.	.	.	2.449^{ab}
	high		0.030^{bc}	0.333^{a}	0.757^{ab}	.	0.913^{ab}	.	.	0.719^{a}	.	.	.	.	.	.	.	2.470^{a}
$CaCO_3$+	low		0.033^{b}	0.311^{b}	0.743^{bc}	.	0.876^{cd}	.	.	0.720^{a}	.	.	.	.	.	.	.	2.406^{cd}
formic acid	high		0.033^{b}	0.322^{ab}	0.747^{bc}	.	0.917^{a}	.	.	0.731^{a}	.	.	.	.	.	.	.	2.442^{abc}
Ca-formate	low		0.033^{b}	0.316^{b}	0.740^{c}	.	0.878^{cd}	.	.	0.710^{a}	.	.	.	.	.	.	.	2.420^{cd}
	high		0.040^{a}	0.282^{c}	0.709^{d}	.	0.844^{d}	.	.	0.676^{b}	.	.	.	.	.	.	.	2.385^{d}
SEM			0.0018	0.0055	0.0062		0.0122			0.0101								
Significant main effects[3]																		
$CaCO_3$			.	.	.	0.966^{b}	.	.	0.078^{a}	.	0.114^{ab}	0.262^{a}	.	0.277^{a}	0.073^{a}	0.094^{ab}	0.109^{a}	.
$CaCO_3$+ formic acid			.	.	.	0.997^{a}	.	.	0.060^{b}	.	0.109^{b}	0.237^{b}	.	0.259^{b}	0.054^{b}	0.088^{b}	0.083^{b}	.
Ca-formate			.	.	.	0.978^{ab}	.	.	0.069^{ab}	.	0.120^{a}	0.243^{b}	.	0.256^{b}	0.057^{b}	0.102^{a}	0.099^{ab}	.
SEM						0.0091					0.0037	0.0057		0.0053	0.0062		0.0088	
	low		.	.	.	0.969^{b}	.	.	.	.	.	0.253^{a}	.	0.274^{a}	0.068^{a}	.	.	.
	high		.	.	.	0.992^{a}	.	.	.	.	.	0.242^{b}	.	0.254^{b}	0.054^{b}	.	.	.
	SEM					0.0079						0.0051		0.0047	0.0054			
		-	.	0.321^{a}	.	0.996^{a}	0.905^{a}	.	.	.	0.110^{b}	0.241^{b}	.	0.255^{b}	.	0.089^{b}	.	2.449^{a}
		+	.	0.311^{b}	.	0.965^{b}	0.865^{b}	.	.	.	0.118^{a}	0.254^{a}	.	0.273^{a}	.	1.000^{a}	.	2.409^{b}
		SEM		0.0032		0.0079	0.0070				0.0032	0.0051		0.0047				0.0082

Table continued on next page.

Supplemental Table 4. Continuation.

ANOVA																
ABC	<0.001	<0.001	<0.001	0.024	0.005	0.25	0.008	<0.001	0.037	0.001	0.07	0.002	0.036	0.033	0.030	<0.001
Ca concentration	0.007	0.10	0.017	0.014	0.18	0.42	0.18	0.19	0.48	0.036	0.21	<0.001	0.027	0.29	0.35	0.50
Phytase	0.39	0.019	0.97	0.001	<0.001	0.16	0.31	0.50	0.032	0.024	0.10	<0.001	0.13	0.010	0.51	<0.001
ABC × Ca concentration	0.037	<0.001	0.007	0.49	0.006	0.57	0.82	0.038	0.20	0.65	0.49	0.87	0.92	0.11	0.82	0.027
ABC × Phytase	0.54	0.17	0.96	0.73	0.29	0.94	0.98	0.84	0.86	0.88	0.94	0.23	0.93	0.78	0.96	0.15
Ca concentration × Phytase	0.30	0.92	0.60	0.62	0.71	0.72	0.70	0.55	0.87	0.47	0.55	0.20	0.40	0.74	0.72	0.98
ABC × Ca concentration × Phytase	0.71	0.65	0.43	0.70	0.85	0.33	0.46	0.60	0.43	0.49	0.35	0.81	0.43	0.49	0.41	0.69

[1] Values are least square means, n = 6 pens/treatment with 15 birds/pen. [a,b] Labeled means of a comparison without a common lowercase superscript letter differ significantly, P≤0.050; ABC, acid-binding capacity; KEGG pathways: 01230 protein digestion and absorption, 00220 arginine biosynthesis, 00250 alanine, aspartic acid, and glutamic acid metabolism, 00260 glycine, serine, and threonine metabolism, 00270 cysteine and methionine metabolism, 00280 valine, leucine, and isoleucine degradation, 00290 valine, leucine, and isoleucine biosynthesis, 00300 lysine biosynthesis, 00310 lysine degradation, 00330 arginine and proline metabolism, 00340 histidine metabolism, 00350 tyrosine metabolism, 00360 phenylalanine metabolism, 00380 tryptophan metabolism, 00400 phenylalanine, tyrosine, and tryptophan biosynthesis, 04974 biosynthesis of amino acids; Ca, calcium; SEM, standard error of the means.

[2] Only presented if significant ($P \leq 0.05$) and if the three-way interaction is not significant ($P > 0.05$).

[3] Only presented if significant ($P \leq 0.05$) and if the three-way interaction and no 2-way interaction including the respective trait are not significant ($P > 0.05$).

Supplemental Table 5. Percent of genes assigned to enzymes related to protein digestion and absorption as well as amino acid metabolism listed in the KEGG database in ileum content of broiler chickens fed with differently acidified diets with low and high Ca concentrations without (-) and with (+) supplementation of 1,500 FTU phytase/kg.[1]

ABC	Ca con-centration	Phytase	01230	00220	00250	00260	00270	00280	00290	00300	00310	00330	00340	00350	00360	00380	00400	04974
Treatments																		
$CaCO_3$	low	-	2.46	0.305	0.712	1.059	0.912	0.120	0.046	0.743	0.098	0.212	0.030	0.241	0.045	0.072	0.072	0.027
		+	2.45	0.319	0.737	1.028	0.911	0.125	0.050	0.734	0.102	0.223	0.026	0.246	0.040	0.078	0.071	0.030
	high	-	2.51	0.333	0.722	1.064	0.968	0.127	0.052	0.719	0.098	0.214	0.022	0.215	0.034	0.069	0.080	0.034
		+	2.49	0.318	0.737	1.022	0.920	0.129	0.059	0.725	0.102	0.231	0.036	0.243	0.043	0.078	0.086	0.029
$CaCO_3$+	low	-	2.44	0.299	0.721	1.055	0.926	0.128	0.049	0.726	0.101	0.209	0.022	0.223	0.032	0.074	0.069	0.037
formic acid		+	2.40	0.294	0.730	1.010	0.868	0.126	0.050	0.723	0.106	0.223	0.029	0.251	0.037	0.086	0.067	0.032
	high	-	2.49	0.327	0.730	1.064	0.960	0.126	0.048	0.737	0.096	0.211	0.018	0.225	0.035	0.068	0.071	0.033
		+	2.45	0.310	0.724	1.045	0.915	0.124	0.046	0.731	0.100	0.215	0.024	0.237	0.036	0.075	0.068	0.031
Ca-formate	low	-	2.46	0.316	0.723	1.048	0.933	0.126	0.047	0.719	0.100	0.209	0.021	0.222	0.027	0.075	0.070	0.035
		+	2.41	0.290	0.717	1.033	0.878	0.123	0.044	0.726	0.104	0.214	0.027	0.241	0.032	0.082	0.063	0.032
	high	-	2.44	0.298	0.708	1.050	0.911	0.127	0.045	0.706	0.103	0.205	0.022	0.215	0.020	0.079	0.068	0.037
		+	2.47	0.299	0.708	1.047	0.911	0.126	0.053	0.708	0.103	0.210	0.033	0.214	0.026	0.077	0.085	0.035
	SEM		0.026	0.0111	0.0069	0.0143	0.0239	0.0025	0.0037	0.0081	0.0020	0.0054	0.0050	0.0112	0.0058	0.0039	0.0058	0.0026
Significant two-way interactions[2]																		
ABC × Ca concentration																		
$CaCO_3$	low		.	.	.	.	.	.	.	0.738[a]	.	.	.	.	.	.	.	.
	high		.	.	.	.	.	.	.	0.722[ab]	.	.	.	.	.	.	.	.
$CaCO_3$+	low		.	.	.	.	.	.	.	0.724[a]	.	.	.	.	.	.	.	.
formic acid	high		.	.	.	.	.	.	.	0.734[a]	.	.	.	.	.	.	.	.
Ca-Formate	low		.	.	.	.	.	.	.	0.722[ab]	.	.	.	.	.	.	.	.
	high		.	.	.	.	.	.	.	0.707[b]	.	.	.	.	.	.	.	.
SEM										0.0058								
Significant main effects[3]																		
$CaCO_3$			.	.	0.727[a]	.	.	.	.	.	.	0.220[a]	.	.	0.041[a]	.	.	0.030[b]
$CaCO_3$+ formic acid			.	.	0.726[a]	.	.	.	.	.	.	0.214[ab]	.	.	0.035[a]	.	.	0.033[ab]
Ca-formate			.	.	0.714[b]	.	.	.	.	.	.	0.209[b]	.	.	0.026[b]	.	.	0.035[a]
SEM					0.0035							0.0033			0.0035			0.0013
	low		2.44[b]	.	.	.	.	.	.	.	.	.	.	.	.	.	0.069[b]	.
	high		2.47[a]	.	.	.	.	.	.	.	.	.	.	.	.	.	0.076[a]	.
	SEM		0.011														0.0029	
		-	.	.	.	1.057[a]	0.935[a]	.	.	.	0.099[b]	0.210[b]	0.022[b]	0.224[b]	.	0.073	.	.
		+	.	.	.	1.031[b]	0.901[b]	.	.	.	0.103[a]	0.219[a]	0.029[a]	0.239[a]	.	0.079	.	.
		SEM				0.0069	0.0102				0.0008	0.0029	0.0024	0.0053		0.0017		

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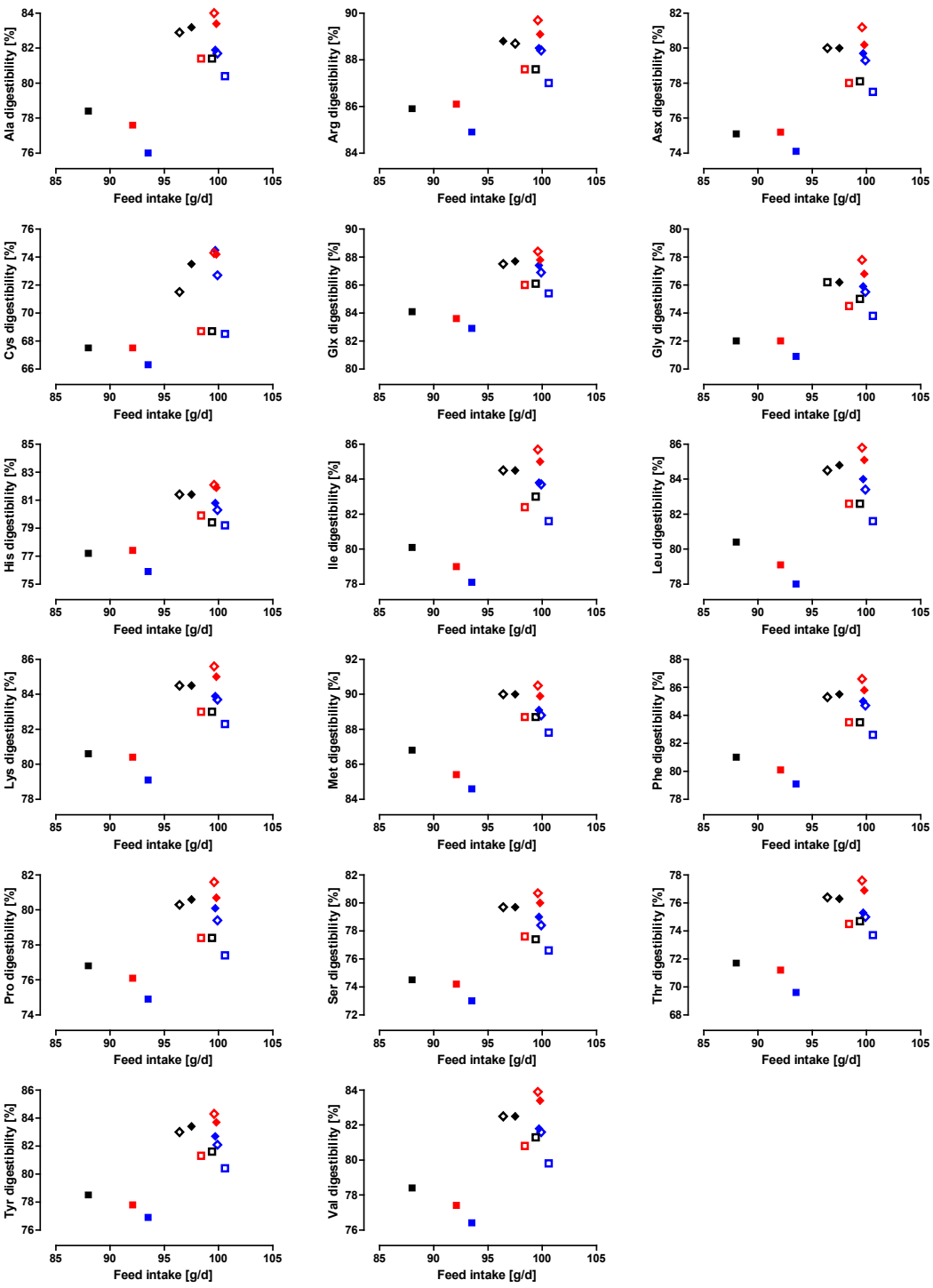
Supplemental Table 5. Continuation.

ANOVA																
ABC	0.13	0.10	0.019	0.99	0.53	0.96	0.17	0.019	0.20	0.023	0.30	0.22	0.003	0.39	0.08	0.046
Ca concentration	0.012	0.11	0.62	0.21	0.06	0.22	0.22	0.14	0.28	0.83	0.95	0.05	0.32	0.13	0.025	0.35
Phytase	0.09	0.22	0.14	0.002	0.016	0.79	0.21	0.94	0.004	0.002	0.019	0.020	0.26	0.005	0.68	0.18
ABC × Ca concentration	0.73	0.24	0.22	0.50	0.57	0.10	0.14	0.038	0.08	0.47	0.50	0.74	0.61	0.27	0.35	0.28
ABC × Phytase	0.62	0.73	0.05	0.33	0.67	0.18	0.44	0.74	0.56	0.47	0.85	0.78	0.91	0.44	0.64	0.63
Ca concentration × Phytase	0.34	0.72	0.40	0.57	0.81	0.94	0.28	0.75	0.43	0.89	0.16	0.80	0.56	0.48	0.11	0.71
ABC × Ca concentration × Phytase	0.35	0.21	0.53	0.61	0.34	0.76	0.36	0.63	0.82	0.49	0.34	0.32	0.51	0.53	0.29	0.35

[1] Values are least square means, n = 6 pens/treatment with 15 birds/pen. [a,b] Labeled means of a comparison without a common lowercase superscript letter differ significantly, P≤0.050; ABC, acid-binding capacity; KEGG pathways: 01230 protein digestion and absorption, 00220 arginine biosynthesis, 00250 alanine, aspartic acid, and glutamic acid metabolism, 00260 glycine, serine, and threonine metabolism, 00270 cysteine and methionine metabolism, 00280 valine, leucine, and isoleucine degradation, 00290 valine, leucine, and isoleucine biosynthesis, 00300 lysine biosynthesis, 00310 lysine degradation, 00330 arginine and proline metabolism, 00340 histidine metabolism, 00350 tyrosine metabolism, 00360 phenylalanine metabolism, 00380 tryptophan metabolism, 00400 phenylalanine, tyrosine, and tryptophan biosynthesis, 04974 biosynthesis of amino acids; Ca, calcium SEM, standard error of the means.

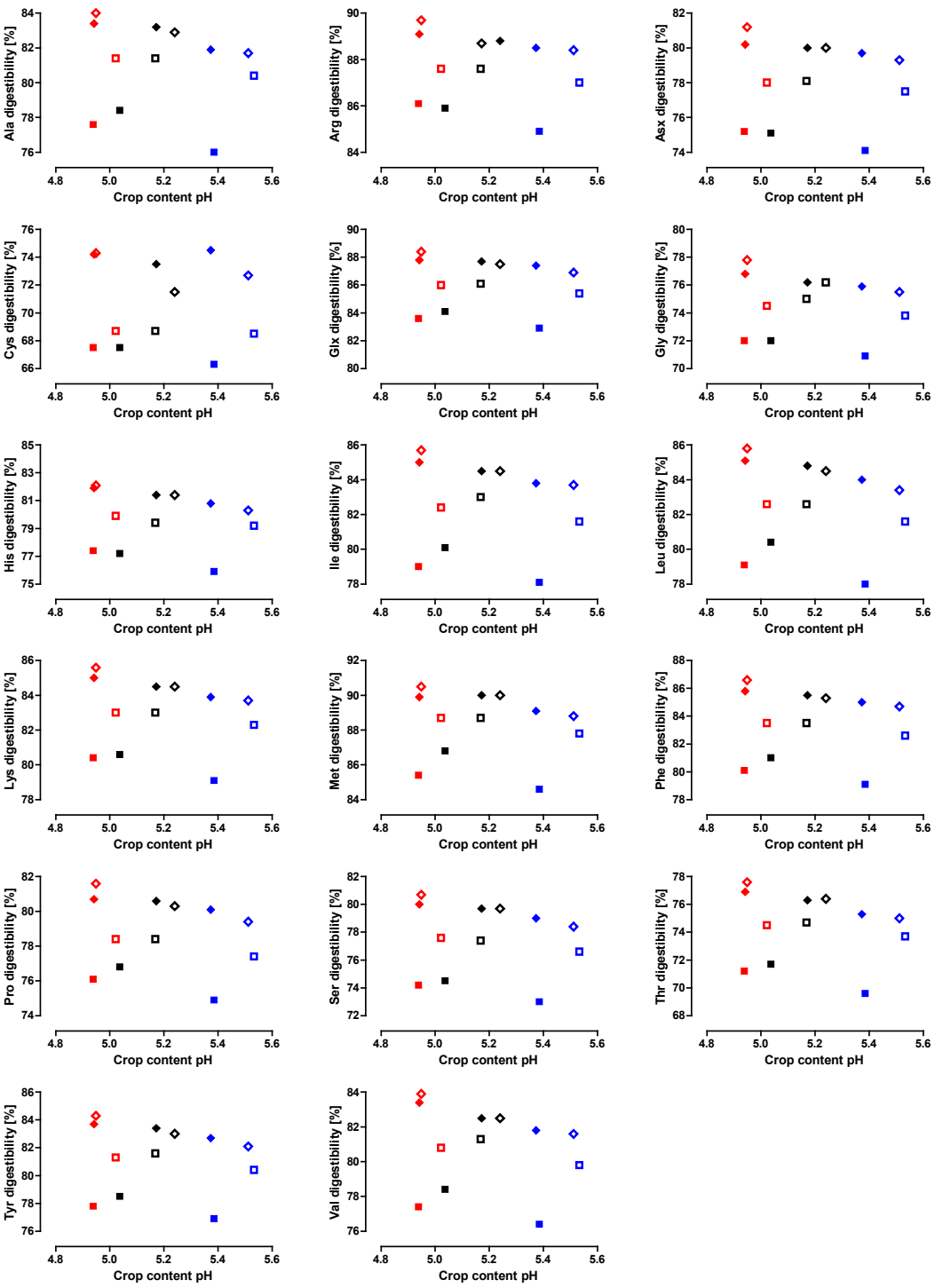
[2] Only presented if significant ($P \leq 0.05$) and if the three-way interaction is not significant ($P > 0.05$).

[3] Only presented if significant ($P \leq 0.05$) and if the three-way interaction and no 2-way interaction including the respective trait are not significant ($P > 0.05$).

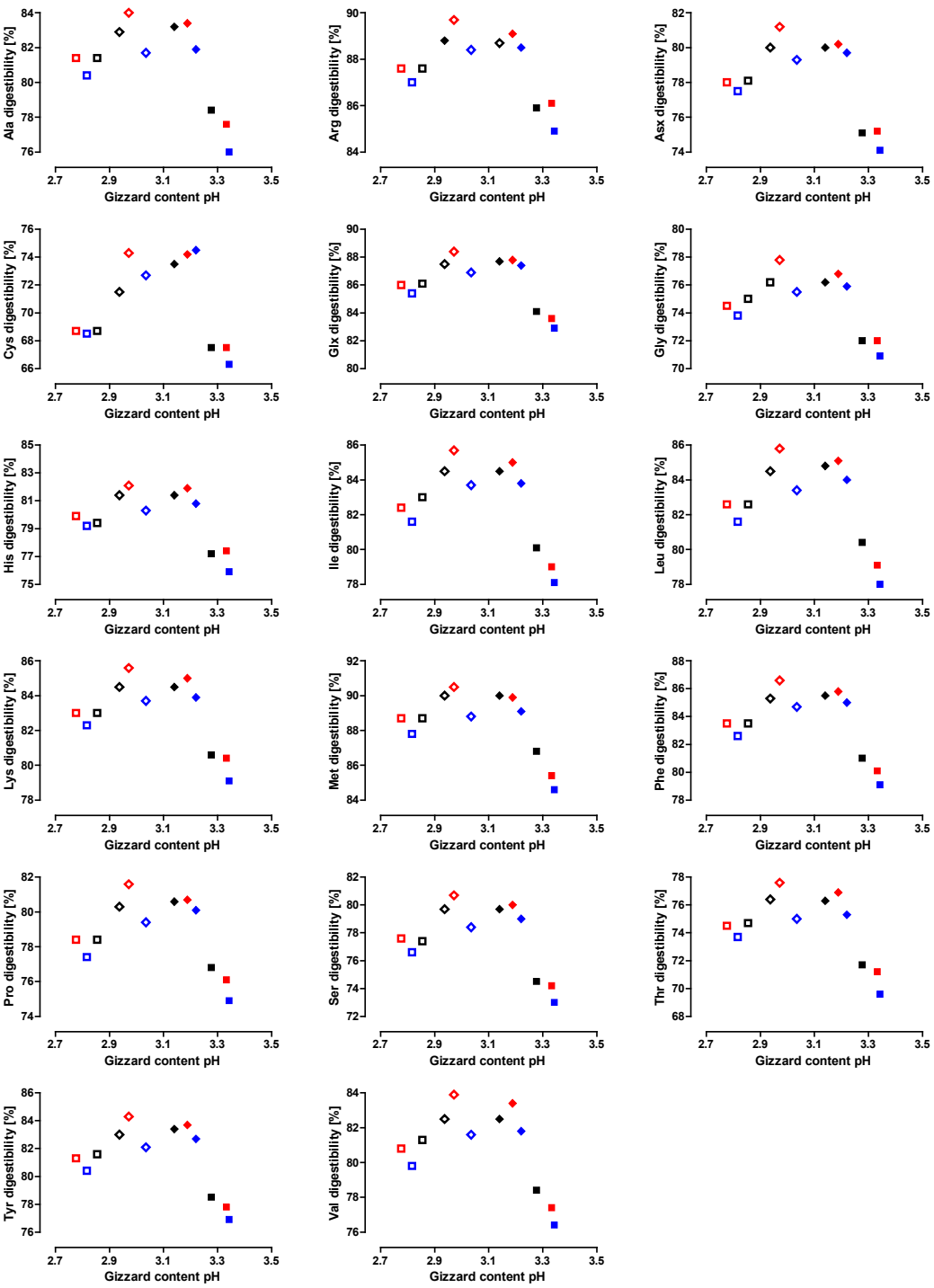


Symbols													
Phytase	-	+	-	+	-	+	-	+	-	+	-	+
Ca concentration	low		high		low		high		Low		High	
Acid-binding capacity	$CaCO_3$				$CaCO_3$ + formic acid				Ca-formate			

Supplemental Figure 1. Relationship between prececal amino acid digestibility and feed intake, symbols represent least square means, details of the statistical evaluation of feed intake are shown in the companion paper (1), n = 6 pens/treatment with 15 birds/pen.

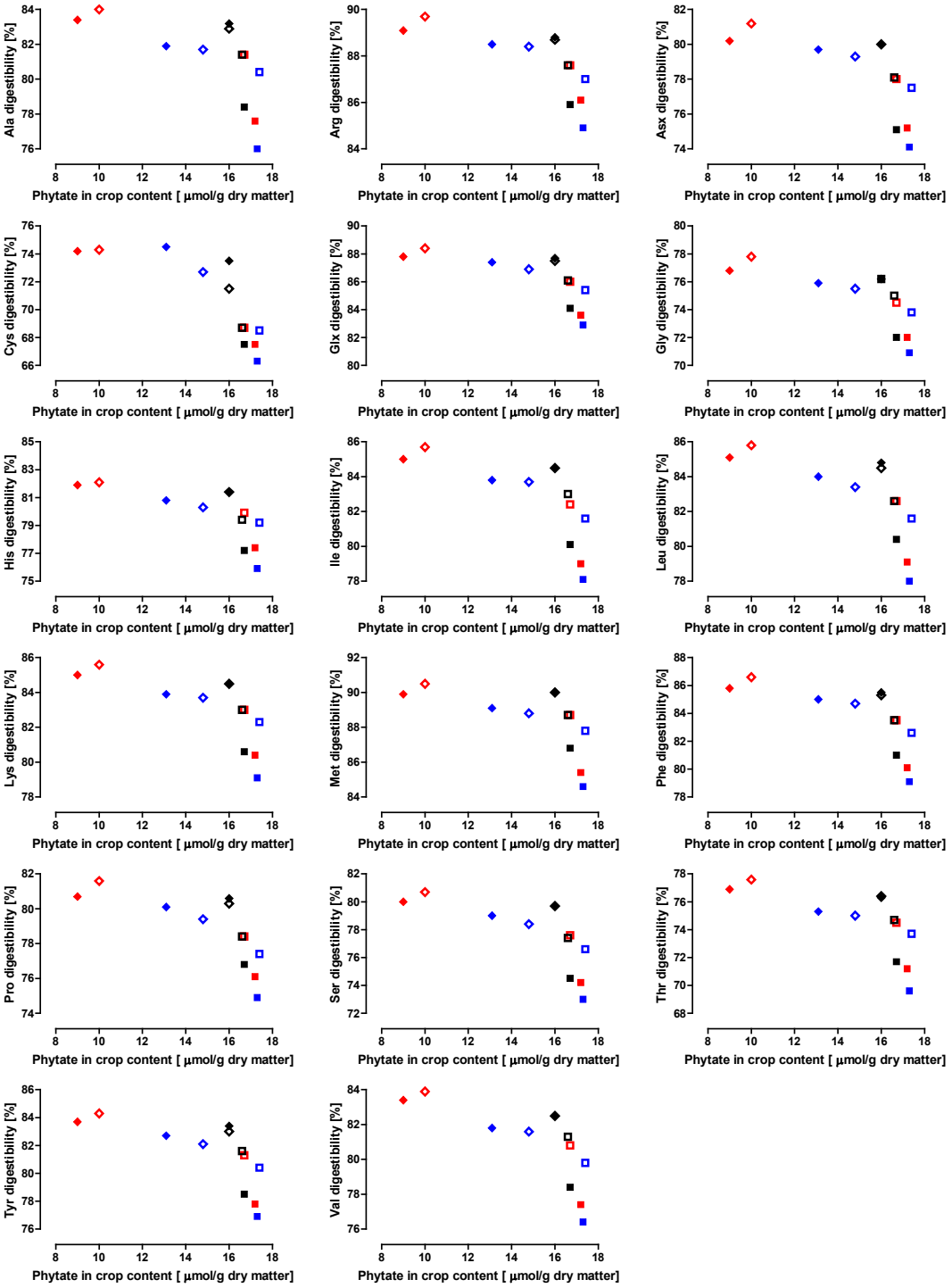


Supplemental Figure 2. Relationship between prececal amino acid digestibility and crop content pH, symbols represent least square means, details of the statistical evaluation of crop content pH are shown in the companion paper (1), n = 6 pens/treatment with 15 birds/pen.

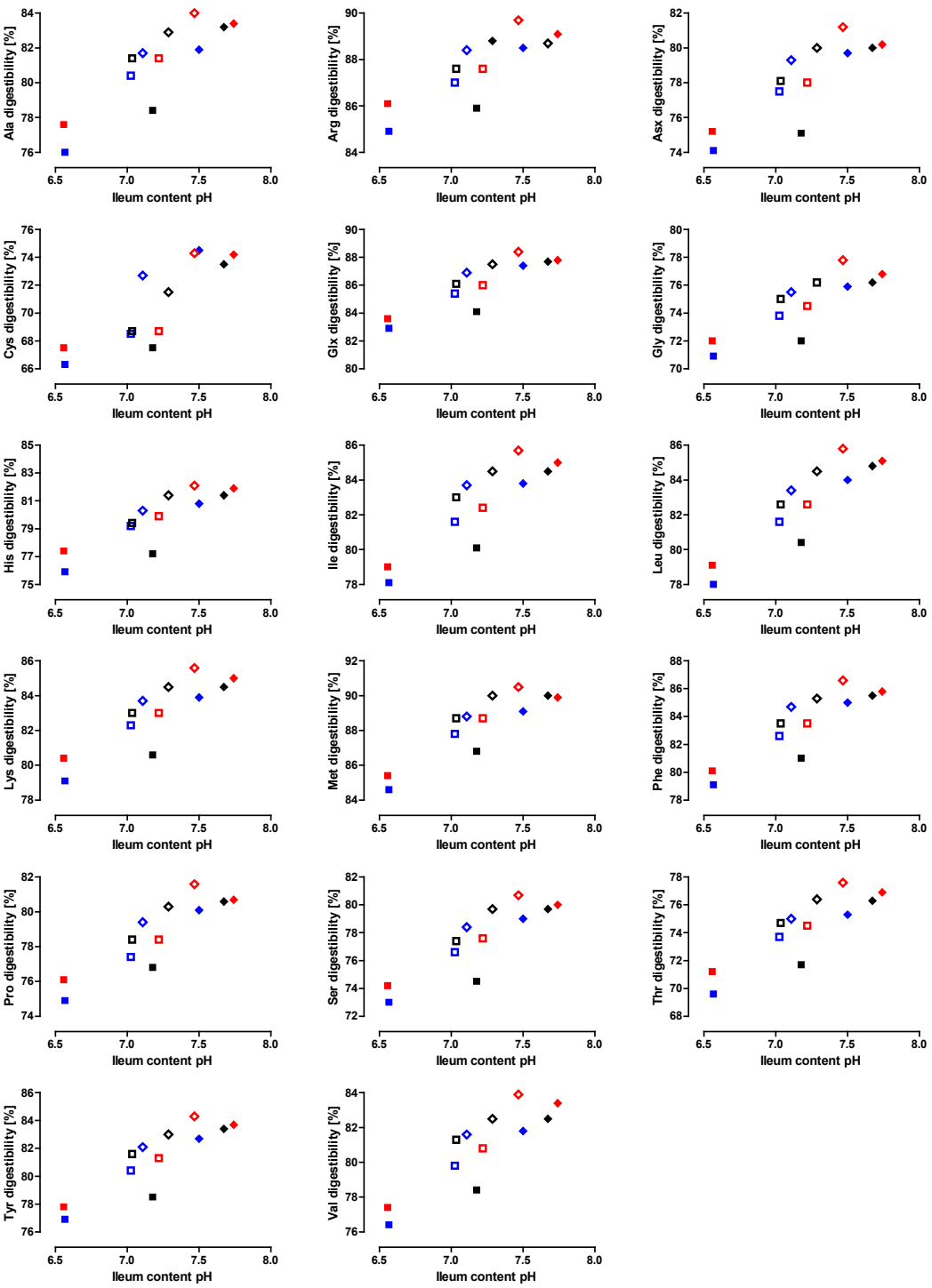


Symbols														
Phytase	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Ca concentration	low		high		low		high		Low		High			
Acid-binding capacity	$CaCO_3$				$CaCO_3$ + formic acid				Ca-formate					

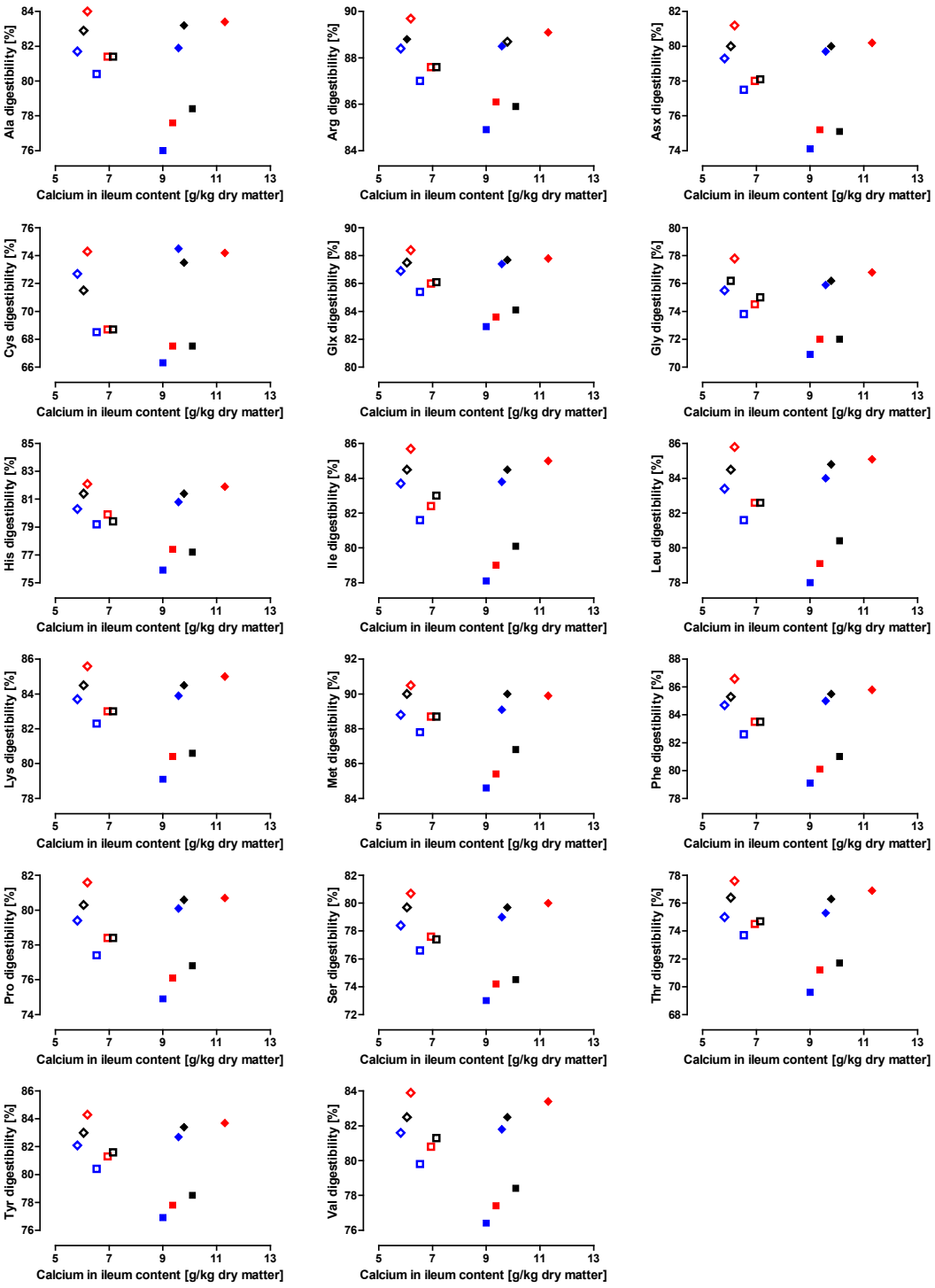
Supplemental Figure 3. Relationship between prececal amino acid digestibility and gizzard content pH, symbols represent least square means, details of the statistical evaluation of gizzard content pH are shown in the companion paper (1), n = 6 pens/treatment with 15 birds/pen.



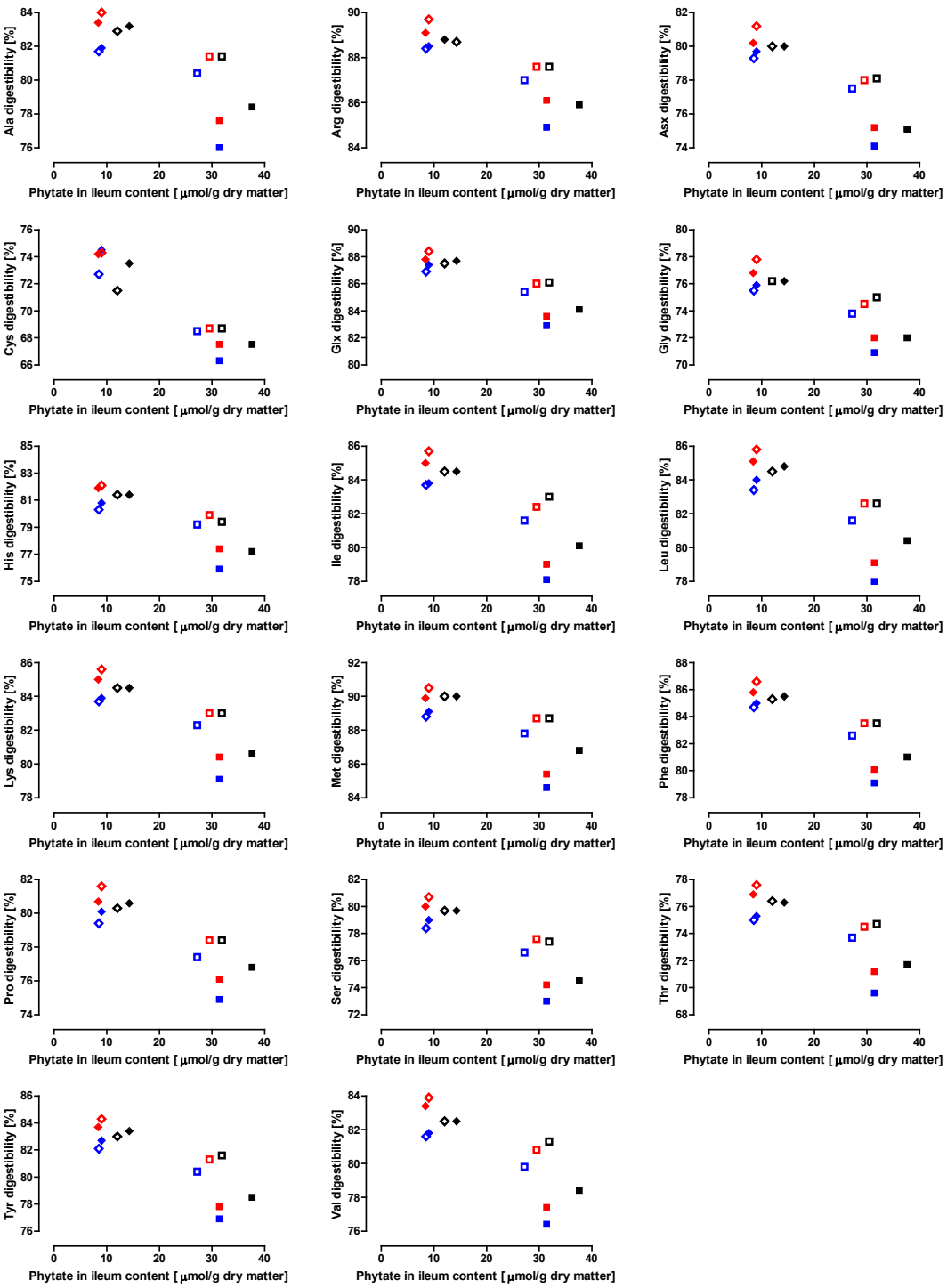
Supplemental Figure 4. Relationship between prececal amino acid digestibility and phytate concentration in the crop content, symbols represent least square means, details of the statistical evaluation of phytate in crop content are shown in the companion paper (1), n = 6 pens/treatment with 15 birds/pen.



Supplemental Figure 5. Relationship between prececal amino acid digestibility and pH in the ileum content, symbols represent least square means, details of the statistical evaluation of ileum content pH are shown in the companion paper (1), n = 6 pens/treatment with 15 birds/pen.

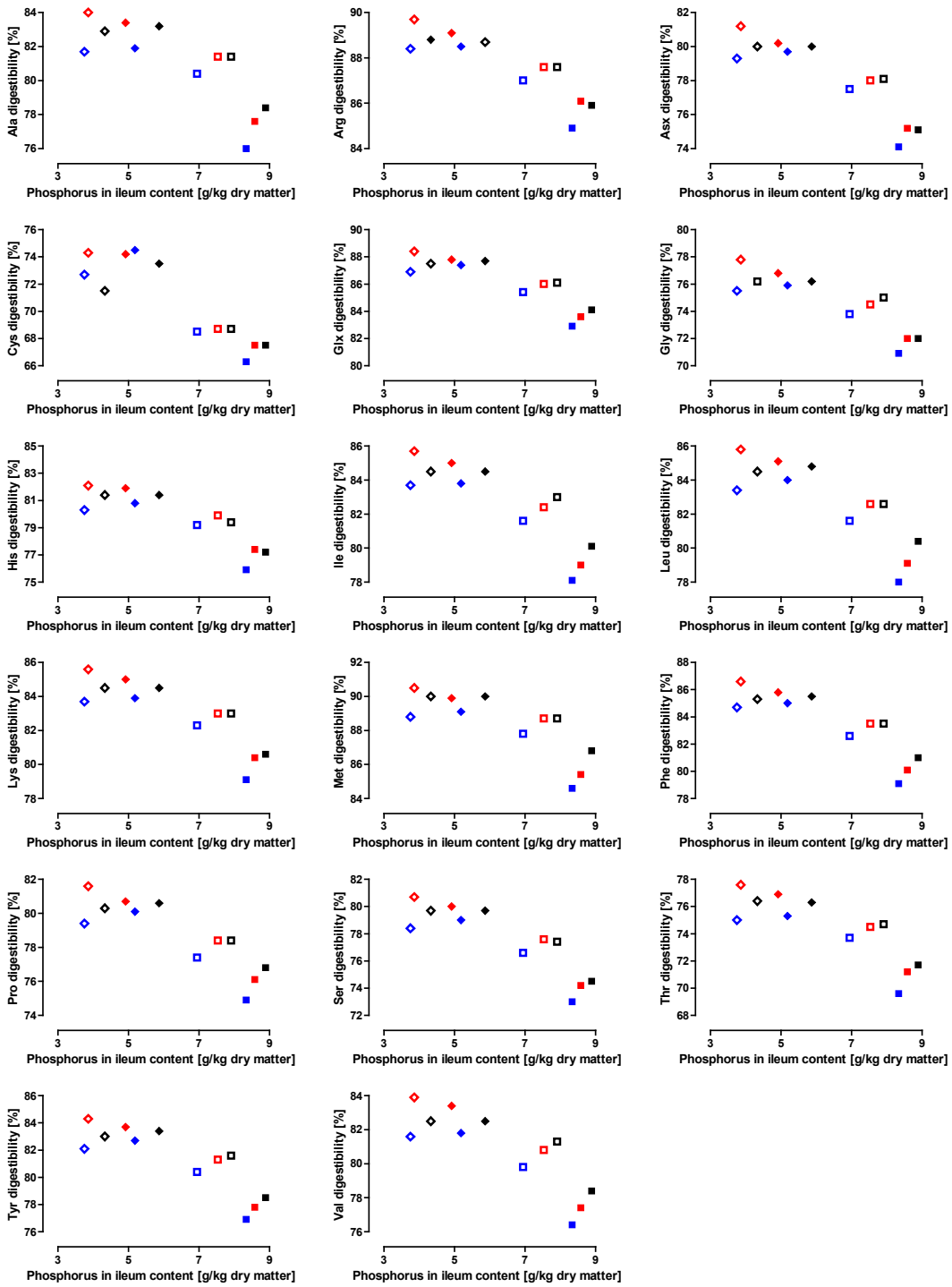


Supplemental Figure 6. Relationship between prececal amino acid digestibility and calcium in the ileum content, symbols represent least square means, details of the statistical evaluation of calcium in ileum content are shown in the companion paper (1), n = 6 pens/treatment with 15 birds/pen.



Symbols	□	◇	■	◆	□	◇	■	◆	□	◇	■	◆
Phytase	-	+	-	+	-	+	-	+	-	+	-	+
Ca concentration	low		high		low		high		Low		High	
Acid-binding capacity	$CaCO_3$				$CaCO_3$ + formic acid				Ca-formate			

Supplemental Figure 7. Relationship between prececal amino acid digestibility and phytate in the ileum content, symbols represent least square means, details of the statistical evaluation of phytate in ileum content are shown in the companion paper (1), n = 6 pens/treatment with 15 birds/pen.



Symbols	□	◇	■	◆	□	◇	■	◆	□	◇	■	◆
Phytase	-	+	-	+	-	+	-	+	-	+	-	+
Ca concentration	low		high		low		high		Low		High	
Acid-binding capacity	$CaCO_3$				$CaCO_3$ + formic acid				Ca-formate			

Supplemental Figure 8. Relationship between prececal amino acid digestibility and phosphorus in the ileum content, symbols represent least square means, details of the statistical evaluation of phosphorus in ileum content are shown in the companion paper (1), n = 6 pens/treatment with 15 birds/pen.

Reference

1. Krieg J, Borda-Molina D, Siegert W, Sommerfeld V, Chi Y-P, Taheri HR, Feuerstein D, Camarinha-Silva A, Rodehutscord M. Effects of calcium level and source, formic acid, and phytase on phytate degradation and the microbiota in the digestive tract of broiler chickens. Anim Microbiome 2021;3:23. *(doi: 10.1186/s42523-021-00083-7)*