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Effects of phytase supplementation on growth performance, plasma biochemistry, bone mineralisation and phosphorus utilisation in pre-lay pullets fed various levels of phosphorus

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Supplementary Material

Table S1. Plasma biochemistry of pre-lay pullets on various diets^{A,B}

Variables	Factors	<i>P</i> value ^D	Diet means						SEM
			L-	L+	M-	M+	H-	H+	
<i>mmol/L</i>									
Sodium	Diet	0.818	144	144	144	145	144	144	0.4
	Age	< 0.001							
	Diet × age	0.811							

	Wk 8		143	143	143	144	143	142	0.4
	Wk 16		146	145	145	146	145	145	0.6
Potassium	Diet	0.862	4.92	4.96	4.92	4.87	5.00	4.90	0.07
	Age	0.345							
	Diet × age	0.372							
	Wk 8		4.83	5.04	4.92	4.95	5.04	4.91	0.09
	Wk 16		5.00	4.89	4.91	4.79	4.95	4.89	0.09
Chloride	Diet	0.684	113	113	114	113	113	113	0.4
	Age	< 0.001							
	Diet × age	0.515							
	Wk 8		112	112	112	112	112	111	0.4
	Wk 16		114	114	115	114	114	114	0.6
Glucose	Diet	0.092	12.5	12.3	12.8	12.6	12.6	12.5	0.10
	Age	< 0.001							
	Diet × age	0.801							
	Wk 8		12.7	12.6	13.0	12.9	13.0	12.8	0.1
	Wk 16		12.3	12.1	12.6	12.2	12.2	12.2	0.1
Cholesterol	Diet	0.635	2.70	2.68	2.57	2.64	2.68	2.69	0.06
	Age	< 0.001							
	Diet × age	0.548							
	Wk 8		2.56	2.60	2.42	2.54	2.57	2.54	0.05
	Wk 16		2.83	2.76	2.72	2.74	2.78	2.84	0.07

<i>U/L</i>									
AST ^C	Diet	0.505	182	183	180	183	176	183	2.9
	Age	< 0.01							
	Diet × age	0.351							
	Wk 8		176	179	178	182	176	178	3.6
	Wk 16		187	187	181	184	176	187	3.6
CK ^C	Diet	0.625	1326	1365	1264	1378	1270	1499	104
	Age	< 0.001							
	Diet × age	0.324							
	Wk 8		1032	1017	1098	1109	1101	1085	41
	Wk 16		1619	1713	1430	1648	1439	1913	189
<i>g/L</i>									
Total protein	Diet	0.183	43.1	44.0	43.1	44.6	43.1	43.6	0.5
	Age	< 0.001							
	Diet × age	0.926							
	Wk 8		40.0	41.3	40.6	42.0	40.6	41.1	0.4
	Wk 16		46.2	46.6	45.6	47.1	45.6	46.2	0.8
Globulin	Diet	0.247	24.5	24.6	24.5	25.4	24.5	25.0	0.3
	Age	< 0.001							
	Diet × age	0.713							
	Wk 8		22.9	23.6	23.4	24.0	23.3	23.8	0.3
	Wk 16		26.1	25.7	25.6	26.9	25.7	26.1	0.5

^AThe birds on treatment L-, M-, and H- were fed diets containing 2.75-2.50-2.25 g/kg, 3.75-3.50-3.25 g/kg, and 4.75-4.50-4.25 g/kg non-phytate P (NPP) for the age period of 0-4 (starter), 4-8 (grower), and 8-16 wk (developer) respectively, without the supplementation of phytase. The birds on treatment L+, M+, and H+ were fed diets containing 1.75-1.50-1.25 g/kg, 2.75-2.50-2.25 g/kg, and 3.75-3.50-3.25 g/kg NPP for the starter, grower, and developer respectively, with the supplementation of 1000 U/kg phytase.

^BData are presented as least squares means and pooled SEM ($n = 8$).

^CAST: aspartate transaminase; CK: creatine kinase.

^DProbability for F tests of main effects and interaction. Means within a row with different lowercase letters are statistically different ($P < 0.05$).

Table S2a. Phosphorus (P) and calcium intake, excretion and retention (mg/bird.day) of pre-lay pullets on various diets^{A,B}

Variables	Factors	<i>P</i> value	Diet means						SEM
			L-	L+	M-	M+	H-	H+	
Total P									
Intake	Diet	< 0.001 ^{C,E}	266c	209d	319b	264c	386a	329b	3.6
	Age	< 0.001 ^C							
	Diet × age	< 0.001 ^C							
	Wk 8	< 0.001 ^{D,E}	243C	184D	277B	234C	331A	292B	4.8
	Wk 16	< 0.001 ^{D,E}	289C	235D	361B	294C	442A	367B	4.8
	Excretion	Diet	< 0.001 ^{C,E}	186d	150e	231bc	211cd	258a	251ab
Age		< 0.001 ^C							
Diet × age		< 0.05 ^C							
Wk 8		< 0.001 ^{D,E}	130C	93D	165AB	148BC	176AB	195A	8.4
Wk 16		< 0.001 ^{D,E}	243CD	207D	296B	273BC	339A	306AB	8.2
Retention		Diet	< 0.001 ^{C,E}	80bc	54c	88b	53c	129a	78bc
	Age	< 0.001 ^C							
	Diet × age	0.413 ^C							
	Wk 8		113	81	111	86	154	95	8.7
	Wk 16		47	27	65	21	103	61	8.6
	Phytate P								
Intake	Diet	< 0.001 ^{C,E}	106bc	110ab	104c	108abc	113a	110abc	1.4
	Age	< 0.001 ^C							
	Diet × age	< 0.001 ^C							

	Wk 8	< 0.001 ^{D,E}	101A	99A	87B	93AB	97A	97A	1.8
	Wk 16	< 0.001 ^{D,E}	112C	121B	120B	123AB	129A	122B	1.8
Excretion	Diet	< 0.001 ^{C,E}	77a	44c	77a	55b	77a	56b	2.4
	Age	< 0.001 ^C							
	Diet × age	< 0.05 ^C							
	Wk 8	< 0.001 ^{D,E}	77A	45B	72A	52B	69A	54B	3.1
	Wk 16	< 0.001 ^{D,E}	77A	44C	82A	58B	84A	58B	3.1
Retention	Diet	< 0.001 ^{C,E}	29cd	66a	27d	53b	36c	54b	2.3
	Age	< 0.001 ^C							
	Diet × age	0.202 ^C							
	Wk 8		24	55	15	40	28	43	3.0
	Wk 16		35	77	38	65	45	64	3.0
Calcium									
Intake	Diet	0.574 ^C	585	577	568	580	575	585	7.6
	Age	< 0.001 ^C							
	Diet × age	< 0.001 ^C							
	Wk 8	< 0.05 ^{D,E}	526A	497AB	481AB	483AB	477B	496AB	9.7
	Wk 16	0.132 ^D	645	657	654	677	673	673	9.7
Excretion	Diet	0.067 ^C	368	379	372	395	402	393	9.1
	Age	< 0.001 ^C							
	Diet × age	0.238 ^C							
	Wk 8		274	283	263	279	288	290	11.8
	Wk 16		462	475	480	511	515	495	11.8

Retention	Diet	< 0.05 ^{C,E}	217a	198ab	196ab	185ab	173b	192ab	8.7
	Age	< 0.001 ^C							
	Diet × age	0.686 ^C							
	Wk 8		252	214	217	204	189	206	12.9
	Wk 16		182	181	174	166	158	178	12.9

Retention = Intake – Excretion.

^AThe birds on treatment L-, M-, and H- were fed diets containing 2.75-2.50-2.25 g/kg, 3.75-3.50-3.25 g/kg, and 4.75-4.50-4.25 g/kg non-phytate P (NPP) for the age period of 0-4 (starter), 4-8 (grower), and 8-16 wk (developer) respectively, without the supplementation of phytase. The birds on treatment L+, M+, and H+ were fed diets containing 1.75-1.50-1.25 g/kg, 2.75-2.50-2.25 g/kg, and 3.75-3.50-3.25 g/kg NPP for the starter, grower, and developer respectively, with the supplementation of 1000 U/kg phytase.

^BData are presented as least squares means and pooled SEM ($n = 8$).

^CProbability for F tests of main effects and interaction. Means within a row with different lowercase letters are statistically different ($P < 0.05$).

^DProbability for F tests and comparisons of means for diet at different ages, carried out when the $P_{\text{diet} \times \text{age}}$ is significant. Means within a row with different uppercase letters are statistically different ($P < 0.05$).

^EContrasts are performed when the main effect of diet or the effect of diet at each age is significant, and the results are presented in Table S2b.

Table S2b. Contrast analysis of the effect of supplemental phytase and dietary non-phytate P (NPP) level on P and calcium flow (mg/bird.day)^A

Variables		Effect of supplemental phytase (phytase present vs. phytase absent)		Effect of dietary NPP ^B			
		Average of L+, M+ and H+ vs. average of L-, M- and H-		Phytase (-)		Phytase (+)	
				Linear	Quadra	Linear	Quadra
Total P							
Intake	Main	< 0.001	<i>0.234^C</i>	< 0.001	0.097	< 0.001	0.240
	Wk 8	< 0.001	<i>0.526^C</i>	< 0.001	0.098	< 0.001	0.517
	Wk 16	< 0.001	<i>0.244^C</i>	< 0.001	0.389	< 0.001	0.259
Excretion	Main	< 0.001	< <i>0.01^C</i>	< 0.001	0.262	< 0.001	0.194
	Wk 8	0.093	< <i>0.01^C</i>	< 0.001	0.232	< 0.001	0.692
	Wk 16	< 0.001	< <i>0.05^C</i>	< 0.001	0.603	< 0.001	0.112
Retention	Main	< 0.001	< <i>0.01^C</i>	< 0.001	< 0.05	< 0.05	0.117
	Wk 8	-	-	-	-	-	-
	Wk 16	-	-	-	-	-	-
Phytate P							
Intake	Main	0.239		< 0.01	< 0.01	0.924	0.250
	Wk 8	0.452		0.154	< 0.001	0.399	< 0.05
	Wk 16	0.274		< 0.001	0.884	0.487	0.481
Excretion	Main	< 0.001		0.970	0.957	< 0.01	0.125
	Wk 8	< 0.001		0.075	0.681	< 0.05	0.486
	Wk 16	< 0.001		0.084	0.620	< 0.01	0.088
Retention	Main	< 0.001		< 0.05	< 0.05	< 0.001	< 0.05
	Wk 8	-		-	-	-	-
	Wk 16	-		-	-	-	-
Calcium							
Intake	Main	-		-	-	-	-
	Wk 8	0.760		< 0.01	0.090	0.949	0.238
	Wk 16	-		-	-	-	-
Retention	Main	0.586		< 0.001	0.957	0.646	0.358
	Wk 8	-		-	-	-	-
	Wk 16	-		-	-	-	-

^AAs a follow-up analysis, planned contrasts are performed when the main effect of diet or the effect of diet at each age is significant shown in Table S2a, to further identify the distinct effects of phytase supplementation and dietary NPP on the observations, given the fact that the diet effect involved two factors including phytase and NPP level. The *P* values of contrast analysis are provided.

^BSince there were three graded levels of dietary NPP with or without phytase supplementation, orthogonal polynomial contrasts were made separately to examine both linear and quadratic trends.

^CItalics are *P* values of planned contrasts made from the average of M+ and H+ vs. the average of L- and M-.