Comparison of Natuphos and Phyzyme as Phytase Sources for Commercial Layers Fed Corn-Soy Diet

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ABSTRACT The objective of this experiment was to compare the effects of 2 sources of phytase on performance of commercial Leghorns fed corn-soy diets. Seven diets were fed to Hy-line W-36 hens (n = 840; 8 replicates of 15 hens per treatment) from 21 to 33 wk of age. The treatments consisted of a control diet containing 0.38% nonphytate P (NPP) and a 2 × 3 factorial arrangement of 2 dietary NPP concentrations (0.11 and 0.26%) with 2 phytase sources [Natuphos (BASF, Mt. Olive, NJ) and Phyzyme (Danisco Animal Nutrition, Carol Stream, IL)] and without phytase. Dietary NPP had significant effects on feed intake, NPP intake, total P intake, egg production, egg weight, egg mass, egg specific gravity, and excreta P. The addition of Phyzyme or Natuphos significantly increased egg production and egg mass of hens fed the P-deficient diet (0.11% NPP) to levels that were similar to hens fed the control diet containing 0.38% NPP. Feed intake of hens fed the diets supplemented with Phyzyme or Natuphos was significantly less than that of hens fed the control diet containing 0.38% NPP. Phyzyme or Natuphos supplementation in the diets containing 0.11% NPP had significantly reduced excreta P of the control diet (approximately 58 and 54%, respectively) with no adverse effect on egg production and egg mass. There were no significant differences in feed intake, NPP intake, total P intake, egg production, egg weight, egg mass, feed conversion, egg specific gravity, mortality, BW, and excreta P between the diets supplemented with Natuphos and the diets supplemented with Phyzyme. In conclusion, Phyzyme had the same positive effects on performance of commercial Leghorns fed corn-soy diets as Natuphos.

Key words: hen, phytase, nonphytate phosphorus

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INTRODUCTION

Phosphorus is an essential mineral for laying hens in the formation of eggshell and metabolism (Frost and Roland, 1991; Summers, 1995; Usayran and Balnave, 1995, Sohail and Roland, 2002). Only 20 to 50% of plant-derived P is available to broilers, and the rest of P is in the form of phytate (myo-inositol hexaphosphate), which is poorly used by broilers (Ravindran et al., 1998). Ravindran et al. (1998) and Sebastian et al. (1998) reported that poultry cannot produce enough endogenous phytase to hydrolyze and release P from phytate. To meet dietary P requirement of laying hens, inorganic P such as dicalcium phosphate and monocalcium phosphate or exogenous phytase enzymes are commonly added to commercial corn-soy layer diets. However, inorganic P supplementation is not only expensive but also leads to environmental problems by over-supplementation. Excess P from the excreta of hens can easily add to the P loading of ground water, rivers, lakes, and oceans and can contribute to eutrophication of aquatic systems and stimulate algae growth, resulting in the mortality of aquatic animals (Ryden et al., 1973).

Many researchers have demonstrated that phytase supplementation [from 100 to 2,000 phytase units (**FTU**)/kg of feed] to diets containing 0.1% dietary nonphytate P (**NPP**) has positive effects on egg production, egg mass, egg weight, egg specific gravity, bone ash, and eggshell quality by improving P use (Van der Klis et al., 1996; Gordon and Roland, 1997, 1998; Boling et al., 2000a,b; Jalal and Scheideler, 2001; Roland et al., 2003; Keshavarz, 2003). Phytase supplementation decreased P excretion in manure and reduced the potential environmental problems (Jalal and Scheideler, 2001).

There are several commercial phytase products including Natuphos (BASF Corp., Mt. Olive, NJ) and Ronozyme (Roche Vitamins, Parsippany, NJ) in the market. Natuphos phytase originates from *Aspergillus niger* and is extensively used in the poultry industry. Recently, a new bacterial phytase, Phyzyme (Danisco Animal Nutrition, Carol Stream, NJ), which originates from the bacteria *Escherichia coli* and is produced by *Schizosacchromyces pombe*, has been introduced into market. Phytases from different sources may have different biochemical and biophysical properties such as pH activity profile and sensi-

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Table 1. Ingredient and nutrient content of the experimental diets

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Control diet
Corn, %	62.54	62.54	62.54	61.84	61.84	61.84	61.30
Soybean meal, %	25.66	25.66	25.66	25.72	25.72	25.72	25.76
$CaCO_3$, %	7.95	7.95	7.95	7.48	7.48	7.48	7.11
Hardshell,[1] %	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate, %	0.00	0.00	0.00	0.84	0.84	0.84	1.49
Poultry oil, %	0.88	0.88	0.88	1.15	1.15	1.15	1.36
Salt, %	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Vitamin premix,[2] %	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix,[3] %	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine, %	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Natuphos,[4] FTU/kg of feed		300			300		
Phyzyme,[5] FTU/kg of feed			300			300	
Calculated analysis							
CP, %	17.38	17.38	17.38	17.35	17.35	17.35	17.33
ME, kcal/kg	2,816	2,816	2,816	2,816	2,816	2,816	2,816
Ca, %	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Total P, %	0.32	0.32	0.32	0.47	0.47	0.47	0.59
Nonphytate P, %	0.11	0.11	0.11	0.26	0.26	0.26	0.38
Methionine + cystine, %	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Lysine, %	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Chemical analysis							
Ca, %	3.78	4.89	4.57	4.27	4.44	4.39	4.84
Total P, %	0.36	0.35	0.34	0.53	0.54	0.51	0.68

[1]Hardshell = large particle (passing US mesh #4 and retained by US mesh #6) $CaCO_3$ supplied by Franklin Industrial Minerals, Lowell, FL.

[2]Provided (/kg of diet): vitamin A (retinyl acetate), 8,000 IU; cholecalciferol, 2,200 ICU; vitamin E (DL-α-tocopheryl acetate), 8 IU; vitamin B_{12}, 0.02 mg; riboflavin, 5.5 mg; D-pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; vitamin B_1 (thiamin mononitrate), 1 mg; pyridoxine, 2.2 mg; D-biotin, 0.05 mg; and vitamin K (menadione sodium bisulfate complex), 2 mg.

[3]Provided (/kg of diet): manganese, 65 mg; iodine, 1 mg; iron, 55 mg; copper, 6 mg; zinc, 55 mg; and selenium, 0.3 mg.

[4]BASF Corp., Mount Olive, NJ.

[5]Danisco Animal Nutrition, Carol Stream, IL.

tiveness to pepsin, which can affect the in vivo bioefficacy of phytase.

Very little research has been conducted to evaluate the effect of the novel phytase Phyzyme on commercial Leghorns fed corn-soy diets. The objective of this experiment was to compare the effects of 2 sources of phytase (Phyzyme and Natuphos on performance of commercial Leghorns fed corn-soy diets from 21 to 33 wk of age.

MATERIALS AND METHODS

Seven diets were fed to Hy-line W-36 hens from 21 to 33 wk of age. The treatments consisted of a control diet containing 0.38% NPP and a 2 × 3 factorial arrangement of 2 dietary NPP concentrations (0.11 and 0.26%) with 2 phytase sources (Natuphos and Phyzyme) and without phytase (Table 1). The diets were mixed twice, and the feed samples of each mix were analyzed for Ca and P concentrations according to AOAC procedures (1984) by Experimental Station Chemical Laboratories, University of Missouri, Columbia.

One phytase unit (**FTU**) is defined as the amount of enzyme activity that liberates 1 mmol of inorganic P/min from a 0.5 m*M* Na-phytate solution at pH 5.5 and 37°C. Phytases from 2 different sources (Natuphos 600 and Phyzyme XP 5000G) were supplemented at 300 FTU/kg of feed in diets at 2 different P concentrations. The phytase contents of Natuphos and Phyzyme in the premixes and diets were analyzed by Danisco Animal Nutrition (Carol Stream, IL) to confirm enzyme activity.

Hy-line W-36 hens (n = 840) at 21 wk of age were randomly assigned into 7 treatments (8 replicates of 15 hens per treatment). Replicates were equally distributed into upper and lower cages to minimize cage level effect. Three hens were housed in a 40.6- × 45.7-cm^2 cage; 5 adjoining cages consisted of a replicate. All hens were housed in an environmentally controlled house with temperature maintained at approximately 25.6°C (21.1°C during the night and 28.9°C during the day). Pullets were moved into the house at 18 wk of age. Light was increased by 15 min/wk from 12 to 16 h/d. All hens were supplied with feed and water ad libitum. Egg production was recorded daily, egg weight and feed consumption were recorded weekly, and egg specific gravity was recorded biweekly. Egg weight and egg specific gravity were measured using all eggs produced during 2 consecutive days. Feed intake was determined by subtracting the ending feed weight of each trough (each replicate) from the beginning feed weight weekly. Egg specific gravity was determined using 11 gradient saline solutions varying in specific gravity from 1.060 to 1.100 at 0.005-unit increments (Holder and Bradford, 1979). At the middle and end of the experiment, excreta samples were obtained (3 replicates per treatment) by placing a pan under cages for 24 h. Excreta samples were then dried for 48 h at 100°C and were analyzed for P concentration according to AOAC

Table 2. Effects of dietary NPP and phytase on performance of Hy-line W-36 hens from 21 to 33 wk of age

	NPP (%)	Phytase[1]	Feed intake (g of feed/hen per day)	NPP intake[2] (mg/hen per day)	Total P intake[3] (mg/hen per day)	Egg production (%)	Egg weight (g)	Egg mass (g of egg/hen per day)
Diet 1	0.11	0	84.2[b]	93[d]	303[d]	78.1[b]	54.03	42.17[b]
Diet 2	0.11	Natuphos	92.0[a]	101[c]	322[c]	89.0[a]	53.77	47.86[a]
Diet 3	0.11	Phyzyme	91.0[a]	100[c]	309[d]	87.8[a]	54.12	47.51[a]
Diet 4	0.26	0	92.8[a]	241[a]	487[a]	88.7[a]	54.48	48.29[a]
Diet 5	0.26	Natuphos	90.4[a]	235[b]	483[a]	86.3[a]	54.27	46.88[a]
Diet 6	0.26	Phyzyme	92.5[a]	241[a]	472[b]	89.1[a]	54.74	48.76[a]
Main effect (Diets 1 to 6)								
NPP	0.11		89.1	98	312	84.9	53.97[b]	45.84
	0.26		91.9	239	481	88.0	54.49[a]	47.98
Phytase		0	88.5	167	395	83.4	54.25	45.23
		Natuphos	91.2	168	402	87.7	54.02	47.37
		Phyzyme	91.8	171	391	88.4	54.43	48.13
Control diet	0.38	0	94.2	358	641	87.5	54.58	47.77
Pooled SEM			0.58	1.19	3.61	1.09	0.26	0.63
					P			
NPP main effect			0.0006	0.0001	0.0001	0.0010	0.0154	0.0001
Phytase main effect			0.0025	0.0568	0.0072	0.0001	NS	0.0001
NPP × phytase			0.0001	0.0002	0.0056	0.0001	NS	0.0001
Control vs. diets with Natuphos			0.0069	0.0001	0.0001	NS	NS	NS
Control vs. diets with Phyzyme			0.0233	0.0001	0.0001	NS	NS	NS
Control vs. Diet 1			0.0001	0.0001	0.0001	0.0001	NS	0.0001
Control vs. Diet 4			NS	0.0001	0.0001	NS	NS	NS

[a-d]Means within a column with no common superscripts differ significantly.

[1]Both Natuphos (BASF, Mt. Olive, NJ) and Phyzyme (Danisco Animal Health, Carol Stream, IL) were supplemented at 300 FTU/kg of feed. One phytase unit (FTU) is defined as the amount of enzyme activity that liberates 1 mmol of inorganic P/min from a 0.5 m*M* Na-phytate solution at pH 5.5 and 37°C.

[2]NPP (nonphytase P) intake was based on calculated dietary NPP concentration.

[3]Toal P intake was based on chemical-analyzed dietary P concentration.

procedures (1984) by Experimental Station Chemical Laboratories, University of Missouri, Columbia. Mortality was determined daily, and egg production and feed consumption were adjusted to a hen-day basis. Body weight was obtained by randomly weighing 3 hens per group at the beginning and the end of the experiment. Mean value and standard deviation of the beginning BW and egg production was 1.41 ± 0.12 kg and 44.8 ± 9.4%, respectively.

Statistical analyses of data were performed by using the GLM procedure of SAS (SAS Inst., 2000). A 2 × 3 factorial arrangement of 2 dietary NPP concentrations (0.11 and 0.26%) with 2 phytase sources (Natuphos and Phyzyme) and without phytase was used to analyze the main effects of dietary NPP and phytase and their interactions (Diets 1 to 6). If differences in treatment means were detected by ANOVA, orthogonal contrasts were applied to separate means. Four preplanned additional nonorthogonal contrasts were also carried out to compare control diet and several specific diets. Statements of statistical significance are based on a probability of $P \leq 0.05$.

RESULTS AND DISCUSSION

A significant interaction between dietary NPP and phytase on feed intake was observed (Table 2). Dietary NPP had a significant effect on feed intake of hens fed the diets without phytase from wk 1 to the end of trial. As dietary NPP concentration increased from 0.11 to 0.26 in the diets without phytase, feed intake significantly increased from 84.21 to 92.08 g of feed/hen per day, resulting in a 9.35% increase of feed intake. The addition of phytase to the diets containing 0.11% NPP had a significant effect on feed intake, and the addition of phytase to the diets with 0.26% NPP had no effect on feed intake. The addition of phytase prevented the decline of feed intake of hens fed the P-deficient diets (0.11% NPP). These results are in agreement with those of Gordon and Roland (1997), Jalal and Scheideler (2001), and Roland et al. (2003), who reported that the addition of phytase to diets containing 0.1% NPP significantly increased feed intake. Feed intake of hens fed the diets supplemented with Natuphos was similar to that of hens fed the diets supplemented with Phyzyme at both NPP concentrations. Feed intake of hens fed the diets supplemented with Phyzyme or Natuphos was significantly lower than that of hens fed the control diet containing 0.38% NPP. There was no significant difference in feed intake between the control diet containing 0.38% NPP and the diet containing 0.26% NPP without phytase. Hens fed the diets containing 0.11% NPP had significantly lower feed intake than hens fed the control diet containing 0.38% NPP.

A significant interaction between dietary NPP and phytase was observed on NPP intake (Table 2). Dietary NPP concentration had a significant effect on NPP intake. When dietary NPP was 0.11%, NPP intake of hens ranged from 93 to 101 mg/hen per day, which was much lower than dietary NPP requirement of hens (250 mg/hen per

day) (NRC, 1994). Nonphytase P intake of hens fed the diets containing 0.26% NPP was around 240 mg/hen per day, which was close to NRC value of 250 mg/hen per day (NRC, 1994). A wide range of NPP, from 290 to 470 mg/hen per day, is used in the industry (Roland, 1994). The NRC recommended value of dietary NPP has declined from 350 mg/hen per day (NRC, 1984) to 250 mg/hen per day (NRC, 1994). Sohail and Roland (2002) reported that dietary NPP requirement of young laying hens for maximum performance ranged from 250 to 325 mg/hen per day, and a higher margin of safety for dietary P might be necessary. Dietary NPP intake in hens fed the control diet containing 0.38 NPP was 358 mg/hen per day, which was close to the NRC value of 350 mg/hen per day (NRC, 1984). Total P intake of hens fed the diets containing 0.11% NPP was significantly lower than that of hens fed diets containing 0.26% NPP and hens fed the control diet containing 0.38% NPP (Table 2).

A significant interaction between dietary NPP and phytase on egg production was observed (Table 2). Egg production significantly increased when dietary NPP of the diets without phytase increased from 0.11 to 0.26%. Phytase supplementation in diets containing 0.11% NPP significantly improved egg production from wk 3 to the end of trial; phytase supplementation in diets containing 0.26% NPP had no effect on egg production. The different egg production response to phytase supplementation can be attributed to the fact that 0.26% NPP concentration in the diets or 241 mg of NPP intake/hen per day has fulfilled the requirement of laying hens, and 0.11% NPP concentration in the diets or 93 mg of NPP intake/hen per day was not enough for laying hens. There was no significant difference in egg production between Natuphos and Phyzyme at both dietary NPP concentrations. Egg production of hens fed the diets supplemented with Natuphos or Phyzyme was similar to that of hens fed the control diet containing 0.38% NPP. These results are consistent to those of Gordon and Roland (1997, 1998), Jalal and Scheideler (2001), and Roland et al. (2003), who reported that supplementing diets containing 0.1% NPP with phytase significantly increased egg production to the level of hens fed adequate P diets. Egg production of hens fed the control diet containing 0.38% NPP was similar to that of hens fed the diet containing 0.26% NPP without phytase. Hens fed the control diet containing 0.38% NPP had significantly higher egg production than hens fed the P-deficient diet (0.11% NPP).

Phytase supplementation had no significant effect on egg weight (Table 2). Egg weight in hens fed the diets supplemented with Natuphos was similar to that in hens fed the diets with Phyzyme. Dietary NPP had a significant effect on egg weight. Egg weight significantly increased when dietary NPP increased from 0.11 to 0.26%. There were no significant differences in egg weight between the control diet containing 0.38% NPP and the diets supplemented with Natuphos or Phyzyme.

A significant interaction between dietary NPP and phytase on egg mass was observed (Table 2). As dietary NPP increased from 0.11 to 0.26% in the diets without phytase, egg mass significantly increased from 42.17 to 48.29 g of egg/hen per day, resulting in a 14.51% increase of egg mass. Phytase supplementation significantly increased egg mass in the diets containing 0.11% NPP, but had no effect on egg mass in the diets containing 0.26% NPP. Egg mass in hens fed the diets supplemented with Natuphos was similar to that in hens fed the diets with Phyzyme. There were no significant differences between the control diet containing 0.38% NPP and the diets supplemented with Phyzyme or Natuphos. Similarly, Jalal and Scheideler (2001) reported that phytase supplementation significantly increased egg mass of hens fed the 0.10% NPP diet. Egg mass of hens fed the control diet was significantly higher than that of hens fed the P-deficient diet without phytase (0.11% NPP), but was similar to that of hens fed the diet containing 0.26% NPP without phytase.

Dietary NPP had no significant effect on feed conversion (Table 3). Phytase effect on feed conversion was approaching significance ($P < 0.08$). There was no significant difference in feed conversion between Natuphos and Phyzyme. Feed conversion of hens fed the diets supplemented with Phyzyme was significantly lower than that of hens fed the control diet containing 0.38% NPP.

Although feed intake of hens fed the diets supplemented with Phyzyme or Natuphos was significantly less than that of hens fed the control diet, there were no significant differences in egg mass and egg production between the control diet containing 0.38% NPP and the diets supplemented with Phyzyme or Natuphos. Also, feed conversion of hens fed the diets supplemented with Phyzyme was significantly lower than that of hens fed the control diet. Similarly, Roland et al. (2003) reported that even though less feed was consumed, there was no difference in egg production between P-adequate diets and the P-deficient diets supplemented with phytase. Therefore, phytase supplementation might have improved not only P availability but also the availabilities of some other nutrients, such as energy and amino acids. This conclusion was supported by those of Namkung and Lesson (1999), who reported that phytase supplementation improved AME and digestibilities for some amino acids such as Val and Ile in broilers.

Dietary NPP had a significant effect on egg specific gravity (Table 3). As dietary NPP concentration increased from 0.11 to 0.26%, egg specific gravity significantly decreased. Phytase had no significant effect on egg specific gravity. Egg specific gravity in hens fed the diets supplemented with Natuphos was similar to that in hens fed the diets supplemented with Phyzyme. Egg specific gravity of hens fed the diets supplemented with Phyzyme was significantly higher than that of hens fed the control diet. Both dietary NPP and phytase had no effect on final BW and mortality (Table 3).

Dietary NPP had significant effect on excreta P content (Table 3). When NPP concentration decreased from 0.38 to 0.11%, a 58 or 54% reduction in excreta P was obtained by supplementing the diets containing 0.11% NPP with Phyzyme or Natuphos, respectively. These results are in

Table 3. Effects of dietary NPP (nonphytase P) and phytase on performance of Hy-line W-36 hens from 21 to 33 wk of age

	NPP (%)	Phytase[1]	Feed conversion (g of feed/g of egg)	Egg specific gravity (unit)	Mortality (%)	Final BW (kg)	P content in excreta (%)
Diet 1	0.11	0	2.00	1.0884[a]	0.00	1.44	0.94[b]
Diet 2	0.11	Natuphos	1.92	1.0872[b]	0.83	1.47	0.98[b]
Diet 3	0.11	Phyzyme	1.92	1.0876[ab]	0.00	1.48	0.88[b]
Diet 4	0.26	0	1.92	1.0868[b]	0.00	1.52	1.63[a]
Diet 5	0.26	Natuphos	1.93	1.0866[b]	1.67	1.45	1.62[a]
Diet 6	0.26	Phyzyme	1.90	1.0867[b]	0.83	1.54	1.67[a]
Main effect (Diets 1 to 6)							
NPP	0.11		1.95	1.0877[a]	0.28	1.46	0.93[b]
	0.26		1.92	1.0867[b]	0.83	1.50	1.64[a]
Phytase		0	1.96	1.0876	0.00	1.48	1.28
		Natuphos	1.93	1.0869	1.25	1.46	1.30
		Phyzyme	1.91	1.0871	0.42	1.51	1.28
Control diet	0.38	0	1.97	1.0862	0.83	1.48	2.11
Pooled SEM			0.015	0.0004	0.59	0.02	0.03
					P		
NPP main effect			NS	0.0001	NS	NS	0.0001
Phytase main effect			0.0769	0.0578	NS	NS	NS
NPP × phytase			NS	NS	NS	NS	NS
Control vs. diets with Natuphos			NS	0.0729	NS	NS	0.0001
Control vs. diets with Phyzyme			0.0269	0.0144	NS	NS	0.0001
Control vs. Diet 1			NS	0.0001	NS	NS	0.0001
Control vs. Diet 4			NS	NS	NS	NS	0.0001

[a,b]Means within a column with no common superscripts differ significantly.

[1]Both Natuphos (BASF, Mt. Olive, NJ) and Phyzyme (Danisco Animal Health, Carol Stream, IL) were supplemented at 300 FTU/kg of feed. One phytase unit (FTU) is defined as the amount of enzyme activity that liberates 1 mmol of inorganic P/min from a 0.5 m*M* Na-phytate solution at pH 5.5 and 37°C.

agreement with those of Boling et al. (2000a), who reported that phytase supplementation decreased excreta P concentration approximately 50%. Phytase supplementation can greatly reduce potential P environmental pollution problems caused by inorganic P in feed.

In conclusion, the addition of Phyzyme or Natuphos significantly increased egg production and egg mass of hens fed the P-deficient diet (0.11% NPP) to levels that were similar to hens fed the control diet containing 0.38% NPP. Feed intake of hens fed the diets supplemented with Phyzyme or Natuphos was significantly less than that of hens fed the control diet containing 0.38% NPP. Feed conversion and egg specific gravity of hens fed the diets supplemented with Phyzyme were significantly better than those of hens fed the control diet. Phyzyme or Natuphos supplementation in the diets containing 0.11% NPP had significantly reduced excreta P of the control diet (approximately 58 and 54%, respectively) with no adverse effect on egg production and egg mass. There were no significant differences in feed intake, NPP intake, total P intake, egg production, egg weight, egg mass, feed conversion, egg specific gravity, mortality, BW, and excreta P between the diets supplemented with Natuphos and the diets supplemented with Phyzyme. Phyzyme had the same positive effects on performance of commercial Leghorns fed corn-soy diets as Natuphos.

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REFERENCES

AOAC. 1984. Official Methods of Analysis. 14th ed. Assoc. Offic. Anal. Chem., Gaithersburg, MD.

Boling, S. D., M. W. Douglas, M. L. Johnson, X. Wang, C. M. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. 2000a. The effect of dietary available phosphorus and phytase on performance of young and old laying hens. Poult. Sci. 79:224–230.

Boling, S. D., M. W. Douglas, R. B. Shirley, C. M. Parsons, and K. W. Keolkebeck. 2000b. The effect of various dietary concentration of phytase and available phosphorus on performance of laying hens. Poult. Sci. 79:535–538.

Frost, T. J., and D. A. Roland, Sr. 1991. The influence of various calcium and phosphorus concentrations on tibia strength and eggshell quality of pullets during peak production. Poult. Sci. 70:963–969.

Gordon, R. W., and D. A. Roland, Sr. 1997. Performance of commercial laying hens fed various phosphorus concentrations with and without supplemental phytase. Poult. Sci. 76:1172–1177.

Gordon, R. W., and D. A. Roland, Sr. 1998. Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. Poult. Sci. 77:290–294.

Holder, D. P., and M. V. Bradford. 1979. Relationship of specific gravity of chicken eggs to number of cracked eggs and percent shell. Poult. Sci. 58:250–251.

Jalal, M. A., and S. E. Scheideler. 2001. Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. Poult. Sci. 80:1463–1471.

Keshavarz, K. 2003. The effect of different concentrations of nonphytate phosphorus with and without phytase on the performance of four strains of laying hens. Poult. Sci. 82:71–91.

Namkung, H., and S. Lesson. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy

and the ileal digestibility of nitrogen and amino acids in broiler chicks. Poult. Sci. 78:1317–1319.
National Research Council. 1984. Nutrient Requirements of Poultry. 8th rev. ed. Natl. Acad. Press, Washington, DC.
National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
Ravindran, V., W. L. Bryden, S. Cabahug, and P. H. Selle. 1998. Impact of microbial phytase on the digestibility of protein, amino acids and energy in broilers. Pages 156–165 in Proc. Maryland Nutr. Conf. Feed Manufact., Baltimore. College of Agric. Life Sci., Univ. Maryland, College Park.
Roland, D. A., Sr. 1994. The Egg Producers' Guide to Optimum Calcium and Phosphorus Nutrition. Mallinckrodt Feed Ingredients, Mundelein, IL.
Roland, D. A., Sr., H. A. Ahmad, S. S. Yadalam, and T. Sefton. 2003. Effect of nongenetically modified phytase supplementation on commercial Leghorns. J. Appl. Poult. Res. 12:257–263.
Ryden, J. C., J. K. Syers, and R. F. Harris. 1973. Pages 1–44 in Advances in Agronomy. Vol. 25. Acad. Press, New York, NY.
SAS Institute. 2000. SAS/STAT User's Guide. SAS Inst., Inc., Cary, NC.
Sebastian, S., S. P. Touchburn, and E. R. Chavez. 1998. Implication of phytic acid and supplemental microbial phytase in poultry nutrition: A review. World's Poult. Sci. J. 54:27–47.
Sohail, S. S., and D. A. Roland, Sr. 2002. Influence of dietary phosphorus on performance of Hy-line W36 hens. Poult. Sci. 81:75–83.
Summers, J. D. 1995. Reduced dietary phosphorus concentrations for layers. Poult. Sci. 74:1977–1983.
Usayran, N., and D. Balnave. 1995. Phosphorus requirements of laying hens fed on wheat based diets. Br. Poult. Sci. 36:285–301.
Van der Klis, J. D., H. A. J. Versteegh, and P. C. M. Simons. 1996. Effectiveness of natuphos phytase in improving the bioavailabilities of phosphorus and other nutrients for layers. Pages 295–304 in Phytase in Animal Nutrition and Waster Management. M. Coelho and E. T. Kornegay, ed. BASF Corporation, Mt. Olive, NJ.