RUNNING TITLE: Phyzyme® Effect

TITLE: Influence of Phyzyme® on Commercial Leghorns Fed Corn-soy diets

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SPECIFIC SECTION: METABOLISM AND NUTRITION

Abstract The objective of this experiment was to determine the influence of Phyzyme® on performance and profits of commercial Leghorns fed con-soy diets. Seven diets were fed to Hy-line W-36 hens (n = 840, 8 replicates of 15 hens per treatment) during Phase 1 (from wk 21 to 33). The treatments consisted of a control diet containing 0.38 nonphytate phosphorus (NPP) and a 2 × 3 factorial arrangement with two dietary NPP levels (0.11 and 0.26%) and three phytase sources (no phytase, Natuphos®, Phyzyme®). Results showed that dietary NPP levels had significant effects on egg production, egg mss, egg weight, egg specific gravity, and NPP intake. The addition of Phyzyme® significantly increased egg production and egg mass of hens fed the deficient-phosphorus diet (0.11% NPP) to levels that were similar to hens fed the control diet (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 and the diets supplemented with Phyzyme® or Natuphos®. 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 supplementation had no effect on egg weight, egg specific gravity, body weight, and mortality. There were no significant differences in egg production, feed intake, egg weight, egg mass, feed conversion, egg specific gravity, mortality, and body weight between the diets supplemented with Natuphos® and the diets supplemented with

Phyzyme®. In conclusion, the addition of Phyzyme® had positive influences on performance and profits of commercial Leghorns fed corn-soy diets.

Key words: Hens, phytase, Phyzyme®

INTRODUCTION

Phosphorus is an essential mineral for laying hens in formation of egg shell and metabolism (Frost and Roland, 1991; Summers, 1995; Usayran and Balnave, 1995, Sohail and Roland, 2002). Only 20%-50% of plant 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. 1995). Ravindran et al. (1998) and Sebatian et al. (1998) reported that poultry can not produce enough amounts of endogenous phytase to hydrolyze and release P from phytate. To meet dietary P requirement of laying hens, inorganic P (Dicalcium phosphate) or exogenous phytase enzymes are commonly added to commercial corn-soy layer diets. However, Dicalcium phosphate supplementation is not only expensive but also leads to environmental problems by over-supplementation. Excess P from the feces of hens is easy to access ground water, rivers, lakes, and oceans, and can lead to mortality of aquatic animals by stimulating algae growth (Ryden et al., 1973).

Considerable research has demonstrated that phytase supplementation (from 100 to 300 phytase unit (FTU)/kg of feed) to diets containing 0.1% dietary nonphytate phosphorus (NPP) has positive effects on egg production, egg mass, egg weight, egg specific gravity, bone ash, and eggshell quality by improving P utilization (Gordon and Roland, 1997, 1998; Boiling et al., 2000a, b; Jalal and Scheideler, 2001; and Roland et al., 2003; Keshavarz, 2003). Phytase supplementation decreased P excretion in the maure and reduced the potential environmental problems (Jalal and Scheideler, 2001).

There are several commercial phytase products including Natuphos®[1] and

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

Ronozyme®[2] in the market. Natuphos® is an extensively used phytase (fungal phytase), which originated from Aspergillus niger. Recently a new bacterial phytase Phyzyme®[3], which originated from the yeast Schizosacchromyces pombe, has been introduced into market. Phytases from different sources may have different biochemical and biophysical properties such as pH-activity profile and sensitiveness to pepsin, which can affect the effect of phytase in varying conditions.

Very little research has been conducted to evaluate the effect of this novel phytase Phyzyme® on commercial Leghorns fed corn-soy diets. The objective of this experiment was to determine the influence of Phyzyme® on performance and profits of commercial Leghorns fed con-soy diets during Phase 1.

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[2] Roche Vitamins, Parsippany, NJ.
[3] Danisco Animal Nutrition, Carol Stream, IL.

MATERIAL AND METHODS

Seven diets were fed to Hy-line W-36 hens during Phase 1 (from wk 21 to 33). The treatments consisted of a control diet containing 0.38 nonphytate phosphorus (NPP) and a 2 × 3 factorial arrangement with two dietary NPP levels (0.11 and 0.26%) and three phytase sources (no phytase, Natuphos®, Phyzyme®) (Table1). The feed samples were sent for nutrient analysis[4].

Hy-line W-36 hens (n = 840) at 21 weeks of age were randomly divided into 7 treatments (8 replicates of 15 hens per treatment). Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. Three hens were housed in a 40.6 cm × 45.7 cm cage and five adjoining cages consisted of a group. 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). The house had controlled ventilation and lighting (16 hr/day). 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 bi-weekly. Egg weight and egg specific gravity were measured using all eggs produced during two consecutive days. Egg specific gravity was determined using 11 gradient saline solutions varying in specific gravity from 1.060 to 1.100 incremented with 0.005-unit increments [15]. At the middle and end of the experiment, excreta samples were collected for P determination (three replicates per treatment). Excreta samples were then dried and sent for P determination[5]. Mortality was determined daily and egg production and feed consumption were adjusted to a hen-day basis. Body weight was obtained by weighing 3 hens per group at the

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[4, 5] Danisco Animal Nutrition, Carol Stream, IL

beginning and the end of the experiment. Egg mass, feed conversion (g feed/g egg), and dietary NPP intake were calculated from egg production, egg weight, feed consumption, and NPP contents in the diets.

Statistical analyses of data were performed by using the general linear models (GLM) procedure in SAS/STAT [16]. A 2 × 3 factorial design with two NPP levels (0.11 and 0.26%) and three phytase sources (no phytase, Natuphos®, Phyzyme®) was used to analyze the main effects of dietary NPP levels and phytase and interaction between them (Diet 1 to 6). If interaction between dietary NPP levels and phytase 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

Feed consumption

There was a significant interaction between dietary NPP levels and phytase on feed intake (Table 2). Dietary NPP levels had a significant effect on feed intake of hens fed the diets containing 0.11% NPP without phytase from week 1 to the end of trial. As dietary NPP level increased from 0.11 to 0.26 in the diets without phytase, overall feed intake significantly increased from 84.21 to 92.08 g feed/h/d, resulting in a 9.35% increase of feed intake. The addition of phytase to the diets containing 0.11% NPP level had a significant effect on feed intake, while the addition of phytase to the diets with 0.26% NPP level had no effect on feed intake. The addition of phytase prevented the decline of feed intake of hens fed the phosphorus-deficient diets (0.11% NPP). These results are in agreement with those of Cordon 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. There was no significant difference in feed intake between Natuphos® and Phyzyme® at both NPP levels. Feed intake of hens fed the diets supplemented with Phyzyme® or Natuphos® was significantly lower than that of hens fed the control diet. There were no significant difference in feed intake between the control diet and the diet containing 0.26% NPP. Hens fed the diets containing 0.11% NPP had significant lower feed intake than hens fed the control diet.

Egg production

There was a significant interaction between dietary NPP levels and phytase (Table 3). Egg production was significantly increased when NPP level 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 week 3 to the end of trial, while phytase supplementation in diets containing 0.26 NPP had no effect on egg production. There was no significant difference in egg production between Natuphos® and Phyzyme® at both dietary NPP levels. Egg production of hens fed the diets supplemented with Natuphos® or Phyzyme® was similar to that of fed the control diet. These results are consistent to those of Cordon 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-phosphorus diets. There was no significant difference in egg production between the control diet and the diet containing 0.26% NPP without phytase. Hens fed the control diet had significant lower egg production than hens fed the phosphorus-deficient diet.

Egg weight

There was no interaction between dietary NPP levels and phytase on egg weight (Table 4). There was no significant phytase effect on egg weight. Dietary NPP had significant effect on egg weight. Egg weight was significantly increased when dietary NPP level increased from 0.11% to 0.26%. There were no significant differences in egg weight between the control diet and the diets supplemented with phytase.

Egg mass

There was a significant interaction between dietary NPP levels and phytase on egg mass (Table 5). As dietary NPP level increased from 0.11 to 0.26 in the diets without phytase, overall feed intake significantly increased from 42.17 to 48.29 g egg/h/d, 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 difference between the control diet and the diets supplemented with Phyzyme® or Natuphos®. Similarly, Jalal and Scheideler (2001) reported that phytase supplementation significantly increased egg mass of hens fed 0.10% NPP diet. Egg mass of hens fed the control diet was significant higher than hens fed the phosphorus-deficient diet without phytase, but was similar to hens fed the diet containing 0.26% NPP.

Feed conversion

There was no interaction between dietary NPP levels and phytase on feed conversion (Table 6). Dietary NPP levels had no significant effect on overall feed conversion. Phytase effect on feed conversion was approaching significant ($P < 0.0769$). Phytase supplementation significantly improved feed conversion of hens fed the diet containing 0.11 NPP from week 9 to week 12. 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.

Egg specific gravity, NPP intake, beginning body weight, final body weight, and mortality

There was no interaction between dietary NPP levels and phytase on egg specific gravity (Table 7). Dietary NPP levels had a significant effect on egg specific gravity. As dietary NPP level increased from 0.11% to 0.26%, egg specific gravity significantly decreased. Phytase had no significant effect on egg specific gravity. Egg specific gravity of hens fed the diets supplemented with Phyzyme® was significantly higher than that of hens fed the control diet. There was a significant interaction between dietary NPP levels and phytase (Table 8). Dietary NPP levels had a significant effect on NPP intake. When dietary NPP was 0.11%, NPP intake of hens ranged from 93 to 101 mg/h/d, which was much lower than 250 mg/h/d (NRC, 1994). NPP intake of hens fed the diets containing 0.26% NPP was around 240 mg/h/d, which was close to NRC recommendation value (NRC, 1994). Phytase supplementation had a significant effect on NPP intake of hens fed the diets containing 0.11% NPP, but had no effect on NPP intake of hens fed the diets containing 0.26% NPP. NPP intake in hens fed the control diet was 358 mg/h/d, which was close to NRC recommendation value (NRC, 1984). There were no significant interactions between dietary NPP levels and phytase on beginning and final body weight and mortality (Table 8). Both NPP levels and phytase had no effect on beginning and final body weight and mortality. Mean values of beginning body weight, final body weight, and mortality were 1.41 kg, 1.48 kg and 0.60% respectively.

Economics

Economic feeding and management program developed by Roland et al. (1998, 2000) was used to calculate profits of phytase supplementation. Hens fed the diet containing 0.11% NPP without phytase had less profit than hens fed other diets (Table 9). Phyzyme® supplementation to the diet containing 0.26% NPP had the best profit among seven diets. The addition of Phyzyme® at two dietary NPP levels (0.11% and 0.26%) had a positive influence on profits. Hens fed the diets supplemented with Phyzyme® had better profits than hens fed the control diet. Because feed ingredient prices and egg price often change, the economics of each treatment may change. In this experiment, only inclusion rate at 300 phytase unit (FTU)/kg feed was used to test Phyzyme® effect on performance and profits of laying hens. If higher inclusion rates of Phyzyme® have more positive effects on performance of laying hens, more profits may be obtained. More research needs to be conducted to evaluate dose effect of Phyzyme® on profits in commercial Leghorns.

In this experiment, when dietary NPP level was reduced to 0.11%, significant decline in egg production, feed intake and egg mass were observed. The addition of Phyzyme® to the diet containing 0.11% NPP significantly improved egg production and egg mass. 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 and the diets supplemented with Phyzyme® or Natuphos®. Also, feed conversion of hens fed the diets supplemented with Phyzyme® was significantly better than that of hens fed the control diet. Similarly, Roland et al. (2003) reported that even though less feed was consumed, no difference in egg production between adequate-phosphorus diets and the deficient-phosphorus diets with phytase. Therefore, phytase® supplementation might have improved not only phosphorus 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 digetibilities for some amino acids such as Val and Ile in broilers.

In conclusion, the addition of Phyzyme® significantly increased egg production and egg mass of hens fed the deficient-phosphorus diet (0.11% NPP) to levels that were similar to hens fed the control diet (0.38% NPP). Hens fed the diets supplemented with Phyzyme® had better feed conversion and egg specific gravity than hens fed the control diet. There were no significant differences in egg production, feed intake, egg weight, egg mass, feed conversion, egg specific gravity, mortality, and body weight between the diets supplemented with Natuphos® and the diets supplemented with Phyzyme®. The addition of Phyzyme® had positive influences on performance and profits of commercial Leghorns fed corn-soy diets.

ACKNOWLEGMENTS

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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
NaCl (%)	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 feed)		300			300		
Phyzyme®[5] (FTU/kg feed)			300			300	
Calculated analysis							
Crude protein (%)	17.38	17.38	17.38	17.35	17.35	17.35	17.33
ME (kcal/kg)	2816	2816	2816	2816	2816	2816	2816
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
Metionine+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
Experimental analysis (%)							

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

[2]Provided per kilogram of diet: vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200 ICU; vitamin E (as $_{DL}$-α-tocopheryl acetate), 8 IU; vitamin B_{12}, 0.02 mg; riboflavin, 5.5 mg; $_{D}$-calcium 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; vitamin K (menadione sodium bisulfate complex), 2 mg.

[3]Provided per kilogram of diet: manganese, 65 mg; iodine, 1 mg; ferrous carbonate, 55 mg; copper oxide, 6 mg; zinc oxide, 55 mg; sodium selenium, 0.3 mg.

[4*]Danisco Animal Nutrition, Carol Stream, IL, FTU/kg feed = phytase units/kg feed.

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

TABLE 2. Effects of dietary NPP level and phytase on feed intake (g feed/h/d)

	NPP (%)	Phytase	1-2 wk	3-4 wk	5-6 wk	7-8 wk	9-10 wk	11-12 wk	Overall
Diet 1	0.11	0	65.26^{b}	79.80^{b}	85.60	88.42^{b}	92.19^{b}	93.99^{b}	84.21^{b}
Diet 2	0.11	Natuphos	73.78^{a}	87.67^{a}	95.27	96.26^{a}	99.30^{a}	99.83^{a}	92.02^{a}
Diet 3	0.11	Phyzyme	71.63^{a}	86.53^{a}	93.58	96.39^{a}	98.65^{a}	98.99^{a}	90.96^{a}
Diet 4	0.26	0	74.46^{a}	89.25^{a}	95.83	96.98^{a}	99.33^{a}	100.97^{a}	92.80^{a}
Diet 5	0.26	Natuphos	73.11^{a}	86.44^{a}	92.96	94.49^{a}	96.88^{ab}	98.41^{a}	90.38^{a}
Diet 6	0.26	Phyzyme	74.02^{a}	87.98^{a}	96.26	97.01^{a}	99.29^{a}	100.69^{a}	92.54^{a}
Main effect (Diet 1 to 6)									
NPP level	0.11		70.22^{b}	84.67^{b}	91.48^{b}	93.69^{b}	96.72^{b}	97.60^{b}	89.06^{b}
	0.26		73.86^{a}	87.89^{a}	95.01^{a}	96.16^{a}	98.50^{a}	100.02^{a}	91.91^{a}
Phytase		0	69.88^{b}	84.53^{b}	90.71^{b}	92.70^{b}	95.76^{b}	97.48^{b}	88.51^{b}
		Natuphos	73.45^{a}	87.05^{a}	94.11^{a}	95.37^{a}	98.09^{a}	99.12^{a}	91.20^{a}
		Phyzyme	72.82^{a}	87.26^{a}	94.92^{a}	96.70^{a}	98.97^{a}	99.84^{a}	91.75^{a}
Control diet	0.38	0	76.12	89.57	96.96	100.08	100.47	102.07	94.21
Pooled SEM			0.65	0.67	0.73	0.91	0.82	0.66	0.58
			----------------------Probability----------------------						
NPP main effect			0.0002	0.0007	0.0011	0.0242	0.0926	0.0086	0.0006
Phytase main effect			0.0029	0.0197	0.0032	0.0135	0.0471	0.0819	0.0025
NPP × Phytase			0.0002	0.0001	0.0001	0.0014	0.0035	0.0020	0.0001
Control vs. Diets with Phyzyme			0.0079	0.0595	0.1253	0.0432	0.3027	0.0659	0.0233
Control vs. Diets with Natuphos			0.0269	0.0419	0.0365	0.0068	0.1093	0.0180	0.0069
Control vs. Diet 1			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Control vs. Diet 4			0.2156	0.8149	0.4507	0.1014	0.4926	0.4182	0.2384

$^{a-b}$ Means within a column with no common superscripts differ significantly

TABLE 3. Effects of dietary NPP level and phytase on egg production (%)

	NPP (%)	Phytase	1-2 wk	3-4 wk	5-6 wk	7-8 wk	9-10 wk	11-12 wk	Overall
Diet 1	0.11	0	56.96	86.96^{b}	82.56^{b}	81.55^{b}	81.31^{b}	78.93^{b}	78.05^{b}
Diet 2	0.11	Natuphos	61.25	93.60^{a}	93.07^{a}	95.71^{a}	95.92^{a}	88.98^{a}	88.98^{a}
Diet 3	0.11	Phyzyme	62.02	92.38^{a}	91.49^{a}	94.35^{a}	94.23^{a}	87.79^{a}	87.79^{a}
Diet 4	0.26	0	60.95	92.62^{a}	94.88^{a}	94.70^{a}	93.99^{a}	88.67^{a}	88.67^{a}
Diet 5	0.26	Natuphos	57.50	91.43^{ab}	90.03^{a}	93.01^{a}	93.10^{a}	86.38^{a}	86.38^{a}
Diet 6	0.26	Phyzyme	57.92	93.69^{a}	96.73^{a}	96.25^{a}	94.97^{a}	89.06^{a}	89.06^{a}
Main effect (Diet 1 to 6)									
NPP level	0.11		60.08	90.98	89.04^{b}	90.54^{b}	90.49^{b}	88.51^{b}	84.94^{b}
	0.26		58.79	92.58	93.88^{a}	94.65^{a}	94.02^{a}	94.30^{a}	88.04^{a}
Phytase		0	58.96	89.79^{b}	88.72^{b}	88.13^{b}	87.65^{b}	86.90^{b}	83.36^{b}
		Natuphos	59.37	92.52^{a}	91.55^{ab}	94.36^{a}	94.51^{a}	93.78^{a}	87.68^{a}
		Phyzyme	59.97	93.04^{a}	94.11^{a}	95.30^{a}	94.60^{a}	95.53^{a}	88.42^{a}
Control diet	0.38	0	61.58	92.26	91.67	93.21	93.90	92.53	87.52
Pooled SEM			3.09	1.22	1.67	1.53	1.46	1.26	1.09
			----------------	----------------	----------------	--------Probability--------	----------------	----------------	----------------
NPP main effect			0.5911	0.0979	0.0013	0.0009	0.0063	0.0001	0.0010
Phytase main effect			0.9409	0.0164	0.0124	0.0001	0.0001	0.0001	0.0001
NPP × Phytase			0.3027	0.0062	0.0003	0.0001	0.0001	0.0001	0.0001
Control vs. Diets with Phyzyme			0.6733	0.6067	0.2380	0.2723	0.6981	0.5205	0.5048
Control vs. Diets with Natuphos			0.5633	0.8659	0.9543	0.5446	0.7352	0.4207	0.9047
Control vs. Diet 1			0.2963	0.0034	0.0003	0.0001	0.0001	0.0001	0.0001
Control vs. Diet 4			0.8870	0.8363	0.1793	0.4958	0.9664	0.1921	0.4615

$^{a-b}$ Means within a column with no common superscripts differ significantly

TABLE 4. Effects of dietary NPP level and phytase on egg weight (g)

	NPP (%)	Phytase	1-2 wk	3-4 wk	5-6 wk	7-8 wk	9-10 wk	11-12 wk	Overall
Diet 1	0.11	0	48.37	51.07	53.70	55.88	56.74	58.41	54.03
Diet 2	0.11	Natuphos	48.71	51.08	53.35	55.25	56.44	57.78	53.77
Diet 3	0.11	Phyzyme	48.35	51.20	53.95	55.69	56.86	58.69	54.12
Diet 4	0.26	0	48.89	51.75	54.41	56.09	57.39	58.32	54.48
Diet 5	0.26	Natuphos	48.77	51.57	54.08	55.81	56.92	58.45	54.27
Diet 6	0.26	Phyzyme	49.12	51.89	54.44	56.16	57.29	59.55	54.74
Main effect (Diet 1 to 6)									
NPP level	0.11		48.48^{b}	51.11^{b}	53.36^{b}	55.61	56.68	58.30	53.97^{b}
	0.26		48.93^{a}	51.74^{a}	54.31^{a}	56.02	57.20	58.77	54.49^{a}
Phytase		0	48.63	51.41	54.05	55.99	57.06	58.37	54.25
		Natuphos	48.74	51.32	53.71	55.53	56.88	58.12	54.02
		Phyzyme	48.73	51.55	54.19	55.93	57.07	59.12	54.43
Control diet	0.38	0	49.13	51.72	54.28	56.14	57.30	58.95	54.58
Pooled SEM			0.25	0.27	0.29	0.34	0.33	0.48	0.26
		--Probability--							
NPP main effect			0.0316	0.0041	0.0080	0.1439	0.0507	0.2544	0.0154
Phytase main effect			0.8849	0.6706	0.2243	0.3540	0.2544	0.1308	0.2676
NPP × Phytase			0.3556	0.9021	0.8962	0.8608	0.1308	0.6126	0.9411
Control vs. Diets with Phyzyme			0.2101	0.6087	0.8170	0.6203	0.5722	0.7649	0.6388
Control vs. Diets with Natuphos			0.2165	0.2361	0.1173	0.1551	0.1254	0.1673	0.0823
Control vs. Diet 1			0.0389	0.0929	0.1656	0.6038	0.2253	0.4388	0.1328
Control vs. Diet 4			0.5173	0.9214	0.7499	0.9217	0.8510	0.3631	0.7696

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

TABLE 5. Effect of dietary NPP level and phytase on egg mass (g egg/h/d)

	NPP (%)	Phytase	1-2 wk	3-4 wk	5-6 wk	7-8 wk	9-10 wk	11-12 wk	Overall
Diet 1	0.11	0	27.54	44.40^{b}	44.34^{b}	45.58^{b}	46.12^{b}	46.13^{b}	42.17^{b}
Diet 2	0.11	Natuphos	29.84	47.82^{a}	49.68^{a}	52.90^{a}	54.16^{a}	54.53^{a}	47.86^{a}
Diet 3	0.11	Phyzyme	29.99	47.29^{a}	49.36^{a}	52.53^{a}	53.57^{a}	54.12^{a}	47.51^{a}
Diet 4	0.26	0	29.83	47.93^{a}	51.62^{a}	53.12^{a}	53.93^{a}	55.32^{a}	48.29^{a}
Diet 5	0.26	Natuphos	28.04	47.15^{a}	48.66^{a}	51.92^{a}	53.00^{a}	54.49^{a}	46.88^{a}
Diet 6	0.26	Phyzyme	28.47	48.63^{a}	52.66^{a}	54.06^{a}	54.40^{a}	56.44^{a}	48.76^{a}
Main effect (Diet 1 to 6)									
NPP level	0.11		29.12	46.50^{b}	47.79^{b}	50.34^{b}	51.28^{b}	51.59^{b}	45.84^{b}
	0.26		28.78	47.90^{a}	50.98^{a}	53.03^{a}	53.78^{a}	55.42^{a}	47.98^{a}
Phytase		0	28.68	46.17^{b}	47.98^{b}	49.35^{b}	50.02^{b}	50.73^{b}	45.23^{b}
		Natuphos	28.94	47.48^{a}	49.17^{a}	52.41^{a}	53.58^{a}	54.51^{a}	47.37^{a}
		Phyzyme	29.23	47.96^{a}	51.01^{a}	53.29^{a}	53.99^{a}	55.28^{a}	48.13^{a}
Control diet	0.38	0	30.23	47.71	49.76	52.36	53.80	54.55	47.77
Pooled SEM			1.52	0.66	0.98	0.97	0.87	0.87	0.63
			--Probability--						
NPP main effect			0.7723	0.0093	0.0003	0.0005	0.0015	0.0001	0.0001
Phytase main effect			0.9310	0.0189	0.0146	0.0001	0.0001	0.0001	0.0001
NPP × Phytase			0.2951	0.0070	0.0007	0.0001	0.0001	0.0001	0.0001
Control vs. Diets with Phyzyme			0.5921	0.7618	0.3036	0.4398	0.8640	0.4972	0.6443
Control vs. Diets with Natuphos			0.4904	0.7794	0.6231	0.9709	0.8368	0.9702	0.5982
Control vs. Diet 1			0.2166	0.0009	0.0003	0.0001	0.0001	0.0001	0.0001
Control vs. Diet 4			0.8513	0.8155	0.1845	0.5840	0.9191	0.5346	0.5620

$^{a\text{-}b}$ Means within a column with no common superscripts differ significantly

TABLE 6. Effects of dietary NPP level and phytase on feed conversion (g feed/g egg).

	NPP (%)	Phytase	1-2 wk	3-4 wk	5-6 wk	7-8 wk	9-10 wk	11-12 wk	Overall
Diet 1	0.11	0	2.38	1.80	1.93	1.95	2.01^{a}	2.04^{a}	2.00
Diet 2	0.11	Natuphos	2.53	1.84	1.92	1.82	1.84^{b}	1.83^{b}	1.92
Diet 3	0.11	Phyzyme	2.39	1.83	1.90	1.84	1.84^{b}	1.83^{b}	1.92
Diet 4	0.26	0	2.52	1.86	1.86	1.83	1.84^{b}	1.83^{b}	1.92
Diet 5	0.26	Natuphos	2.61	1.83	1.91	1.82	1.83^{b}	1.81^{b}	1.93
Diet 6	0.26	Phyzyme	2.61	1.81	1.83	1.80	1.83^{b}	1.79^{b}	1.90
Main effect (Diet 1 to 6)									
NPP level	0.11		2.43	1.82	1.92	1.87^{b}	1.89^{b}	1.90^{b}	1.95
	0.26		2.58	1.83	1.87	1.82^{a}	1.83^{a}	1.81^{a}	1.92
Phytase		0	2.45	1.83	190	1.89^{b}	1.92^{b}	1.93^{b}	1.96
		Natuphos	2.57	1.83	1.91	1.82^{a}	1.83^{a}	1.82^{a}	1.93
		Phyzyme	2.50	1.82	1.86	1.82^{a}	1.83^{a}	1.81^{a}	1.91
Control diet	0.38	0	2.58	1.88	1.95	1.92	1.87	1.87	1.97
Pooled SEM			0.105	0.017	0.024	0.025	0.023	0.020	0.015
			-------------------	-------------------	-------------------	Probability	-------------------	-------------------	-------------------
NPP main effect			0.1802	0.4969	0.0602	0.0383	0.0386	0.0010	0.1371
Phytase main effect			0.6446	0.8606	0.2732	0.0383	0.0225	0.0007	0.0769
NPP × Phytase			0.8633	0.2276	0.4938	0.1184	0.0510	0.0077	0.1939
Control vs. Diets with Phyzyme			0.6803	0.0592	0.0434	0.0302	0.3980	0.0793	0.0269
Control vs. Diets with Natuphos			0.9730	0.1391	0.3832	0.0384	0.3980	0.1421	0.1174
Control vs. Diet 1			0.3592	0.0233	0.7134	0.5904	0.0070	0.0005	0.3841
Control vs. Diet 4			0.7786	0.5709	0.0624	0.0881	0.5634	0.2562	0.1396

$^{a-b}$ Means within a column with no common superscripts differ significantly

TABLE 7. Effects of dietary NPP level and phytase on egg specific gravity (unit)

	NPP (%)	Phytase	1-2 wk	3-4 wk	5-6 wk	7-8 wk	9-10 wk	11-12 wk	Overall
Diet 1	0.11	0	1.0900	1.0907^{a}	1.0900^{a}	1.0868^{a}	1.0857^{a}	1.0874^{a}	1.0884^{a}
Diet 2	0.11	Natuphos	1.0890	1.0900^{ab}	1.0885^{ab}	1.0858^{ab}	1.0836^{b}	1.0860^{ab}	1.0872^{b}
Diet 3	0.11	Phyzyme	1.0900	1.0903^{ab}	1.0893^{ab}	1.0857^{ab}	1.0841^{ab}	1.0863^{ab}	1.0876^{ab}
Diet 4	0.26	0	1.0891	1.0893^{ab}	1.0885^{ab}	1.0855^{ab}	1.0834^{b}	1.0850^{b}	1.0868^{b}
Diet 5	0.26	Natuphos	1.0892	1.0894^{ab}	1.0882^{b}	1.0848^{b}	1.0831^{b}	1.0851^{b}	1.0866^{b}
Diet 6	0.26	Phyzyme	1.0896	1.0892^{b}	1.0879^{b}	1.0853^{ab}	1.0829^{b}	1.0850^{b}	1.0867^{b}
Main effect (Diet 1 to 6)									
NPP level	0.11		1.0897	1.0903^{a}	1.0893^{a}	1.0861^{a}	1.0845^{a}	1.0866^{a}	1.0877^{a}
	0.26		1.0893	1.0893^{b}	1.0882^{b}	1.0852^{b}	1.0832^{b}	1.0850^{b}	1.0867^{b}
Phytase		0	1.0896	1.0900	1.0892	1.0862	1.0845^{a}	1.0862	1.0876
		Natuphos	1.0891	1.0897	1.0884	1.0853	1.0834^{b}	1.0855	1.0869
		Phyzyme	1.0898	1.0897	1.0886	1.0855	1.0835^{b}	1.0857	1.0871
Control diet	0.38	0	1.0890	1.0883	1.0876	1.0849	1.0828	1.0849	1.0862
Pooled SEM			0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
			---------------------	---------------------	----------	Probability	----------	---------------------	---------------------
NPP main effect			0.2833	0.0004	0.0025	0.0094	0.0009	0.0001	0.0001
Phytase main effect			0.2178	0.6663	0.1012	0.1185	0.0270	0.2804	0.0578
NPP × Phytase			0.4818	0.5049	0.3090	0.5651	0.1384	0.2046	0.2000
Control vs. Diets with Phyzyme			0.0893	0.0018	0.0451	0.2018	0.1991	0.1060	0.0144
Control vs. Diets with Natuphos			0.7722	0.0029	0.1313	0.3881	0.3151	0.1709	0.0729
Control vs. Diet 1			0.0728	0.0001	0.0002	0.0017	0.0001	0.0001	0.0001
Control vs. Diet 4			0.7497	0.0651	0.1229	0.2772	0.3672	0.8419	0.1817

$^{a\text{-}b}$ Means within a column with no common superscripts differ significantly

TABLE 8. Effects of dietary NPP level and phytase NPP intake, P content in excreta, mortality, and final body weight

	NPP (%)	Phytase	NPP intake (mg/h/d)	P content in excreta (%)	Mortality (%)	Final body weight (kg)
Diet 1	0.11	0	93[d]		0.00	1.44
Diet 2	0.11	Natuphos	101[c]		0.83	1.47
Diet 3	0.11	Phyzyme	100[c]		0.00	1.48
Diet 4	0.26	0	241[a]		0.00	1.52
Diet 5	0.26	Natuphos	235[b]		1.67	1.45
Diet 6	0.26	Phyzyme	241[ab]		0.83	1.54
Main effect (Diet 1 to 6)						
NPP level	0.11		98[b]		0.28	1.46
	0.26		239[a]		0.83	1.50
Phytase		0	167		0.00	1.48
		Natuphos	168		1.25	1.46
		Phyzyme	171		0.42	1.51
Control diet	0.38	0	358		0.83	1.48
Pooled SEM						
		----------	----------	Probability	----------	----------
NPP main effect			0.0001		0.4247	0.0851
Phytase main effect			0.0568		0.3338	0.1630
NPP × Phytase			0.0002		0.8476	0.1121
Control vs. Diets with Phyzyme			0.0001		0.6866	0.3397
Control vs. Diets with Natuphos			0.0001		0.6875	0.4965
Control vs. Diet 1			0.0001		0.4876	0.2720
Control vs. Diet 4			0.0001		0.4876	0.3022

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

TABLE 9. Influence of phytase on profits

Treatment	NPP (%)	Phytase	Cost of feed[1] ($/ton)	Profit returns[2] (cents/dozen)
Diet 1	0.11	0	175.05	2.8
Diet 2	0.11	Natuphos	175.51	3.2
Diet 3	0.11	Phyzyme	175.51	3.9
Diet 4	0.26	0	177.00	3.9
Diet 5	0.26	Natuphos	177.44	3.7
Diet 6	0.26	Phyzyme	177.44	4.6
Control diet	0.38	0	178.50	3.2

[1] Corn price = $5.52/100lb, soy price = $18.06/100lb, $CaCO_3$ = $1.30/100lb, hard shell = $1.60/100lb, Dicalcium phosphate = $245/ton, poultry oil = $10.00/100lb, salt = $2.88/100lb, Vitamin premix = $102.00/100lb, mineral premix = $28.00/100lb, DL-methionine = $110.18/lb, Phyzyme XP = $0.44/ton of feed, Natuphos = $0.44/ton feed.

[2]The egg price spread between medium and large eggs was 11 cents, profit returns (R) were calculated using the equation: R = UBEP - NR -PC - FdC, where UBEP = Urner Barry Egg Price, NR = nest run into package product delivered, PC = production cost, and FdC = feed cost, as described by Roland et al. (1998, 2000).