A Demonstration of Postpellet Application of Dry Phytase to Broiler Diets

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Primary Audience: Nutritionists, Production Managers, Feed Mill Managers, Researchers

Summary

A comparison of performance and P reduction in litter and manure from broilers fed rations with and without phytase enzyme was made with chickens reared on litter or in cages. Low-activity phytase supplemented as a dry powder to mash diets did not affect performance, but litter P accumulation was decreased 14 to 21% in the finisher phase. Manure P content of broilers in cages was reduced 14 to 19% during the starter and grower phases. Liquid phytase applied postpelleting to diets with 0.5% total P (0.3% available P) reduced litter P accumulation (14 to 19%) and reduced manure P content in starter, grower, and finisher phases of the study. Phosphorus content in raw manure was decreased by 55% during the finisher phase. Feed conversions of broilers in cages and on conventional litter-covered floors were improved significantly with postpellet application of liquid phytase. Feed conversion improvement was greater in birds in cages. Postpellet application of dry phytase was shown to be feasible based upon performance and reduction in litter P accumulation. Feed conversions were improved by 7 to 8 points ($P < 0.05$) when compared to those of birds given a normal P level (0.72% total P) diet without phytase. Litter P accumulation was reduced between 20.5 and 28.5% with dry phytase applied postpelleting. With liquid phytase applied postpelleting, litter P accumulation was reduced 26.6%. These reductions in litter P content represent about 4 lb of P per ton of litter.

Key words: broiler, phytase, postpellet, application, phosphorus, litter, manure
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Description of Problem

On a regional and national level, disposal of manure and litter from poultry production facilities is a critical issue because the total P (tP) being produced in some production areas exceeds the amount that can be used by crops and other plants. Phosphorus and N in animal manure often are applied to land, and excessive levels can be subject to leaching into and polluting ground and surface water. If manure is applied to course-textured, permeable soils, such as that found in the coastal plains, rapid water

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filtration allows for vertical and subsurface movement of P (principally inorganic and to a lesser degree organic P [1]) and N into ground and surface water systems [2]. Concerns raised by some environmental activist groups about potential over-application of nutrients have led to considerable debate about the appropriate application of manure and litter. The Water Quality Improvement Act of 1998 and the Nutrient Management Practices Act of 1998 in the State of Maryland mandate that all feeds for monogastric animals must be supplemented with a phytase enzyme or other additives that reduce P in animal wastes. These laws require that this supplementation be done to the maximum extent that is commercially and biologically feasible. Other states may follow this precedent.

Poultry manure P can be significantly reduced by reducing dietary inorganic P and supplementing the diets with a microbial phytase [3, 4, 5]. Significant reductions in caged layer manure P content has been reported to be between 16 and 38%, but these manure P reductions cannot be achieved without reductions in the concentration of inorganic P (P_i) that is normally supplemented to the diet [3, 4, 5, 6, 7, 8, 9]. Similarly, decreased P excretion in phytase-supplemented market turkeys has been reported [10]. Fecal P reductions from laying hens, market turkeys, broilers and swine have been reported when feeds were supplemented with phytase enzymes [3, 11, 12, 13, 14].

There is no question about the efficacy of phytase use in feeds for poultry and swine. Nevertheless, due to cost and application difficulties, there is resistance from the animal industries to use this technology. Several reasons prevail, but they most often focus on the cost of using postpelleting, liquid phytase application and the cost of liquid phytase. If the feasibility of using postpellet application of dry phytase could be demonstrated, poultry growers could benefit from reduced cost of enzyme, the positive effects of phytase usage on performance, and decreased P levels in manure and litter, thereby allowing them to spread more litter per acre of land. Thus, the objective of this investigation was to compare the efficacy of postpellet liquid phytase application and postpellet dry phytase application in broiler chickens.

Materials and Methods

ANIMAL WELFARE

This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee, which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

ANIMALS AND HUSBANDRY

Four separate experiments were conducted. In each experiment, Arbor Acres × Arbor Acres sex-linked, slow-feathering, high-yielding males were used. After the hatchling chicks had been sexed, chicks were assigned randomly to groups of 40 for weighing and placement into each pen. The ambient temperature at placement was 32°C. At 7-d intervals after placement, ambient temperature was reduced by 4°C until a final minimum ambient temperature of 24°C was reached and maintained until the end of the experiment (42 d). Upper limits on the ambient temperature were not set but were allowed to fluctuate with the daily maximum. The photoperiod at placement was set at 24 h for the first 5 d, and thereafter, the photoperiod was set at 23 h light:1 h darkness. Feed and water were provided ad libitum.

HOUSING

In Experiments 1 and 2, a 2 × 4 factorially arranged, completely randomized experimental design was used to test the effects of phytase on male broiler chickens reared in replicate pens in conventional litter-covered floor pens and replicate pens in cage rows in the Broilermatic cage-rearing system [15, 16]. There were 40 chickens in each of the 64 pens (e.g., 8 rows of 8 cages with 320 birds per cage row) in the Broilermatic cage-rearing system, and 40 chickens in each of the 32 pens in the conventional broiler house. With this design, a total of 3,840 broiler chickens was used. A total of 1,280 chickens was housed in the conventional house and 2,560 were assigned to the Broilermatic cage-rearing system.

Experiments 3 and 4 were conducted to address the use of postpelleting dry phytase vs. liquid phytase application. This phase of the investigation was conducted in the conventional

TABLE 1. Composition of North Carolina Agricultural Research Service basal broiler diets

	INGREDIENTS AS A PERCENTAGE OF DIET COMPOSITION		
INGREDIENT	Starter	Grower	Finisher
Corn	59.08	68.61	76.62
Soybean meal (48%)	26.95	19.30	15.38
Limestone	0.70	0.70	0.40
Dicalcium phosphate (22% Ca, 18.7% P)[A]	0.70	0.80	
Poultry fat	3.49	1.80	
Poultry meal	7.98	8.00	6.79
DL-Methionine	0.18	0.04	
Lysine-HCl	0.07	0.09	0.05
NaCl	0.40	0.20	0.30
Choline chloride	0.20	0.20	0.20
Minerals[B] (TM-90)	0.20	0.20	0.20
Vitamins[C] (NCSU-90)	0.05	0.05	0.05
Selenium premix (0.02% Se)	0.04	0.04	0.04

[A]Basal diets were modified to provide 0.5, 0.4, or 0.3% available inorganic P (P_i) in the control diet by manipulating the amount of dicalcium phosphate in the diets for the phytase studies. The Ca:available P_i was also modified to yield 2:1 in the diets in the phytase studies.
[B]Trace mineral (TM-90) premix provided in milligrams per kilogram of diet: manganese, 120; zinc, 120; iron, 80; copper, 10; iodine, 2.5; cobalt, 1.0. Selenium premix as sodium selenite was provided to each diet at a level to assure a concentration of 0.15 ppm.
[C]Vitamin premix (NCSU-90) provided per kilogram of diet: vitamin A, 6,600 IU; cholecalciferol, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 μg; riboflavin, 6.6 mg; niacin, 55 mg; pantothenic acid, 11 mg; vitamin K, 2 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; biotin, 126 mg.

housing environment described above. As previously described, after chicks had been sexed, females were assigned randomly and were weighed in groups of 40 birds. They were then placed in one of 36 floor pens containing five inches of blended fresh and used litter. A total of 1,440 chicks was used in each of Experiments 3 and 4. Feed and water were provided ad libitum, and ambient temperatures were maintained as described above.

DIETS

The broilers were given North Carolina Agricultural Research Service diets (Table 1) on the following schedule: starter diet: 3,177 kcal ME/kg, 22.5% crude protein (available to 18 d of age); grower diet: 3,168 kcal ME/kg, 19.5% crude protein (18 to 35 d of age); and finisher diet: 3,160 kcal ME/kg, 17.4% crude protein (35 to 42 d of age). In Experiments 1 and 2, there were four dietary treatments for each of the starter, grower, and finisher diets, consisting of (1) negative control, (2) phytase control, (3) phytase test 1 with a 0.1% reduction in tP, and (4) phytase test 2 with 0.2% reduction in tP. These treatments are described in Table 2. In Experiment 3 and Experiment 4, there also were four dietary treatments for each of the starter, grower, and finisher diets as described in Table 3. The dietary treatments consisted of the following: starter, grower, and finisher treatments (1) control, (2) negative control (6 lb corn oil per 1,000 lb applied postpelleting and 0.2% decrease in tP), (3) phytase and 0.2% decrease in tP, and (4) phytase and 0.2% decrease in tP + oil spray. The finisher diets in all four experiments had no supplemental P, but phytase was retained in the phytase treatment groups. The recommended [17] dietary Ca and available P (aP) for broiler starter, grower, and finisher diets are 1.0% Ca and 0.45% aP, 0.9% Ca and 0.35% aP, and 0.8% Ca and 0.30 aP, respectively. Broiler starter, grower, and finisher diets were analyzed by the North Carolina Department of Agriculture's Food and Drug Protection Division Forage Testing Laboratory in Raleigh, NC, and the Ca and tP concentrations are shown in Tables 2 and 3. Basal diets without supplemental P contained roughly 0.21% tP.

PHYTASE

Allzyme phytase [18] is an enzyme preparation derived from a solid-state fermentation process with *Aspergillus niger*, and in addition to

TABLE 2. Calculated dietary Ca, total P, available P, and analyzed phytase distribution of treatments among Experiments 1 (dry phytase mixed into mash) and 2 (liquid phytase sprayed onto pellets)

	EXPERIMENT 1 FEEDS			EXPERIMENT 2 FEEDS		
TREATMENT	Starter	Grower	Finisher	Starter	Grower	Finisher
Negative control						
Ca, %	1.00	1.00	0.46	1.00	1.00	0.46
Total P, %	0.72	0.72	0.41	0.72	0.72	0.41
Available P, %	0.52	0.52	0.21	0.52	0.52	0.21
Phytase, PTU[A]/kg	0	0	0	0	0	0
Phytase control						
Ca, %	1.00	1.00	0.46	1.00	1.00	0.46
Total P, %	0.72	0.72	0.41	0.72	0.72	0.41
Available P, %	0.52	0.52	0.21	0.52	0.52	0.21
Phytase, PTU/kg	1,240	1,240	1,240	10,478	10,478	10,478
Phytase test 1						
Ca, %	0.80	0.80	0.46	0.80	0.80	0.46
Total P, %	0.62	0.62	0.41	0.62	0.62	0.41
Available P, %	0.42	0.42	0.21	0.42	0.42	0.21
Phytase, PTU/kg	1,240	1,240	1,240	10,478	10,478	10,478
Phytase test 2						
Ca, %	0.60	0.60	0.46	0.60	0.60	0.46
Total P, %	0.52	0.52	0.41	0.52	0.52	0.41
Available P, %	0.32	0.32	0.21	0.32	0.32	0.21
Phytase, PTU/kg	1,240	1,240	1,240	10,478	10,478	10,478

[A]PTU = phytase units.

the phytase enzyme, there are several additional side-enzyme activities that include cellulase, protease, xylanase, and acid phosphatase [19]. In Experiment 1, phytase was dry-blended into Diets 2, 3 and 4, and activity was determined by chemical analysis to be 1,240 phytase units (PTU)/kg, an activity that is roughly 10.7% the recommended activity, in all three diets. In Experiment 2, due to susceptibility of the enzyme to degrade at the temperatures necessary for pelleting (82°C), it was applied postpelleting as a liquid onto Diets 2, 3 and 4, and activity was determined by chemical analysis to be 10,478 PTU/kg, an activity that is roughly 91.1% the recommended activity, in all three diets. In Experiments 3 and 4, postpelleting dry phytase application was accomplished as follows: diets were pelleted at 82 C and were run through the cooling tower. The pellets were then returned to a horizontal ribbon blender where one operator dusted the dry phytase over the blending pellets, and a second operator sprayed corn oil over the pellets. Complete blending for postpelleting dry phytase application required approximately 1 min. The pellets were then moved to the dispensing bin where the feed was bagged in 40-lb lots. During the bagging process, a total of 20 different 1-lb samples were collected randomly and were sealed in plastic bags until analyzed for phytase activity by biochemists at Alltech Biotechnology Center [18]. Phytase activity was determined chemically to be 11,500 PTU/kg.

ASSESSMENT OF DIETARY PHYTASE INFLUENCE ON PERFORMANCE

The influences of dietary phytase on serum concentration of P_i and total Ca, weight gain, feed conversion, livability, and manure and litter P were determined in each of the four experiments. Phosphorus was determined on the basis of pounds per ton in the litter and on the basis of grams tP per kilogram of dry matter intake in manure in Experiment 1 and 2. In Experiments 3 and 4, accumulation of P was determined on the basis of pounds per ton of litter. Litter samples were collected prior to placement of birds in each of the experiments so that a baseline litter P level could be determined. Blood sampling for determination of serum P_i [20] and total Ca [21] was done at 21 and 42 d of age; fecal P was determined weekly on fresh feces collected from belts under the cages in the Broilermatic cage-

TABLE 3. Calculated dietary Ca, total P, Available P, and analyzed phytase distribution of treatments among Experiments 3 and 4 (dry phytase applied post pelleting)

	EXPERIMENT 3 FEEDS			EXPERIMENT 4 FEEDS		
TREATMENTS	Starter	Grower	Finisher	Starter	Grower	Finisher
Control						
Ca, %	1.00	1.00	0.46	1.00	1.00	0.46
Total P, %	0.72	0.72	0.41	0.72	0.72	0.41
Available P, %	0.52	0.52	0.21	0.52	0.52	0.21
Phytase, PTU[A]/kg	0	0	0	0	0	0
Oil spray, %	0	0	0	0	0	0
Negative control						
Calcium, %	0.60	0.60	0.46	0.60	0.60	0.46
Total P, %	0.52	0.52	0.41	0.52	0.52	0.41
Available P, %	0.32	0.32	0.21	0.32	0.32	0.21
Phytase, PTU/kg	0	0	0	0	0	0
Oil spray, %	0.6	0.6	0.6	0.6	0.6	0.6
Phytase Test 1						
Ca, %	0.60	0.60	0.46	0.60	0.60	0.46
Total P, %	0.52	0.52	0.41	0.52	0.52	0.41
Available P, %	0.32	0.32	0.21	0.32	0.32	0.21
Phytase, PTU/kg	11,500	11,500	11,500	11,500	11,500	11,500
Oil spray, %	0	0	0	0	0	0
Phytase Test 2						
Ca, %	0.60	0.60	0.46	0.60	0.60	0.46
Total P, %	0.52	0.52	0.41	0.52	0.52	0.41
Available P, %	0.32	0.32	0.21	0.32	0.32	0.21
Phytase, PTU/kg	11,500	11,500	11,500	11,500	11,500	11,500
Oil spray, %	0.6	0.6	0.6	0.6	0.6	0.6

[A]PTU = phytase units.

rearing system and in litter that was collected from 10 random sites within each sampled pen in the conventional house. An AOAC-certified commercial laboratory [22] was used to analyze the litter and manure for P by colorimetric analysis.

Analyses of variance were conducted for each parameter, and the level of significance was set at $P \leq 0.05$, depending upon the F values generated by the GLM procedure. When differences among treatments were found, the means were separated by least-significant difference procedures of SAS software [23]. Percentage data were converted to arc sins and analyzed with the GLM procedure of SAS software [23].

Results and Discussion

PHYTASE AND PERFORMANCE

Mixed results have been observed with regard to BW response of broilers fed phytase. In Experiment 1 (a late summer study with dry phytase in mash feed) and Experiment 2 (a winter study with liquid phytase sprayed onto post-pelleted feed), phytase supplementation had minimal effects on 42-d BW of broilers grown in cages or in conventional housing (Table 4). However, there was a seasonal effect with winter-grown broilers having a heavier BW. In Experiment 2, there was a housing effect on BW with broilers in the conventional litter-covered floor pens showing a slight advantage. In young broiler chickens, a phytase product, different from that used in this study, was associated with improved digestibility of amino acids [24]. Kornegay [25] did not find improved BW in broilers fed a finisher diet with phytase from 3 to 7 wk of age. However, McKnight [26] summarized the work of several scientific studies and reported that the use of phytase reversed the negative impact of reducing dietary P_i supplementation, which appeared to be the condition in this study as well. Qian et al. [27] reported that broilers fed very low levels of dietary P had depressed

TABLE 4. Influence of phytase applied dry in broiler mash (experiment 1) or as a liquid to postpelleted broiler feeds (Experiment 2) on production parameters of chickens reared in conventional litter-covered floor pens and cage environments (Broilermatic cage system)

		BODY WEIGHT[B] (kg)		FCR		MORTALITY (%)	
HOUSING[A]	DIET	Exp. 1[C]	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Broilermatic	(1) 0.5% P	2.09[a]	2.28[b]	1.73[b]	1.82[ab]	2.66[a]	5.32[ab]
Cage	(2) 0.5% P + phytase	2.01[ab]	2.25[b]	1.81[b]	1.84[ab]	2.81[a]	5.79[ab]
	(3) 0.4% P + phytase	1.98[ab]	2.31[b]	1.74[b]	1.81[ab]	2.03[a]	4.07[b]
	(4) 0.3% P + phytase	1.97[ab]	2.34[ab]	1.78[b]	1.72[c]	2.03[a]	2.04[c]
Conventional	(1) 0.5% P	2.00[ab]	2.40[a]	2.06[a]	1.86[a]	2.19[a]	7.82[a]
Litter floor	(2) 0.5% P + phytase	1.96[ab]	2.34[ab]	2.05[a]	1.87[a]	1.56[a]	5.94[ab]
	(3) 0.4% P + phytase	1.92[b]	2.40[a]	2.04[a]	1.84[ab]	2.10[a]	9.07[a]
	(4) 0.3% P + phytase	2.01[ab]	2.41[a]	2.01[a]	1.80[b]	2.10[a]	6.57[ab]
	SEM	0.06	0.04	0.02	0.02	0.73	1.02

[a–c]Within a column, means with unlike superscripts differ significantly ($P < 0.05$).
[A]n = 640 birds per treatment in the Broilermatic cage system and 320 birds per treatment in the conventional house.
[B]Body weights were based on average pen weights for each treatment group.
[C]Exp. = experiment; FCR = feed conversion ratio.

weight gain, and even with an excess activity of supplemental phytase, weight gain was not equivalent to controls with NRC-recommended P levels [17] in their diets. However, this reduced weight gain, even with phytase, was related to the ratio of dietary Ca to total aP in the diet. Calcium:tP (Ca:tP) ratios greater than 1.5 were associated with reduced weight gain [27].

In Experiments 1 and 2, the Ca:tP ratios for Diets 1, 2, 3, and 4 were 1.43, 1.43, 1.33, and 1.20, respectively, for starter and grower feeds, but in the finisher feed, the Ca:tP ratios were all 1.12. Nevertheless, broilers in the conventional house demonstrated slight reductions in BW when they were given 0.5 or 0.4% aP diets with supplemental phytase, but broilers given a 0.3% aP diet with supplemental phytase had 6-wk BW that was slightly greater than controls given the 0.5% aP diet without supplemental phytase. In the conventional house, it was possible that the broilers were engaging in coprophagy and were obtaining additional nutrients that were unavailable to the birds in the cage rearing facility.

In Experiment 1, feed conversion ratios (FCR) within rearing environment were not affected significantly by phytase supplementation to the diets, but in Experiment 2, in both housing environments, phytase supplementation to diets with lower P_i concentrations resulted in improved FCR. However, there was a clear trend in cages and conventional houses that showed an improving FCR with addition of phytase in all treatment groups with the best FCR in groups with the lowest levels of available P_i. In Experiment 1, which was a summer study with tunnel ventilation in the cage house but not in the conventional house, the overall FCR in the cage facility was improved by 28 points in comparison to the conventional house. In Experiment 2, which was a winter study with relatively even temperatures and ventilation in both facilities, the overall FCR in the cage facility was improved by only 4 points in comparison to the conventional house. In this study, the improved FCR in broilers given Allzyme phytase was consistent with observations made when broilers were given another phytase enzyme, Natuphos [2, 23]. In Experiment 1, there were no differences among treatment mortality rates. Yet in Experiment 2, in the cage facility, there was a tendency for phytase supplementation to reduce mortality, which resulted in an environment × phytase interaction ($P < 0.05$).

In Experiments 3 and 4, dry phytase was applied postpelleting. The data shown in Table 5 indicate that this method of phytase application to feed had no adverse effect on BW. Furthermore, it appeared that the use of an oil spray to facilitate adherence of the dry phytase to the pellets was not necessary, if BW was used as a criterion of efficacy. Nevertheless, reduction of tP from 0.72 to 0.52% without phytase supplementation resulted in birds with smaller body weights (significant in Experiment 4), which was

TABLE 5. Influence of dry phytase applied to postpelleted broiler feeds (Experiments 3 and 4) on production parameters of broiler chickens reared in conventional litter-covered floor pens

	BODY WEIGHT[A] (kg)		FCR		MORTALITY (%)	
TREATMENT	Exp. 3[B]	Exp. 4	Exp. 3	Exp. 4	Exp. 3	Exp. 4
0.72% tP, no oil spray, no phytase	2.18[a]	2.20[a]	1.86[b]	1.86[b]	2.24[a]	4.17[a]
0.52% tP, oil spray, no phytase	2.13[a]	2.15[b]	1.91[a]	1.89[a]	1.39[a]	1.96[a]
0.52% tP, no oil spray, phytase	2.18[a]	2.19[ab]	1.82[c]	1.84[bc]	3.61[a]	2.50[a]
0.52% tP, oil spray, phytase	2.17[a]	2.22[a]	1.83[c]	1.82[c]	1.96[a]	1.94[a]
SEM	0.03	0.02	0.01	0.01	1.42	0.82

[a–c]Within a column, means with unlike superscripts differ significantly ($P < 0.05$).
[A]Body weights are based on average pen weights for each treatment group.
[B]Exp. = experiment; FCR = feed conversion ratio; tP = total P.

consistent with observations made earlier [27]. Yet on a practical basis, an average of a 0.1-lb depression in BW of birds given a low-aP diet is highly significant, as it would require approximately one additional day for growout to the same body weight. The postpellet application of dry phytase to diets containing 0.52% tP (0.3% aP) corrected this problem.

Reduction of tP in the diets used in these experiments without supplementation of phytase resulted in a significant increase in FCR (Table 5). However, FCR for those diets with reduced tP and with dry phytase applied postpelleting were improved significantly compared to the control (0.72% tP, no oil spray, no phytase diet and 0.52% tP, no oil spray diet). These results were consistent with the results from Experiment 2, in which liquid phytase was applied postpelleting and with observations made by other scientists working with phytase [4, 26].

Mortality was not influenced by the phytase treatment. Therefore, if BW and FCR are to be used as criteria for efficacy of dry phytase applied to postpelleted feed, one must conclude that this method of application is effective in overcoming the negative effects of simply reducing tP in diets as a means to reduce fecal P.

PHYTASE INFLUENCE ON SERUM CA AND P_i CONCENTRATIONS

Serum concentrations of P_i and total Ca are presented in Table 6. Phytase and lower dietary levels of aP did not cause a decrease in serum P_i levels at either 21 or 42 d of age. To the contrary, there was evidence at 42 d of age in the lowest aP treatment that phytase supplementation had actually elevated serum P_i in both Experiment 1 and 2 (Table 6). Serum total Ca concentrations were also not affected by phytase supplementation in the feed in which the Ca levels were decreased along with P_i. The Ca:aP ratios were maintained at 2:1 for each of the experimental diets in this study, but serum Ca:P_i ratios at 21 d of age ranged between 1.02 and 1.26 in Experiment 1 and between 1.16 and 1.44 in Experiment 2. This finding was not a departure from ratios normally reported [28] even though values for serum Ca and P_i were elevated at 21 d of age in this study. At 42 d of age, the serum Ca and P_i concentrations were lower than those found at 21 d of age, and this finding might have been a reflection of lower dietary levels of aP and Ca in the finisher diets. The higher serum Ca and P_i concentrations associated with the phytase treatment of the finisher diets is an important observation. There was a very low dietary aP level in the basal finisher formulation, and the provision of phytase in the diet apparently catalyzed the hydrolysis of phytate P, resulting in increased P for absorption and less for elimination in feces. Serum Ca:P_i ratios at 42 d of age were higher than at 21 d of age and ranged between 1.04 and 1.70 in Experiment 1 and between 1.41 and 1.86 in Experiment 2, suggesting that there was freeing of these minerals from phytate in the feed.

The data in Table 7 indicate that postpelleting application of dry phytase had no adverse effects on serum P_i and Ca concentrations. At 21 d of age in Experiments 3 and 4, serum P_i in phytase-treated groups were not different from the control diet (0.72% tP). In Experiment 4, the 0.52% tP group was found to have significantly lower serum P_i concentrations than the

TABLE 6. Influence of phytase applied dry in broiler mash (Experiment 1; Exp. 1) or as a liquid to postpelleted broiler feeds (Experiment 2; Exp. 2) on serum inorganic phosphorus (P_i) and total Ca concentrations in chickens reared in conventional litter-covered floor pens and cage environments (Broilermatic cage system)

		SERUM CONCENTRATION (mg/dL)							
		3 Wk				6 Wk			
		P_i		Ca		P_i		Ca	
HOUSING[A]	DIET[B]	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Cage	0.5% aP	8.3^{b}	7.6^{bc}	9.0^{c}	9.2^{bc}	5.3^{b}	5.5^{b}	5.5^{d}	8.4^{c}
	0.5% aP + phytase	7.7^{c}	7.8^{bc}	9.7^{bc}	11.2^{a}	6.2^{a}	5.8^{b}	7.8^{c}	10.8^{a}
	0.4% aP + phytase	8.0^{bc}	8.1^{ab}	9.9^{b}	10.6^{ab}	6.1^{a}	5.8^{b}	7.4^{c}	10.6^{a}
	0.3% aP + phytase	8.7^{ab}	7.9^{b}	10.1^{ab}	9.3^{c}	6.1^{a}	5.8^{b}	8.0^{bc}	10.4^{a}
Floor									
	0.5% aP	9.1^{a}	7.4^{c}	10.1^{ab}	9.0^{c}	5.4^{b}	5.7^{b}	9.2^{a}	8.7^{bc}
	0.5% aP + phytase	8.6^{ab}	7.8^{bc}	9.2^{c}	9.4^{bc}	5.8^{ab}	5.9^{b}	8.5^{b}	9.2^{b}
	0.4% aP + phytase	9.1^{a}	8.6^{a}	10.6^{a}	10.1^{b}	5.8^{ab}	6.2^{ab}	8.7^{ab}	9.4^{b}
	0.3% aP + phytase	9.6^{a}	7.9^{b}	9.8^{b}	9.2^{bc}	6.0^{a}	6.6^{a}	7.8^{c}	9.3^{b}
	SEM	0.3	0.2	0.3	0.3	0.2	0.3	0.2	0.4

[a–d]Within a column, means with unlike superscripts differ significantly ($P < 0.05$).
[B]n = 20 for each treatment mean in both the Broilermatic cage system and in the conventional house.
[B]NRC dietary requirement of 0.45% available P_i (aP) and 1.0% Ca in starter; 0.35% aP and 0.8% Ca in grower; 0.3% aP and 0.6% Ca in finisher.

0.52% tP, no oil spray, phytase group; a similar condition that was not statistically significant was observed in Experiment 3. At 42 d of age in Experiment 3, there were no differences among treatment groups for serum P_i even though the P_i concentrations in the phytase-treated groups were numerically higher than the two non-supplemented groups. In Experiment 4 at 42 d of age, serum P_i concentrations in the 0.72% tP group was significantly less than the P_i in the two phytase-treated groups.

Generally, low aP_i in the finisher diet caused a depression in serum P_i concentrations, but phytase supplementation increased the serum P_i concentrations to a control level or even exceeded the control level. The reason for this response is that supplemental phytase in the finisher diet facilitated hydrolysis of phytate P making more P available for absorption. At 21 and 42 d of age, serum total Ca concentrations were not affected by lower tP levels in the diet, and supplementation with phytase did not alter that condition. Nevertheless, at 42 d of age, there was a nonsignificant tendency for those birds given phytase treatment to have a slightly higher serum total Ca concentration.

TABLE 7. Influence of dry phytase applied to postpelleted feeds (Experiments 3 and 4) on serum inorganic P (P_i) and total Ca concentrations in broilers reared in conventional litter-covered floor pens[A]

	SERUM CONCENTRATIONS (mg/dL)							
	3 Wk				6 Wk			
	P_i		Ca		P_i		Ca	
DIET	Exp. 3	Exp 4	Exp. 3	Exp. 4	Exp. 3	Exp. 4	Exp. 3	Exp. 4
0.72% tP, no oil spray, no phytase	8.1^{a}	7.4^{ab}	10.3^{a}	11.0^{a}	7.2^{a}	6.9^{b}	8.4^{a}	10.6^{a}
0.52% tP, oil spray, no phytase	7.6^{a}	6.8^{b}	10.8^{a}	10.6^{a}	7.3^{a}	7.2^{ab}	8.6^{a}	10.7^{a}
0.52% tP, no oil spray, phytase	7.9^{a}	7.9^{a}	10.5^{a}	11.8^{a}	7.7^{a}	7.4^{a}	9.0^{a}	11.1^{a}
0.52% tP, oil spray, phytase	8.1^{a}	7.4^{ab}	10.1^{a}	10.9^{a}	7.7^{a}	7.5^{a}	9.0^{a}	11.4^{a}
SEM	0.2	0.2	1.3	1.1	0.2	0.2	1.2	1.4

[a,b]Within a column, means with unlike superscripts differ significantly ($P < 0.05$).
[A]Exp. = experiment; tP = total P.

TABLE 8. Effect of low phytase activity applied in starter, grower, and finisher mash diets on changes in litter P content in a conventional house and raw manure P content from birds in a cage facility[A]

	LITTER			MANURE		
TREATMENT	Starter	Grower	Finisher	Starter	Grower	Finisher
NRC	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]
NRC + phytase	96.6[a]	107.2[a]	100.0[a]	95.9[a]	94.3[a]	94.1[a]
0.4% aP + phytase	102.4[a]	99.1[a]	85.9[b]	95.2[a]	92.3[a]	107.1[a]
0.3% aP + phytase	104.3[a]	97.4[a]	78.9[b]	85.9[b]	80.6[b]	104.7[a]
SEM	2.2	2.4	2.7	2.9	2.7	2.8

[a,b]Within a column, means with unlike superscripts differ significantly ($P < 0.05$).
[A]All cells are expressed as a percentage of the NRC basal diet (set to an equivalent of 100%) without added phytase.

These data for serum P_i and total Ca were consistent with the data from Experiment 2. The results suggest that even when tP is at 0.52% (0.32% aP), the phytase can hydrolyze sufficient phytate P to meet the needs of broiler chickens. In fact, if one uses serum P_i as an indicator of efficacy of the enzyme in the feed, it might be concluded that 0.32% aP is excessive in the diet because phytase, liquid or dry applied postpelleting, was sufficient to maintain serum P_i at a control level. Serum total Ca was not affected significantly by the phytase treatment, but it was interesting to note the numerically higher serum total Ca levels at 42 d of age in the phytase treated broilers. Higher serum P_i and total Ca concentrations can be related to improved bone ash in chickens.

PHYTASE INFLUENCE ON P CONTENT IN RAW MANURE AND LITTER

The phytase activity added to diets in Experiment 1 was about 10% of the recommended activity. Therefore, it was not very surprising to find that the amount of P in litter and manure was not very different from that found in litter and manure from control birds (Table 8). However, in the cage facility, phytase supplementation resulted in significantly lower P content in the 0.3% aP diet during feeding of starter and grower feeds. During the feeding of finisher feed there was significantly less P accumulation in the litter under birds fed a 0.3% aP diet. These reductions in litter and manure P are not due entirely to the phytase activity in the finisher feeds but may simply reflect the lower levels of P in the diets. Nevertheless, even with the inadequate phytase activity in the finisher feed, serum P and Ca concentrations suggest that the phytase was hydrolyzing some phytate P and Ca that caused a slight increase in serum concentrations.

In Experiment 2, liquid phytase was applied postpelleting, and the activity of the phytase was applied at a rate chemically determined to be 91.1% of the recommended activity level. In this experiment, when tP was reduced in the diet and phytase was supplemented, there was significantly less P in litter and manure (Table 9). Reduced litter and manure P was most evident in those groups that had been fed diets with 0.3% aP. Reductions in litter and manure P content were apparent in all three feeding regimes (starter, grower, and finisher). Phytase-associated reductions in litter ranged between 15 and 19%, whereas in the raw manure from the cage facility, P was reduced between 12 and 18% in the 0.4% aP-fed birds and in the 0.3% aP diets the range was between 25 and 55%.

In Experiments 3 and 4, phytase was applied dry to postpelleted feeds. In both experiments, significant decreases in litter P accumulation were determined at the end of the study (Table 10). Reductions in litter P under birds fed the phytase diet without oil spray were between 28.5 and 20.5% in Experiments 3 and 4, respectively. Similarly, in Experiments 3 and 4, litter P under birds fed the phytase diet with oil spray was reduced between 22.9 and 23.1%, respectively. There was no difference between Experiments 3 and 4 for the phytase-associated reduction in litter P. These data compared favorably with the results obtained in Experiment 2 when a liquid phytase was applied to feed postpelleting. In Experiment 2, the 0.3% aP diet was comparable with the phytase diets in Experiments 3 and 4. In Experiment 2, a 26.1% reduction in litter P

TABLE 9. Effect of liquid phytase activity applied postpelleting in starter, grower, and finisher diets on changes in litter P content in the conventional house and raw manure P content from birds in the cage facility[A]

	LITTER			MANURE		
TREATMENT[A]	Starter	Grower	Finisher	Starter	Grower	Finisher
NRC	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]
NRC + phytase	107.9[a]	100.9[a]	103.1[a]	91.0[a]	97.8[a]	101.0[a]
0.4% aP + phytase	92.3[b]	92.8[b]	100.7[a]	82.1[b]	88.0[b]	84.6[b]
0.3% aP + phytase	81.2[c]	82.4[c]	85.7[b]	74.6[c]	74.9[c]	45.0[c]
SEM	2.1	2.2	2.4	2.0	2.2	2.9

[a–c]Within a column, means with unlike superscripts differ significantly ($P < 0.05$).
[A]All cells are expressed as a percentage of the NRC basal diet (set to an equivalent of 100%) without added phytase.
[B]aP = available P.

was determined at 42 d of age, similar to the observations made in Experiments 3 and 4. Therefore, it appears that application of dry phytase to postpelleted feeds can be accomplished with results comparable with results associated with liquid phytase application postpelleting.

The efficacy of phytase use in feeds to decrease litter P can be variable. A 30% P reduction in poultry manure was reported in early studies with a microbial phytase [3, 4, 5]. Later, it was reported that a 38% reduction in laying hen manure P could be achieved with microbial phytase [9]. In 21-d-old broilers, the availability of dietary P could be increased up to 65% by means of supplemental dietary phytase while reducing fecal P by 50% [3]. The phytase used in the current study yielded significant 16 and 25% reductions in fecal P, similar to reductions in fecal P from laying hens fed P_i at 80 or 60% of the NRC requirements [6]. The same phytase enzyme has been shown to reduce manure P in market turkeys [10]. Fecal P reductions from laying hens [4], market turkeys [10], and broilers given the phytase used in the current study were similar to the fecal P reductions found in pigs [3], layers [11, 12], and broilers [13, 14] given another phytase feed supplement.

At the termination of these studies, litter P accumulation was reduced by 20.5 to 28.5% in pens containing broilers given the diet with 0.5% tP (0.3% aP) and supplemental phytase. Similar reductions in litter P accumulation were not obtained with dietary tP at 0.6% (0.4% aP) or higher. Qian et al. [27] reported that phytase, vitamin D_3, and Ca:tP ratios are important factors in degrading phytate P and improving di-

TABLE 10. Comparison of the influence of postpellet application of dry phytase (Experiments 3 and 4) and postpellet application of liquid phytase (Experiment 2) on the accumulation of P in pine wood shavings litter[A] under 6-wk-old broiler chickens

	Exp. 2		Exp. 3		Exp. 4	
TREATMENTS[B]	Litter P (lb/t)	Control (%)	Litter P (lb/t)	Control (%)	Litter P (lb/t)	Control (%)
0.72% tP, no oil spray, no phytase (control)			18.0	100.0[a]	15.6	100.0[a]
0.52% tP, oil spray, no phytase			17.3	–3.73[a]	14.4	–5.1[a]
0.52% tP, phytase, no oil spray			12.9	–28.5[b]	12.4	–20.5[b]
0.52% tP, phytase, oil spray			13.9	–22.9[b]	12.0	–23.1[b]
SEM			1.4		1.1	
NRC (0.7% tP, control)	13.8	100.0[a]				
NRC + phytase (0.7% tP)	14.6	+5.8[a]				
0.4% aP + phytase (0.6% tP)	14.0	+1.5[a]				
0.3% aP + phytase (0.5% tP)	10.2	–26.1[b]				
SEM	1.2					

[a,b]Within a column, means with unlike superscripts differ significantly ($P < 0.05$).
[A]n = 9 for each cell mean.
[B]tP = total P; Exp. = experiment.

etary P and Ca use in broilers. Dietary Ca in excess of 1.25% [29] can precipitate phytate as an insoluble Ca-phytate in the intestine and inhibit release of phytate P by phytase [30, 31, 32], but in this study dietary Ca was 1% or less. However, it has been demonstrated that broiler chickens have the ability to degrade phytate P as they approach market age [7, 8, 33]. The digestibility of phytate P can increase from 31% at 14 d of age to 38.2% at 25 d of age in broilers [7, 8]. At low levels of dietary P_i, phytate P becomes more available due to an adaptive increase in phytate digestibility [34]. It has been noted that low levels of dietary P result in increased activity of intestinal phytase in chicks [30, 35], explaining the observations made by Edwards [33] and Van der Klis and Versteegh [7, 8]. Therefore, an increase in endogenous intestinal phytase in response to decreased dietary P (0.6% tP; 0.4% aP) and supplemental dietary phytase activity might have liberated phytate Ca that was sufficiently high to precipitate Ca-phytate. Thus, when dietary tP is higher than 0.5%, even with phytase supplemented to the diet, litter P accumulation may be higher than expected.

Conclusions and Applications

1. The use of phytase at less than recommended activities does not reduce fecal P when dietary tP is high, but it is possible to reduce fecal P at the expense of performance if there is low tP in the diet.
2. Feed conversions are improved significantly with the use of properly formulated diets with adequate phytase activity.
3. Dry phytase applied to postpelleted broiler feeds resulted in significant reductions in litter P accumulation comparable to the reductions associated with postpellet liquid phytase application.
4. It is feasible to apply dry phytase postpelleting; revenue savings can be realized due to reduced costs of purchasing and shipping liquid phytase.
5. Use of dry phytase applied postpelleting provides for a safety factor associated with the bactericidal effect of high pelleting temperature.
6. Dry phytase is more stable than liquid phytase and requires less warehouse space for storage.

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