Using Enzyme Supplemented, Reduced Protein Diets to Decrease Nitrogen and Phosphorus Excretion of Broilers

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ABSTRACT : An experiment was conducted to investigate the effect of dietary protein levels and supplementation of phytase and pentosanase in wheat-soybean meal diet on the performance and output of N and P in broilers. Addition of phytase alone or in combination with pentosanase to reduced or control protein diets did not affect average final body weight of mixed sexes. However, addition of phytase and pentosanase in combination to reduced protein diets in male broilers significantly depressed body weights. Intestinal viscosity of 21d broilers was significantly decreased by addition of phytase and pentosanase alone or in combination. Tibia ash content was significantly increased by phytase supplementation. Supplementation of phytase alone and in combination with pentosanase to reduced protein diets significantly decreased P in manure and daily output of P. Daily N output was lowest in the reduced protein diet supplemented with phytase and pentosanase combination. The retention of DM, N and P was highest in the reduced protein diet supplemented with phytase and pentosanase combination. In conclusion, supplementation of phytase alone or in combination with pentosanase to reduced protein diets can decrease output of N and P. But the combination of the enzymes has no beneficial effects on the performance of broilers, especially those on wheat-soybean meal diet with reduced protein level. ***(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 11 : 1561-1567)***

Key Words : Broilers, Enzymes, Phytase, Pentosanase, Reduced-Protein Diets, Nitrogen, Phosphorus

INTRODUCTION

Environmental pollution from nitrogen (N) and phosphorus (P) in poultry manure is becoming a serious problem in many areas of intensive animal production. Research conducted to date indicates that it is possible, with the use of crystalline amino acids, to formulate diets with a reduced protein content and still meet the animal's requirement for essential amino acids. Estimates of the reduction in N excretion that can be attained with laying hens range from 20% to over 50% (Blair et al., 1976; Han et al., 1992; Klasing, 1993). For broilers, the estimates range from 10% to 30% (Nelson, 1967; Newman, 1994).

Phytate represents from 50 to 80% of the total P content in cereal grains (Parr and Summers, 1991). Phytate P is poorly utilized by poultry (Raboy, 1990) and other nonruminants (Kornegay and Qian, 1994). Microbial phytase can be added to animal feeds to increase the availability of phytate P, reducing the amount of inorganic P supplementation required and decreasing P excretion (Paik, 2000).

When wheat, rye, and triticale are used in poultry feeds there is a reduction in bird performance and sticky excreta are produced (Annison, 1991). This is attributed to a lack of polysaccharidase such as ß-glucanase and pentosanase in the digestive tract of these birds. The soluble non-starch polysaccharides (NSPs) are believed to increase the viscosity of digesta. The increasingly gel-like environment reduces normal movement and limits nutrient absorption from the intestinal tract (Schutte et al., 1993). Enzymes can be added to digest the NSPs, decrease intestinal viscosity, and improve nutrient utilization.

The use of enzymes in poultry feed is increasing. As environmental concerns about poultry waste disposal increase, enzymes are frequently considered as a tool for reducing the excretion of nutrients, especially nitrogen and phosphorus. Odor control is a major concern also being addressed by supplementation with feed enzymes. Feed enzymes have also increased the nutritive value of a number of different grains, such as wheat and barley, increasing their use in poultry feeds. As the number of commercially available feed enzymes increases, it is important to consider the interaction between enzymes when they are combined in a single feed.

The objective of this study was to evaluate the possible additive effects of reduced protein diets and supplementation with commercial phytase and/or pentosanase on the excretion of N and P of broilers.

MATERIALS AND METHODS

The study was composed of two parts - a growth trial and a balance trial. Both trials were run con-

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currently for a period of six weeks. The broiler chicks were sexed at the hatchery and vaccinated against Marek's disease. Control and reduced protein diets (23% CP in starter; 21% CP in grower vs 21% CP in starter; 17.5% CP in grower) were compared with and without phytase and/or pentosanase supplementation. Phytase supplemented diets (diet 2, 3, 6 and 7) were formulated to have 0.57% less dicalcium phosphate (equivalent to 0.1% available P) than other diets. Starter diets (table 1) were fed as crumbles from 0-21 days of age and the grower diets (table 2) were fed as pellets from 22-42 days of age. All the diets were wheat/soybean meal based. The phytase (Natuphos 5.000 L, BASF Canada Inc.) and pentosanase

Table 1. Composition of the starter diets

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
	%							
Wheat	60.13	60.10	60.03	60.06	67.69	67.66	67.60	67.62
Soybean meal (46%)	26.84	26.84	26.87	26.86	19.57	19.58	19.60	19.59
Meat meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
DL-Methionine	0.28	0.28	0.28	0.28	0.31	0.31	0.31	0.31
L-Lysine	0.08	0.08	0.07	0.08	0.34	0.34	0.34	0.34
L-Threonine	-	-	-	-	0.01	0.01	0.01	0.01
Fat Blend[1]	4.60	4.60	4.62	4.62	3.95	3.96	3.98	3.97
Dicalcium phosphate	0.87	0.30	0.30	0.87	0.88	0.31	0.31	0.88
Limestone	0.86	1.10	1.10	0.86	0.88	1.12	1.12	0.88
Sand	0.50	0.83	0.81	0.48	0.50	0.83	0.81	0.48
NaCl	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Coccidiostat[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Growth promoter[3]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Liquid choline 70%	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Vitamin premix[4]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Mineral premix[5]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phytase[6]	-	0.01	0.01	-	-	0.01	0.01	-
Pentosanase[7]	-	-	0.05	0.05	-	-	0.05	0.05
Determined composition (air dry basis):								
ME, kcal/kg[8]	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Dry matter, %	89.50	88.95	89.15	89.57	89.28	89.65	89.64	89.74
Crude protein, %	23.78	23.69	24.12	23.68	21.63	21.69	21.17	21.31
Methionine, %	0.33	0.33	0.34	0.33	0.32	0.32	0.33	0.32
Met+cys, %	0.74	0.72	0.75	0.73	0.71	0.64	0.67	0.69
Lysine, %	1.20	1.15	1.20	1.16	1.25	1.10	1.08	1.21
Tryptophan, %	0.28	0.27	0.28	0.27	0.26	0.25	0.23	0.24
Threonine, %	0.76	0.73	0.77	0.76	0.72	0.62	0.62	0.71
Crude fat, %	6.23	6.94	6.93	7.12	5.84	5.76	5.29	5.89
Crude fibre, %	2.72	2.79	2.58	2.74	2.60	2.81	2.77	2.61
Ash, %	4.81	4.98	5.06	4.75	5.10	5.21	4.85	5.15
Calcium, %	0.97	0.98	0.94	0.99	0.96	0.90	0.95	0.97
Phosphorus (total), %	0.76	0.76	0.71	0.69	0.71	0.65	0.74	0.75
Phosphorus (available), %[8]	0.40	0.34	0.32	0.37	0.38	0.29	0.33	0.39

[1] Commercial blend of vegetable and animal fat.
[2] Coxistac 6% (salinomycin sodium), Cyanamid Canada Inc.
[3] Stafac 44 (virginiamycin), SmithKline Beecham Animal Health Products.
[4] Supplied per kg diet: vitamin A 12,000 IU; cholecalciferol 3,600 IU; vitamin E 50 IU; vitamin K 2.25 mg; cobalamin 0.023 mg; thiamin 1.5 mg; riboflavin 7.5 mg; folic acid 0.75 mg; biotin 0.12 mg; pantothenic acid 12 mg; niacin 28 mg; pyridoxine 7.5 mg.
[5] Supplied per kg diet: Mn 90 mg; Cu 125 mg; Zn 80 mg; I 0.4 mg; Se 0.3 mg; Fe 80 mg.
[6] Natuphos 5.000 L, BASF Canada Inc., supplying 600 FTU phytase per kg diet.
[7] Bio-Feed™ Plus 50 L, Novo Nordisk, Denmark, supplying 25,000 FBG beta-glucanase, 6,250 VHCU hemicellulase, 325 NCU cellulase and 825 PTU pentosanase per kg diet.
[8] Calculated value.

Table 2. Composition of the grower diets

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
	%							
Wheat	68.59	68.56	68.48	68.51	78.38	78.35	78.28	78.30
Soybean meal (46%)	17.42	17.43	17.45	17.44	7.94	7.94	7.97	7.96
Meat meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Methionine	0.24	0.24	0.24	0.24	0.30	0.30	0.30	0.30
Lysine	0.23	0.23	0.23	0.23	0.57	0.57	0.57	0.57
Threonine	-	-	-	-	0.14	0.14	0.14	0.14
Fat Blend[1]	4.00	4.00	4.00	4.00	3.69	3.70	3.71	3.71
Tallow	1.57	1.58	1.60	1.59	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.79	0.22	0.22	0.79	0.80	0.23	0.23	0.80
Limestone	0.80	1.04	1.04	0.80	0.83	1.07	1.07	0.83
Sand	0.50	0.83	0.81	0.48	0.50	0.83	0.81	0.48
NaCl	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Coccidiostat[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Growth promoter[3]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Liquid choline 70%	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Vitamin premix[4]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Mineral premix[5]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phytase[6]	-	0.01	0.01	-	-	0.01	0.01	-
Pentosanase[7]	-	-	0.05	0.05	-	-	0.05	0.05
Determined composition (air dry basis):								
ME, kcal/kg[8]	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100
Dry matter, %	89.38	89.54	89.44	89.65	89.52	89.76	89.53	89.74
Crude protein, %	20.27	20.72	20.91	20.84	17.13	16.85	16.54	17.06
Methionine, %	0.28	0.29	0.27	0.27	0.26	0.24	0.25	0.26
Met+cys, %	0.64	0.65	0.60	0.61	0.58	0.56	0.58	0.59
Lysine, %	1.06	1.05	1.02	1.03	1.07	1.07	1.08	0.92
Tryptophan, %	0.23	0.23	0.23	0.24	0.21	0.17	0.18	0.20
Threonine, %	0.65	0.65	0.63	0.63	0.63	0.65	0.60	0.66
Crude fat, %	7.36	7.15	7.22	7.19	6.54	6.30	6.28	6.61
Crude fibre, %	2.66	2.58	2.71	2.65	2.49	2.81	2.38	2.52
Ash,%	5.12	5.30	4.98	4.95	5.06	5.15	4.99	5.20
Calcium, %	0.91	0.89	0.93	0.90	0.89	0.94	0.92	0.90
Phosphorus (total), %	0.69	0.72	0.64	0.60	0.61	0.66	0.71	0.71
Phosphorus (available), %[8]	0.36	0.31	0.28	0.33	0.33	0.39	0.31	0.37

[1,2,3,4,5,6,7,8] As in table 1.

(Bio-Feed™ Plus 50 L, Novo Nordisk, Denmark) enzymes were sprayed on after pelleting.

Growth trial

There was a total of 48 pens, with 50 broilers per pen. Each of the eight experimental diets was fed to three pens of females and three of males using a completely randomized design. Each floor pen was provided with pine wood shavings bedding, a tube feeder and nipple drinkers. Total pen body weights were recorded at the start of the trial. Individual body weights were recorded at 21 and 42 days of age. Pen feed consumption was determined weekly and mortality was recorded daily. At 21 days of age three broilers per pen were sacrificed by cervical dislocation and the contents of the small intestine removed for viscosity determination. Viscosity measurements were taken for two samples obtained from the fluid portion of a pooled sample from the three broilers. Similarly, at 42 days of age two broilers per pen were sacrificed and the contents of the small intestine removed for viscosity determination. Viscosity measurements were taken for a sample from of the fluid portion from each of the two broilers. To obtain the samples for viscosity measurement, the intestinal contents were removed from the end of the duodenal loop to the Meckel's diverticulum, mixed, and spun in a micro-centrifuge. Viscosity was measured using a Brookfield Digital Viscometer, Model DV-11+ version 2.0. at 42.5 sec^{-1} at 40℃.

Balance trial

A total of 192 broilers (96 males and 96 females - wing banded) were used in a balance trial. For the first 21 days the broilers were housed in battery brooders - eight broilers (four females and four males) per pen. A starter diet (table 1) was fed for the entire 3 week period. During the second week of the study, total excreta output, per pen, over a 24 hour period was measured. Feed consumption for the 24 hour period were also recorded. On day 21, 80 broilers (40 males and 40 females) were individually weighed and moved into individual cages and fed a grower diet (table 2). During the fifth week of the study, total excreta output for each broiler was measured over a 48 hour period. Daily feed consumption for the 48 hour period were also recorded. For both excreta collection periods, the excreta was collected every 12 hours and frozen. Later, the excreta samples were pooled per pen (for collection 1) or per bird (for collection 2), dried in a 60℃ oven, ground, and analyzed for total N and P content by the method of AOAC (1990).

A preliminary study at this laboratory (unpublished data) compared the nitrogen content of fresh excreta to that of excreta dried by freeze-drying, oven drying at 60℃ and oven drying at 100℃. There were no significant differences in total nitrogen content.

At 21 and 42 days of age, tibia samples were taken and percent ash determined on a fat-free basis. Fat was extracted from the tibia samples using the soxhlet procedure. The fat-free bone samples were then ashed in a muffle furnace at 600℃ for 18 hours.

Statistical analysis

The data were analyzed as an 8×2 factorial (diet ×sex) using SAS (1996). Unless otherwise indicated, all statements of significance were based on $p=0.05$. Where there was no significant effect of sex, the data for the two sexes were combined and analyzed as a 2 ×2×2 factorial (dietary protein, phytase supplementation, pentosanase supplementation), again using SAS (1996). Where significant interactions were noted, differences between diets were detected using the Least Square Means test.

RESULTS AND DISCUSSION

Birds fed control (normal) protein diet gained more weight than did birds fed reduced protein diet (table 3). As would be expected, the males had higher body weights at both 21 and 42 days of age, consumed more feed in both the starter and grower periods, and, on most diets, had better FCRs. The performance data were separated by sex because there was a statistically significant diet×sex interaction affecting most performance parameters. In both sexes, addition of phytase alone or in combination with pentosanase to reduced protein diets significantly decreased body weight but supplemental pentosanase alone did not decrease body weight, even though the added enzymes significantly decreased intestinal viscosity. Yi et al. (1996) and Kornegay (1996) reported in the studies involving turkey poult and broiler that reducing protein level decreased body weight but body weight was increased by phytase supplementation to reduced protein diets. Therefore, low body weight in birds fed reduced protein diets with phytase alone or in combination with pentosanase is thought to be associated with the levels of dietary protein, amino acids and P. The lowest body weight in birds fed diet 7 which is low protein and low P diet supplemented with pentosanase and phytase seems to be associated with the lowest levels of dietary protein, amino acids (lysine in starter diet and threonine in grower diet) and P.

Paik (2000) reported that wheat contains high level of natural phytase (1120 U/kg) while corn doesn't. Therefore, the beneficial effect of phytase supplementation to corn-soy diets used by Yi et al (1996) and Kornergay (1996) may not appear in wheat-soy diet of the present experiment.

Intestinal viscosity was significantly decreased by addition of phytase and pentosanase alone or in combination (table 4) but this decreased intestinal viscosity did not affect body weight. Birds fed reduced protein diet supplemented with pentosanase alone had comparable body weight to those of control protein diets. Pettersson and Aman (1989) reported that supplementation of enzyme containing pentosanase to diet based on wheat and rye increased body weight by approximately 15% and feed intake by 8% and increased digestibility of organic matter and crude protein. It is uncertain why phytase supplementation also decreased viscosity. As expected, dietary phytase significantly increased (table 6) tibia ash content at 42 d, which agrees with the result of previous study (Qian et al., 1996). Dietary protein levels and pentosanase did not affect tibia ash content.

At two weeks of age, supplementation of phytase alone or in combination with pentosanase significantly decreased P and N in manure (table 7). Supplementation with phytase, however, was only successful in reducing the daily P output of those broilers receiving the reduced protein diets. Phytase supplementation of the control protein diets did not result in a significant reduction in daily P output. Phytase supplementation of the reduced protein diets also significantly increased apparent P retention (table 8).

Table 3. Body weight, feed consumption and feed conversion ratio of broilers fed experimental diets

Diet	Protein	Enzyme added		Average body weights (g/broiler)		Average feed consumption (g/broiler)		Average feed:gain ratio		
		Phytase	Pentosanase	21 days	42 days	Starter	Grower	Starter	Grower	Overall
a) Mixed sex										
1	Control	No	No	643	2,272	758^{ab}	2,942	1.27^{b}	1.81	1.63
2	Control	Yes	No	626	2,277	794^{a}	2,907	1.37^{a}	1.76	1.62
3	Control	Yes	Yes	641	2,326	793^{a}	2,959	1.33^{ab}	1.76	1.61
4	Control	No	Yes	642	2,325	761^{ab}	2,961	1.28^{b}	1.77	1.59
5	Reduced	NO	No	635	2,230	747^{ab}	2,882	1.27^{b}	1.82	1.63
6	Reduced	Yes	No	608	2,138	735^{b}	2,765	1.31^{ab}	1.81	1.63
7	Reduced	Yes	Yes	598	2,106	754^{b}	2,747	1.36^{a}	1.82	1.65
8	Reduced	No	Yes	646	2,250	758^{ab}	2,894	1.26^{b}	1.80	1.61
Overall mean				630	2,241	763	2,882	1.31	1.79	1.62
Overall SEM				4	27	5	33	0.01	0.01	0.01
1	Control	No	No	640^{ab}	2,453^{a}	770	3,143	1.25^{ab}	1.75	1.59^{ab}
2	Control	Yes	No	646^{bc}	2,451^{a}	820	3,096	1.37^{ab}	1.72	1.59^{ab}
3	Control	Yes	Yes	669^{ab}	2,524^{a}	812	3,148	1.30^{ab}	1.70	1.56^{ab}
4	Control	No	Yes	665^{ab}	2,514^{a}	784	3,113	1.27^{ab}	1.68	1.53^{b}
5	Reduced	No	No	655^{ab}	2,407^{ab}	760	3,016	1.25^{b}	1.72	1.56^{ab}
6	Reduced	Yes	No	621^{cd}	2,258^{bc}	746	2,912	1.30^{ab}	1.78	1.61^{a}
7	Reduced	Yes	Yes	609^{d}	2,205^{c}	783	2,896	1.39^{a}	1.82	1.66^{ab}
8	Reduced	No	Yes	680^{a}	2,409^{ab}	812	3,167	1.25^{b}	1.83	1.62
Overall mean				651	2,402	783	3061	1.27	1.75	1.59
Overall SEM				5	25	7	29	0.01	0.01	0.01
1	Control	No	No	626^{a}	2,092^{abc}	746	2,740	1.28^{bc}	1.87	1.67
2	Control	Yes	No	605^{abc}	2,103^{abc}	767	2,718	1.37^{a}	1.82	1.66
3	Control	Yes	Yes	614^{abc}	2,128^{ab}	774	2,770	1.36^{ab}	1.83	1.67
4	Control	No	Yes	619^{ab}	2,137^{a}	738	2,808	1.29^{bc}	1.85	1.65
5	Reduced	No	No	614^{abc}	2,053^{abc}	734	2,747	1.29^{bc}	1.94	1.70
6	Reduced	Yes	No	594^{bc}	2,018^{bc}	724	2,618	1.32^{abc}	1.84	1.66
7	Reduced	Yes	Yes	587^{c}	2,006^{c}	724	2,597	1.33^{abc}	1.83	1.64
8	Reduced	No	Yes	613^{abc}	2,092^{abc}	728	2,620	1.28^{c}	1.77	1.60
Overall mean				609	2,079	742	2,702	1.32	1.84	1.65
Overall SEM				3	12	5	27	0.01	0.02	0.01

[a,b,c] Means within a column with no common superscript differ significantly ($p<0.05$).

Table 4. Average intestinal viscosity measurements

Diet	Protein	Enzymes added		Intestinal viscosity	
		Phytase	Pento-sanase	21 days	42 days[1]
1	Control	No	No	7.1^{a}	5.0
2	Control	Yes	No	4.1^{bcd}	3.9
3	Control	Yes	Yes	4.3^{bc}	3.2
4	Control	No	Yes	3.2^{cd}	3.0
5	Reduced	No	No	6.7^{a}	5.8
6	Reduced	Yes	No	5.2^{b}	5.0
7	Reduced	Yes	Yes	3.1^{d}	3.8
8	Reduced	No	Yes	4.1^{bcd}	4.0
Overall mean				4.7	4.2
Overall SEM				0.2	0.2

[1] Significant ($p<0.05$) diet × sex interaction.
[a,b,c,d] Means within a column with no common superscript differ significantly ($p<0.05$).

Table 5. Intestinal viscosity measurements for males and females at 42 days of age

Diet	Protein	Enzymes added		Intestinal viscosity	
		Phytase	Pento-sanase	Males	Females
1	Control	No	No	4.2^{ab}	5.7^{ab}
2	Control	Yes	No	3.8^{b}	4.0^{abc}
3	Control	Yes	Yes	3.0^{b}	3.4^{bc}
4	Control	No	Yes	2.9^{b}	3.1^{c}
5	Reduced	No	No	6.0^{a}	5.7^{ab}
6	Reduced	Yes	No	3.8^{b}	6.6^{a}
7	Reduced	Yes	Yes	4.0^{b}	3.6^{bc}
8	Reduced	No	Yes	3.7^{b}	4.3^{abc}
Overall mean				3.9	4.5
Overall SEM				0.2	0.3

[a,b,c] Means within a column with no common superscript differ significantly ($p<0.05$).

Table 6. Average tibia ash content

Diet	Protein	Enzymes added: Phytase	Enzymes added: Pentosanase	Tibia ash (%): 21 days	Tibia ash (%): 42 days
1	Control	No	No	54.1	51.1
2	Control	Yes	No	54.1	55.4
3	Control	Yes	Yes	54.7	54.7
4	Control	No	Yes	54.8	51.7
5	Reduced	No	No	53.0	51.4
6	Reduced	Yes	No	55.5	55.8
7	Reduced	Yes	Yes	53.1	53.8
8	Reduced	No	Yes	53.6	53.0
Overall mean				54.1	53.4
Overall SEM				0.4	0.7
Significance:					
Crude protein (CP)				NS	NS
Phytase (Ph)				NS	0.05
Pentosanase (P)				NS	NS
CP × Ph				NS	NS
CP × P				NS	NS
Ph × P				NS	NS
CP × Ph × P				NS	NS

At 5 weeks of age, phytase supplementation alone, but not in combination with pentosanase, resulted in a significant reduction in manure P content (table 7). Supplementation with phytase, either alone or in combination with pentosanase, resulted in a significant reduction in daily P output and a significant increase in apparent P retention (table 8).

Supplementation with either phytase or pentosanase alone had no significant effect on manure N content or daily N output. Daily N output was the lowest, and apparent dry matter and N retention were the highest, for those broilers receiving the reduced protein diets supplemented with both phytase and pentosanase. Unfortunately, the broilers receiving this diet also had the lowest 3 week body weights (table 3).

At five weeks of age, reducing the dietary protein from 20 to 17.5% resulted in a significant reduction in manure N content and daily N output. Again, daily N output was the lowest, and apparent dry matter and N retention the highest, for those broilers receiving the reduced protein diets supplemented with both phytase and pentosanase. These broilers also had the lowest final body weights.

In conclusion, supplementation of phytase alone or in combination with pentosanase to reduced protein diets can increase N and P retention and decrease output of N and P. But the combination of these enzymes had no beneficial effects on the performance of broilers, especially those on wheat-soybean meal diet with reduced protein level.

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Table 7. Nitrogen and phosphorus content of manure (% DM basis) and daily output (g/broiler/day) of phosphorus and nitrogen

	Diet description			Manure composition (DM basis)				Daily output (g/broiler)			
				% phosphorus		% nitrogen		Phosphorus		Nitrogen	
	Protein	Phytase	Pentosanase	2 wk	5 wk	2 wk	5 wk	2 wk	5 wk	2 wk	5 wk
1	Control	No	No	1.12[a]	1.63[b]	5.02[abc]	4.29[ab]	0.15[a]	0.80[a]	0.67[abc]	2.12[a]
2	Control	Yes	No	0.85[bc]	1.43[cd]	5.19[ab]	4.27[ab]	0.12[ab]	0.56[c]	0.73[ab]	1.66[bc]
3	Control	Yes	Yes	0.93[b]	1.52[bc]	5.24[a]	4.69[a]	0.14[a]	0.58[bc]	0.80[a]	1.80[bc]
4	Control	No	Yes	1.16[a]	1.93[a]	5.02[abc]	4.52[ac]	0.14[a]	0.82[a]	0.63[bcd]	1.92[ab]
5	Reduced	No	No	1.13[a]	1.67[b]	4.75[bc]	3.75[cd]	0.14[a]	0.85[a]	0.58[bcd]	1.91[ab]
6	Reduced	Yes	No	0.80[c]	1.28[d]	4.75[bc]	3.67[d]	0.09[b]	0.61[bc]	0.53[cd]	1.74[bc]
7	Reduced	Yes	Yes	0.89[b]	1.56[bc]	4.63[c]	4.37[ab]	0.09[b]	0.50[c]	0.48[d]	1.39[cd]
8	Reduced	No	Yes	1.16[a]	1.98[a]	4.81[abc]	4.18[bc]	0.14[a]	0.74[ab]	0.56[cd]	1.56[bcd]
	Overall mean			1.00	1.63	4.92	4.23	0.13	0.68	0.62	1.76
	Overall SEM			0.03	0.03	0.05	0.05	0.01	0.02	0.02	0.04

[a,b,c,d] Means within a column with no common superscript differ significantly ($p<0.05$).

Table 8. Apparent dry matter, nitrogen and phosphorus retention

	Diet description			Apparent retention (%)					
				Dry matter		Nitrogen		Phosphorus	
	Protein	Phytase	Pentosanase	2 wk	5 wk	2 wk	5 wk	2 wk	5 wk
1	Control	No	No	72.9[bc]	65.6[d]	68.0[abc]	59.3[d]	61.9[cd]	33.9[cd]
2	Control	Yes	No	72.2[c]	70.8[bc]	66.2[bc]	66.5[ab]	65.4[bc]	46.8[a]
3	Control	Yes	Yes	71.5[c]	72.1[ab]	65.6[c]	65.1[ab]	59.5[d]	44.6[ab]
4	Control	No	Yes	74.4[bc]	70.3[bc]	69.7[abc]	63.9[bc]	63.5[bcd]	31.0[cd]
5	Reduced	No	No	74.2[bc]	66.3[d]	69.6[abc]	63.4[cd]	63.8[bcd]	28.9[d]
6	Reduced	Yes	No	75.9[ab]	66.8[cd]	70.6[abc]	64.7[ab]	72.2[a]	38.2[bc]
7	Reduced	Yes	Yes	78.0[a]	76.1[a]	73.3[a]	69.1[a]	67.9[ab]	47.4[a]
8	Reduced	No	Yes	76.0[ab]	73.0[ab]	71.2[ab]	66.4[ab]	63.6[bcd]	32.6[cd]
	Overall mean			74.4	70.2	69.3	64.8	64.7	38.1
	Overall SEM			0.48	0.5	0.6	0.5	0.8	1.0

[a,b,c,d] Means within a column with no common superscript differ significantly ($p<0.05$).

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