Using Enzyme Supplemented, Reduced Protein Diets to Decrease Nitrogen and Phosphorus Excretion of White Leghorn Hens

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ABSTRACT : An experiment was conducted to investigate the effect of supplementation of commercial phytase and β-glucanase to wheat-soybean meal based layer diets. Control (17% CP) and reduced protein (13.5% CP) diets were compared with and without phytase and/or β-glucanase. Reducing dietary crude protein levels reduced the amount of N excreted by laying hens with no adverse affect on egg production or overall feed conversion ratio. There was, however, a slight reduction in average egg weight. When phytase was added to the control protein diets it was possible to reduce the level of dicalcium phosphate in the diet without a loss in performance and daily P output was reduced significantly. When phytase was added to the reduced protein diets, however, there was a dramatic loss in performance in the last four weeks of the study. Supplementation of β-glucanase to wheat based layer diet did not appear to have beneficial affects in terms of laying performance and reducing nitrogen or phosphorus excretion. Combination of phytase and β-glucanase had no positive effects on laying performance or reduction of DM, N and P. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 12 : 1743-1749)*

Key Words : Layer, Enzymes, Phytase, β-glucanase, Dietary Protein, Egg, 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. Run-off and ground-water contamination from ammonia, urea, and to a lesser extent uric acid, is a critical problem with important implications for the future of animal agriculture. A major reduction in the nitrogen content of poultry manure would have a significant and positive impact on the environment and community. Research conducted to date indicates that it is possible, with the use of synthetic amino acids, to formulate diets with a reduced protein content but which meet the layer'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; Klasing, 1993; Summers, 1993). For broilers, the estimates range from 10% to 30% (Han et al., 1992; Parr and Summers, 1991).

Poultry diets are commonly composed of feedstuffs of plant origin and the availability of the phosphorus in such feedstuffs is low. Phytate is the storage form of phosphorus in plants and it is not readily hydrolyzed by the endogenous enzymes of nonruminant animals. As a result, poultry diets are usually supplemented with inorganic phosphorus (e.g., dicalcium phosphate) to meet the phosphorus requirements of the animal. Organic phosphorus present in the diet but unavailable to the nonruminant animal is excreted in the feces. Most animal manure is spread on the land as fertilizer. The phosphorus not taken up by plants accumulates in the root zone and erosion, run-off and leaching can lead to pollution of surface waters. Commercial microbial phytase products are now available which can be added to animal feeds to increase the availability of phytate-P and reduce the need for supplementation with inorganic phosphorus (Paik, 2000).

A wide variety of microbial enzymes are now commercially available that can be added to poultry diets to digest components that nonruminants are intrinsically incapable of digesting. These enzymes are particularly useful for diets containing cereals with a high non-starch polysaccharide (NSP) level, such as wheat and barley. Dietary NSPs are responsible for increasing the viscosity of intestinal contents. A high intestinal viscosity is associated with reduced nutrient availability (Bedford and Classen, 1992).

The objective of this study was to evaluate the possible additive effect of reduced protein diets and supplementation with a microbial phytase and/or β-glucanase on the excretion of N and P of layers.

MATERIALS AND METHODS

The study was composed of two parts - a production trial and a balance trial. Both trials, which were run concurrently for a period of 8 weeks, involved White Leghorn layers (H&N Nick Chick) receiving 15 h of light per day. The layers were 39

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weeks of age at the start of the trial and were at 88% production one week prior to being put on the experimental diets (table 1). Two levels of dietary protein (17 and 13.5% CP) were compared with and without supplementation with phytase (Natuphos®) and/or β-glucanase (Barlican). Natuphos® and Barlican are feed enzymes from BASF Canada. Natuphos® is an enzyme preparation with phytase activity. Barlican is an enzyme preparation obtained by submerged fermentation of a selected strain of *Trichoderma reesei*. The product contains endo-1,3 (4)-β-glucanase as its principal activity which is able to hydrolyze polysaccharides such as β-glucans. In addition, it also includes other carbohydrases, such as hemicellulases and cellulases, as side activities. Phytase supplemented diets (diet 2, 3, 6 and 7) were formulated to have

Table 1. Composition of experimental diets

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
	%							
Wheat	64.34	64.31	64.31	64.34	75.44	75.45	75.45	75.44
Corn	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Tallow	1.92	1.92	1.92	1.92	1.16	1.16	1.16	1.16
Soybean meal (46%)	11.31	11.31	11.31	11.31	-	-	-	-
Canola meal	-	-	-	-	0.50	0.47	0.47	0.50
Meat meal	5.00	5.00	5.00	5.00	3.77	3.78	3.78	3.77
Methionine	0.22	0.22	0.22	0.22	0.29	0.29	0.29	0.29
Lysine	0.09	0.09	0.09	0.09	0.53	0.53	0.53	0.53
Threonine	-	-	-	-	0.20	0.20	0.20	0.20
Tryptophan	-	-	-	-	0.04	0.04	0.04	0.04
Limestone (fine)	4.74	4.98	4.98	4.74	4.95	5.19	5.19	4.95
Limestone (coarse)	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Dicalcium phosphate	0.84	0.26	0.26	0.84	1.05	0.48	0.48	1.05
Sand	0.50	0.80	0.79	0.49	0.50	0.80	0.79	0.49
Salt	0.37	0.37	0.37	0.37	0.38	0.38	0.38	0.38
Grass meal	2.00	2.00	2.00	2.00	2.50	2.50	2.50	2.50
Liquid choline 70%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin premix[1]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase[3]	-	0.04	0.04	-	-	0.04	0.04	-
β-glucanase[4]	-	-	0.01	0.01	-	-	0.01	0.01
Determined composition (air dried basis):								
ME (kcal/kg)[5]	2,700	2,700	2,700	2,700	2,700	2,700	2,700	2,700
Dry Matter, %	89.81	89.89	90.06	89.84	89.39	89.69	89.89	88.87
Crude protein, %	16.45	16.85	16.95	16.55	14.56	14.56	14.56	14.56
Methionine, %	0.26	0.24	0.24	0.26	0.25	0.21	0.21	0.20
Met+cys, %	0.56	0.55	0.59	0.56	0.51	0.49	0.49	0.48
Lysine, %	0.74	0.70	0.77	0.72	0.75	0.79	0.77	0.82
Tryptophan, %	0.17	0.18	0.20	0.19	0.19	0.17	0.19	0.17
Threonine, %	0.52	0.52	0.54	0.53	0.57	0.54	0.55	0.53
Crude fat, %	3.88	3.69	3.72	3.68	3.71	3.80	3.86	3.66
Crude fibre, %	3.13	2.89	2.82	3.11	3.19	2.89	2.94	3.12
Ash, %	9.23	10.68	9.21	9.55	9.73	10.22	10.37	9.93
Calcium, %	3.49	3.99	3.87	3.49	3.75	3.71	3.76	3.64
Phosphorus (total), %[5]	0.73	0.64	0.62	0.72	0.73	0.62	0.63	0.73

[1] 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.

[2] Supplied per kg idet: Mn 90 mg; Cu 125 mg; Zn 80 mg; I 0.4 mg; Se 0.3 mg; Fe 80 mg.

[3] Natuphos® 5.000L, BASF Canada Inc., supplying 600 FTU phytase per kg diet.

[4] Barlican 25%L, BASF Canada Inc., supplying 8,000 BGU ß-glucanase per kg diet, side activities of hemicellulase and cellulase.

[5] Calculated value.

0.57% less dicalcium phosphate (equivalent to 0.17% available P) than those without phytase. The diets were fed in the mash form and the enzymes were added dry during mixing.

Production trial

The eight experimental diets were fed to layers housed in battery cages (12 in. wide × 16 in. deep), with two layers per cage (0.67 ft^2 per layer). One row of 25 cages represented one replication and there were three replications per diet using a completely randomized design. Egg production was recorded daily, by row. Mortality was recorded as it occurred. Feed consumption, by row, was determined weekly. Individual body weights were recorded at the start and end of the trial. Every two weeks all eggs produced during a two day period were individually weighed and specific gravity determined.

Balance trial

In addition to the production study, 48 layers were individually housed in battery cages. At 3 and 6 weeks after the introduction of the test diets, the individual measurement of body weight change, feed intake and excreta output over a 48 hour period were recorded. For both collection periods, the excreta was collected every 12 hours and frozen. Later, the excreta samples were pooled by bird, dried in a 60℃ oven, ground and analyzed for total nitrogen and phosphorus 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.

On the last day of the second excreta collection, six layers per treatment were sacrificed by cervical dislocation and the left tibias removed for bone ash determination. The small intestines were removed for viscosity determinations. Fat was extracted from the tibia samples using the soxhlet apparatus and the fat-free bone samples were then ashed in a muffle furnace at 600℃ for 18 hours. To obtain 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. Only the supernatant is used in viscosity determinations. Viscosity was measured using a Brookfield Digital Viscometer, Model DV-11+version 2.0. at 42.5 sec^{-1} at 40℃.

Statistical analysis

The weekly egg production and feed consumption data from the eight week trial were pooled into two 4 week periods. The performance and balance data were analyzed as a 2×2×2 factorial (dietary protein × phytase supplementation × β-glucanase supplementation) using the Analysis of Variance procedure of SAS (SAS, 1996). Where significant interactions were noted, the data were analyzed by ANOVA using a completely randomized design. Tukeys multiple range test was used to detect differences between means.

RESULTS

Additions of phytase and β-glucanase alone or in combination to control (normal) protein diets did not affect egg production (table 2). Phytase supplementation to low protein diet significantly decreased egg production in Period 2 and overall period. Addition of phytase and β-glucanase to control protein diets did not affect feed conversion in Period 1 and overall period (table 3). In Period 2, Addition of phytase and β-glucanase alone or in combination to control protein diets tended to improve feed conversion at 2nd period but addition of phytase alone to reduced protein diet decreased feed conversion efficiency at 2nd period. Egg weight at Week 2 was significantly higher in control protein diet than in reduced protein diets but there was no effect of phytase or β-glucanase supplementation alone or in combination (table 4). Phytase supplementation to reduced protein diet decreased egg weight at Week 6. Egg specific gravity (table 5) was not different among treatments. Laying hens fed low protein diets with phytase lost final body weight while other laying hens

Table 2. Hen day egg production of birds fed experimental diets

Diet description				% Production (Total eggs)		
No.	Protein[1]	Phytase[2]	β-glucanase[3]	Period 1	Period 2	Overall
1	17	No	No	84.2	87.5^{ab}	85.8^{a}
2	17	Yes	No	84.8	89.3^{ab}	86.9^{a}
3	17	Yes	Yes	86.7	91.7^{a}	89.1^{a}
4	17	No	Yes	84.3	89.2^{ab}	86.6^{a}
5	13.5	No	No	82.7	87.6^{ab}	85.0^{ab}
6	13.5	Yes	No	81.4	75.1^{c}	78.4^{b}
7	13.5	Yes	Yes	80.6	84.7^{b}	82.6^{ab}
8	13.5	No	Yes	82.8	87.2^{ab}	84.9^{ab}
Overall mean				83.4	86.5	84.9
Overall SEM				0.7	1.1	0.8

[1] Percent crude protein.

[2] With (yes) and without (no) Natuphos supplementation.

[3] With (yes) and without (no) Barlican supplementation.

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

Note: Significant (p<0.05) protein × phytase interaction for overall egg production.

gained weight (table 6). Tibia ash was not affected by dietary enzyme supplementation (table 7). Intestinal viscosity was not affected by supplementation of phytase and β-glucanase alone or in combination to control protein diets while it was significantly decreased when the enzymes were added to reduced protein diet (table 7). Manure N and P levels in Collection 1 and 2 were not consistent with treatments. In Collection 2, however, manure P level was low in low P diets and control protein diets. Manure N level was low in reduced protein diets (table 8). Addition of phytase and β-glucanase to diets did not affect manure N content in Collection 1. In Collection 2, however, manure N content was significantly low in birds fed low protein diet with or without β-glucanase supplementation. Daily output of DM, N and P tended to be low in reduced protein diets (table 9). Supplementation of phytase significantly reduced output of P. Supplemenatation of β-glucanase had no significant effects on any of the parameters measured. Combination of two enzymes had negatively associative effect in terms of P output. Apparent DM and N retention was high in reduced protein diets (table 10). Apparent N retention was significantly reduced by supplementation of phytase to reduced protein diets in Collection 2.

DISCUSSION

The results of this study confirm previous findings (Blair et al., 1976; Klasing, 1993; Summers, 1993) that it is possible to reduce the amount of N excreted by layers by reducing dietary crude protein levels. Reducing crude protein levels from 17% to 13.5% significantly reduced the N content of manure and daily N output with no significant effect on egg production or feed efficiency. There was, however, a slight reduction in average egg weight. A 24.8% reduction in daily N output was observed three weeks into the trial (collection 1) and a 35.6% reduction at six weeks (collection 2).

Phytase supplementation to the control protein diets made it possible to reduce the level of dicalcium phosphate in the diet by almost 70%, with no loss in egg production, feed efficiency, average egg weight or egg specific gravity. Daily P output was reduced by 18% and 32% in collections 1 and 2, respectively. This reduction is due to the low P content of diet and supplementation of phytase. The beneficial effects of

Table 3. Feed conversion ratio of layers fed experimental diets

Diet description				kg feed/doz total eggs		
No.	Protein[1]	Phytase[2]	β-glucanase[3]	Period 1	Period 2	Overall
1	17	No	No	1.819	1.812[ab]	1.816
2	17	Yes	No	1.769	1.730[b]	1.750
3	17	Yes	Yes	1.784	1.732[b]	1.759
4	17	No	Yes	1.784	1.730[b]	1.757
5	13.5	No	No	1.816	1.775[ab]	1.795
6	13.5	Yes	No	1.838	1.901[a]	1.867
7	13.5	Yes	Yes	1.836	1.788[ab]	1.812
8	13.5	No	Yes	1.816	1.772[ab]	1.794
Overall mean				1.808	1.780	1.794
Overall SEM				0.012	0.014	0.012

[1,2,3] As in table 2.
[a,b,c] Means within a column with no common superscript differ significantly ($p<0.05$).
Note: Significant ($p<0.05$) protein × phytase × β-glucanase interaction during period 2.

Table 4. Average egg weight

Diet description				Average egg weight (g)			
No.	Protein[1]	Phytase[2]	β-glucanase[3]	Pre-trial	Week 2	Week 4	Week 6
1	17	No	No	62.6	64.3[a]	64.2[a]	63.8[a]
2	17	Yes	No	62.6	64.2[a]	62.8[b]	63.1[a]
3	17	Yes	Yes	63.3	65.6[a]	63.5[ab]	63.8[a]
4	17	No	Yes	62.7	64.5[a]	62.6[b]	63.0[a]
5	13.5	No	No	62.4	62.0[b]	61.3[cd]	61.8[ab]
6	13.5	Yes	No	62.1	61.6[b]	58.2[e]	57.9[b]
7	13.5	Yes	Yes	62.6	62.3[b]	60.9[d]	62.1[a]
8	13.5	No	Yes	63.2	62.5[b]	62.2[bc]	65.1[a]
Overall mean				62.7	63.4	62.0	62.6
Overall SEM				0.11	0.30	0.37	0.48

[1,2,3] As in table 2.
[a,b,c,d] Means within a column with no common superscript differ significantly ($p<0.05$).

Table 5. Average egg specific gravity

Diet description				Average egg specific gravity			
No.	Protein[1]	Phytase[2]	β-glucanase[3]	Pretrial	Week 2	Week 4	Week 6
1	17	No	No	1.089	1.093	1.088	1.089
2	17	Yes	No	1.089	1.092	1.088	1.088
3	17	Yes	Yes	1.089	1.093	1.089	1.089
4	17	No	Yes	1.088	1.093	1.088	1.089
5	13.5	No	No	1.090	1.093	1.088	1.087
6	13.5	Yes	No	1.089	1.093	1.088	1.088
7	13.5	Yes	Yes	1.089	1.092	1.089	1.088
8	13.5	No	Yes	1.089	1.093	1.088	1.088
Overall mean				1.089	1.093	1.088	1.088
Overall SEM				0.0002	0.0002	0.0002	0.0002

[1,2,3] As in table 2.

Table 6. Average body weight

Diet description				Average body weight (g)		
No.	Protein[1]	Phytase[2]	β-glucanase[3]	Initial	Final	Change
1	17	No	No	1878	1981[a]	103[a]
2	17	Yes	No	1838	1924[a]	86[a]
3	17	Yes	Yes	1880	1985[a]	105[a]
4	17	No	Yes	1816	1929[a]	113[a]
5	13.5	No	No	1864	1894[a]	30[a]
6	13.5	Yes	No	1848	1686[b]	-161[b]
7	13.5	Yes	Yes	1869	1907[a]	38[a]
8	13.5	No	Yes	1864	1927[a]	64[a]
Overall mean				1857	1904	47
Overall SEM				6.7	19.2	18.3

[1,2,3] As in table 2.
[a,b] Means within a column with no common superscript differ significantly ($P<0.05$).

Table 7. Average intestinal viscosity (cp=centipoise) and percent tibia ash

Diet description					
No.	Protein[1]	Phytase[2]	β-glucanase[3]	Intestinal viscosity (cp)	Tibia ash (%)
1	17	No	No	8.8[b]	52.6
2	17	Yes	No	9.9[b]	52.0
3	17	Yes	Yes	6.3[b]	51.9
4	17	No	Yes	6.7[b]	53.4
5	13.5	No	No	16.7[a]	53.6
6	13.5	Yes	No	7.0[b]	55.5
7	13.5	Yes	Yes	7.8[b]	55.3
8	13.5	No	Yes	5.5[b]	55.0
Overall mean				8.7	53.7
Overall SEM				0.6	0.43

[1,2,3] As in table 2.
[a,b] Means within a column with no common superscript differ significantly ($p<0.05$).

phytase supplementation appeared to be more dramatic with time. There was a significant reduction in P excretion when phytase was added to the reduced P and protein diets, but it was at the expense of performance. Egg production was significantly lower in the second four-week period for the layers receiving this diet and there was a slight reduction in average egg weight. In addition, those layers receiving the reduced P and protein diet supplemented with phytase alone, on average, lost weight over the course of the eight week trial while the other layers, on average, gained weight. The loss in production and body weight may have been due to the low protein and low P and interactions with phytase. 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 wheat based diets may not have been as good as expected. In addition, a lower apparent N retention was observed in the second collection for the layers receiving this diet. The cause of this is still unclear.

The apparent P retention values are not shown since P excretion is affected by egg production. When egg shells are being deposited, calcium is mobilized from the medullary bone of birds. When bone calcium is mobilized, phosphorus is also released into the blood stream. Since this phosphorus is not required for shell formation, it is excreted in the manure. This phosphorus excretion resulting from the mobilization of bone calcium will affect phosphorus balance data, sometimes resulting in negative phosphorus balance.

The experimental diets used in this study were wheat based. The use of wheat in poultry diets is hampered by the presence of arabinoxylans which can form a viscous solution in the digesta blocking nutrient absorption and causing sticky droppings. Barlican is a commercial multi-carbohydrase preparation that can be added to feeds to breakdown β-glucans and other NSPs, reduce intestinal viscosity and improve nutrient utilization. Supplementation of the reduced protein diets with Barlican resulted in a major reduction in intestinal viscosity although there was no significant effect on apparent DM or N retention. In addition, there was no significant affect on daily N output, and reduced daily P output was observed in the first collection only. Both phytase (Natuphos®) and β-glucanase (Barlican) supplementation improved the intestinal viscosity of the layers receiving the reduced protein diet. The improvement was greatest when the diet was supplemented with Barlican only. Um et al. (1998) reported that multi-carbohydrase preparation (xylanase 37,150 U, glucanase 27,600 U and cellulase 11,150 U/g) was very effective in improving performance of layers when it was supplemented to wheat based diet. It has been known that predominant NSP of wheat is arabinoxylan while that of barley is β-glucan. The lack of response in the present experiment may be due to the addition of β-glucanase instead of arabinoxylanase.

CONCLUSIONS AND IMPLICATIONS

1. Reducing dietary crude protein from 17 to 13.5% resulted in a significant reduction in daily N output with no significant loss in egg production or feed efficiency.

2. Supplementing diets formulated to current industry norms (17% CP) with phytase resulted in a significant reduction in daily P output with no loss in

Table 8. Composition of manure

	Diet description			Manure N (% of dry matter)		Manure P (% of dry matter)	
	Protein[1]	Phytase[2]	β-glucanase[3]	Collection 1	Collection 2	Collection 1	Collection 2
1	17	No	No	4.17	4.41^{ab}	2.12^{abc}	2.11^{bc}
2	17	Yes	No	4.18	4.62^{a}	2.06^{bc}	1.93^{c}
3	17	Yes	Yes	4.55	4.70^{a}	2.10^{abc}	1.99^{bc}
4	17	No	Yes	4.52	4.74^{a}	2.30^{a}	2.24^{abc}
5	13.5	No	No	3.94	3.74^{cd}	2.10^{bc}	2.46^{a}
6	13.5	Yes	No	4.19	4.36^{ab}	2.00^{c}	2.34^{ab}
7	13.5	Yes	Yes	3.90	4.15^{bc}	2.24^{ab}	2.29^{ab}
8	13.5	No	Yes	3.84	3.55^{d}	1.93^{c}	2.52^{a}
	Overall mean			4.16	4.29	2.11	2.23
	Overall SEM			0.09	0.08	0.03	0.04

[1,2,3] As in table 2.
[a,b,c] Means within a column with no common superscript differ significantly ($p<0.05$).

Table 9. Daily outputs of dry matter, nitrogen and phosphorus

	Diet description			Daily output (g/layer)					
				DM		N		P	
	Protein[1]	Phytase[2]	β-glucanase[3]	Collection 1	Collection 2	Collection 1	Collection 2	Collection 1	Collection 2
1	17	No	No	28.99^{a}	31.40^{a}	1.21^{ab}	1.38^{ab}	0.62^{abc}	0.66^{a}
2	17	Yes	No	26.47^{ab}	26.17^{ab}	1.13^{abc}	1.21^{b}	0.54^{cd}	0.50^{bc}
3	17	Yes	Yes	30.24^{a}	30.92^{a}	1.36^{a}	1.45^{a}	0.64^{ab}	0.62^{ab}
4	17	No	Yes	28.74^{a}	29.25^{a}	1.30^{ab}	1.38^{ab}	0.66^{a}	0.65^{a}
5	13.5	No	No	26.79^{ab}	26.17^{ab}	1.06^{bc}	0.98^{c}	0.56^{bc}	0.64^{a}
6	13.5	Yes	No	21.34^{c}	18.67^{c}	0.91^{c}	0.78^{c}	0.43^{e}	0.42^{c}
7	13.5	Yes	Yes	21.92^{c}	22.53^{bc}	0.86^{c}	0.94^{c}	0.49^{cde}	0.52^{bc}
8	13.5	No	Yes	23.78^{bc}	22.51^{bc}	0.92^{c}	0.79^{c}	0.46^{de}	0.56^{abc}
	Overall mean			26.03	26.36	1.09	1.13	0.55	0.58
	Overall SEM			0.65	0.88	0.04	0.05	0.02	0.02

[1,2,3] As in table 2.
[a,b,c] Means within a column with no common superscript differ significantly ($p<0.05$).

egg production or feed efficiency.

3. While phytase supplementation was beneficial for the layers receiving the control protein diets, when the layers were fed the reduced protein diet supplemented with phytase there was a loss in performance.

4. Supplementation of β-glucanase alone did not appear to have beneficial affects in terms of reducing nitrogen or phosphorus excretion.

5. Combination of two enzymes had no beneficial positive effects on laying performance or reduction of DM, N and P.

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