Feed formulation using phytase in laying hen diets[1]

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Primary Audience: Nutritionists, Flock Supervisors, Researchers

SUMMARY

Lohmann LSL-Lite and Classic Brown laying pullets were reared and caged at the Agassiz Research Centre following recommendations of the management guides. At 30 wk of age, these hens were fed 1 of 5 diets per strain following the Lohmann nutrient recommendations. In addition to a control treatment, diets were formulated to contain 300 or 600 U/kg of phytase, with or without enzyme inclusion, where the phytase was assumed to cause the release of P, Ca, energy, and protein. Diets were changed at 45 wk to follow a phase-feeding program, but treatments remained the same. These dietary changes did not result in major changes in measures associated with P deficiency, likely because the management guides suggest P levels that largely exceed the requirements of the birds. Rather than adding high levels of inorganic P to layer feeds, the safety margin currently included in recommended dietary specifications could be provided by the addition of phytase. This would reduce the negative environmental effects of intensive poultry production that are associated with P excretion.

Key words: layer, phosphorus, phytase, feed formulation

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DESCRIPTION OF PROBLEM

Much of the P in poultry feeds is unavailable to chickens because it is bound in phytate, which serves as the storage molecule for P in seeds [1]. The result is that although poultry feeds contain sufficient P for normal growth and production, inorganic P is added to the feed and excess P is excreted, leading to an oversupply of P in manure that is applied to farmland. Over the past 15 yr, phytase enzymes [2] have been introduced to the poultry feed industry to increase the availability of P from phytate to the bird, thus reducing the environmental costs of poultry production (for a recent review see [3]).

Phytate binds nutrients in addition to P, and the addition of phytase to feed causes the release of these nutrients and allows their absorption by the bird. Nutrients affected by phytates include minerals and protein [1, 4], and phytase has been shown to affect the release of energy [5]. Protein and energy may be the most significant because they represent the greatest nutrient costs in poultry diets. Therefore, although most attention has been on the positive effects of phytase on the release of P, energy and protein should

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be considered when evaluating phytase supplementation.

For the benefits of phytase to be realized, formulation changes are needed that reflect the action of the enzyme on nutrient availability. Research on the use of phytase in broiler nutrition has been extensive [3], but that on layer feeds has been much more limited, despite the importance of P for both bone strength and eggshell strength. This trial evaluated the inclusion of phytase enzyme in layer diets, using matrix values for the enzyme that adjust the formulation for the release of energy, protein, and other nutrients from the feed ingredients, in addition to that of P.

MATERIALS AND METHODS

A total of 480 Lohman LSL-Lite and Classic Brown 1-d-old chicks (240 of each strain) were obtained from a local hatchery [6] and raised in pullet-rearing cages at the Agassiz Research Centre. At 16 wk of age, pullets were housed 3 to a cage with a floor space of 2,250 cm^2, providing 750 cm^2 per bird. At 18 wk, day length was increased from 9 to 14 h to initiate sexual maturity and egg production. Six birds in 2 adjacent cages (3 birds/cage) formed an experimental unit, and there were 8 units for each combination of layer strain and diet. Throughout the pretrial and experimental periods, feed and water were available to allow for ad libitum consumption. Care of the birds followed principles described by the Canadian Council of Animal Care [7] and the protocol was approved by the Animal Care Committee of the Agassiz Research Centre.

Formulation for dietary nutrients followed the nutrient requirements described by the production guides [8] and the nutrient profiles of ingredients described by the National Research Council [9]. Ingredients and nutrients for the rearing and early lay periods are not shown here. Experimental diets were fed from 30 wk of age, with the experimental period divided into a mid (30 to 45 wk) and late (46 to 60 wk) cycle. For each strain and period, a control diet (0) was formulated based principally on corn and soybean meal (Table 1). Experimental diets were formulated by using matrix values provided by Danisco Animal Nutrition [10] for phytase additions of 300 and 600 U/kg (**300+**, **600+**) to layer diets (Table 2). Formulation is based on the amount of material added, so although the total nutrient amounts released by 600 U of phytase/kg were greater than with 300 U of phytase/kg, the nutrient amounts released per gram of material added were less for the greater addition, as can be seen in Table 2. Phytase was added as a mixture with Celite [11], an insoluble ash, and negative control diets (**300−**, **600−**) were formulated for enzyme addition, but included an equal amount of Celite without the enzyme. Phytase activity in the diets was analyzed by Danisco Animal Nutrition, following methods described by Engelen et al. [12].

Body weight was measured just before the diet changes at 30 and 45 wk and at the end of the trial. Feed consumption was measured during 1-wk periods at 29, 44, and 59 wk, and FE was calculated as the grams of feed required to produce 1 g of egg. Measuring feed consumption just before diet changes allowed the hens the longest possible time to adjust to changed nutrient amounts so that it most accurately reflected dietary nutrients. Mortality was recorded throughout the trial.

Egg production was measured for 5 d/wk. Egg quality was measured before experimental treatments were applied (29 wk) and at 43 and 58 wk. At each of these times, eggs were collected for 1 d and stored overnight at 4°C. Each egg was weighed and broken onto a flat surface, and albumen height was measured with a tripod micrometer. The yolk was separated from the albumen and weighed, and the shells were washed in warm water, dried at room temperature for several days, then at 100°C for 4 h, and weighed. Albumen weight was determined by the difference.

Statistical Analysis

Response data were analyzed separately by strain and period. Most were analyzed by using the GLM procedure of SAS [13] with ANOVA that included the diet as a fixed effect. When the model was significant at $P < 0.05$, means were separated by using Duncan's multiple range test. The significance of differences in mortality among groups was tested by using contingency chi-square [14].

Table 1. Diet ingredients (%) and calculated nutrient values for the control (0) and low-phosphorus diets with or without 2 concentrations (300 or 600 U/kg) of phytase

	Lohman LSL-Lite						Lohman Classic Brown					
	Weeks 31 to 45			Weeks 46 to 60			Weeks 31 to 45			Weeks 46 to 60		
Item	0	300	600	0	300	600	0	300	600	0	300	600
Ingredient												
Corn	52.52	52.52	52.52	54.15	54.15	54.15	50.08	50.08	50.08	48.40	48.40	48.40
Barley	0	2.23	2.57	0.50	2.76	3.06	2.45	4.69	4.99	5.64	7.87	8.19
Wheat	10.30	10.30	10.30	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Canola meal	5.00	5.00	5.00	7.50	7.50	7.50	10.68	10.68	10.68	13.00	13.00	13.00
Soybean meal	17.79	16.88	16.75	15.08	14.16	14.02	16.55	15.67	15.56	12.08	11.20	11.09
Calcium phosphorus dibasic[1]	1.34	0.76	0.60	1.29	0.71	0.56	1.23	0.65	0.50	1.03	0.45	0.30
Canola oil	2.20	1.40	1.28	2.37	1.54	1.46	1.42	0.60	0.50	1.83	1.00	0.90
Vitamin-mineral premix[2]	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
NaCl	0.38	0.38	0.38	0.38	0.38	0.38	0.33	0.33	0.33	0.33	0.33	0.33
L-Lysine	0	0.01	0.01	0	0	0.01	0	0	0	0	0	0
DL-Methionine	0.10	0.10	0.10	0.08	0.11	0.11	0.10	0.10	0.10	0.06	0.06	0.06
Limestone	10.15	10.14	10.15	10.43	10.41	10.41	8.94	8.92	8.92	9.41	9.41	9.39
Phytase[3]	0	0.06	0.12	0	0.06	0.12	0	0.06	0.12	0	0.06	0.12
Calculated[4]												
ME (kcal/kg)	2,800	2,758	2,753	2,800	2,758	2,756	2,720	2,686	2,682	2,720	2,686	2,682
CP (%)	16.50	16.32	16.29	16.00	15.82	15.79	17.80	17.62	17.60	16.70	16.51	16.50
Calcium (%)	4.20	4.09	4.06	4.30	4.19	4.16	3.75	3.64	3.61	3.90	3.79	3.76
Nonphytate phosphorus (%)	0.39	0.27	0.24	0.38	0.26	0.23	0.38	0.26	0.23	0.34	0.22	0.19
Methionine	0.37	0.37	0.37	0.35	0.38	0.38	0.40	0.40	0.40	0.35	0.34	0.34
Lysine	0.80	0.79	0.79	0.76	0.75	0.75	0.87	0.85	0.85	0.79	0.77	0.77

[1]A mixture of mono- and dicalcium phosphate containing 18% calcium and 21% phosphorus.

[2]The premix contained (per kilogram of diet): vitamin A, 9,600 IU as retinyl acetate; cholecalciferol, 3,120 IU; vitamin E, 36 IU as DL-α-tocopheryl acetate; menadione, 2.4 mg; vitamin B_{12}, 0.018 mg; riboflavin, 7.2 mg; pantothenic acid, 14.4 mg; niacin, 60 mg; thiamine, 1.2 mg; pyridoxine, 2.4 mg; folic acid, 0.72 mg; biotin, 0.10 mg; zinc, 100 mg; iron, 80 mg; manganese, 100 mg; copper, 12 mg; iodine, 1 mg; selenium, 0.3 mg.

[3]Phyzyme [10] mixed 1:10 in Celite [11], an insoluble ash, or Celite without enzyme.

[4]Calculated nutrient values for diets that included enzyme are the same as those for the control (0) diets, and values calculated for diets without enzyme are shown with the assumption of no enzyme activity.

Table 2. Feed formulation matrix values for phytase with 2 amounts of supplementation of layer rations[1]

Item	300 U/kg (60 g/tonne)	600 U/kg (120 g/tonne)
Total phosphorus (%)	2,105	1,340
Available phosphorus (%)	2,000	1,273
Calcium (%)	1,833	1,167
ME (kcal/kg)	637,174	352,525
Protein (%)	3,031	1,705
Lysine (%)	153	83
Methionine (%)	57	31
Cysteine (%)	116	63
Methionine + cysteine (%)	173	95
Threonine (%)	127	70
Tryptophan (%)	40	22
Isoleucine (%)	133	75
Leucine (%)	304	171
Valine (%)	167	94

[1]Supplied by Danisco Animal Nutrition. The enzyme has matrix values greater than 100% because it causes release of nutrients from other ingredients in the diet.

RESULTS AND DISCUSSION

Formulation changes to account for the nutrient release caused by phytase addition allowed the diets to contain slightly less soybean meal because of the protein value of the enzyme, less canola oil because of the change in energy, and substantially less inorganic P (Table 1). The reductions in soybean meal, canola oil, and inorganic P were accompanied by greater amounts of barley. Adding phytase with the matrix values shown in Table 2 allowed a reduction in the calculated value for AME of 34 to 47 kcal/kg and a reduction in the calculated value for protein of 0.18 to 0.21% for the 300+ and 600+ treatments, respectively. Most significantly, the formulation changes resulted in a reduction in available P (before phytase was considered) of 0.12% with 300 U/kg and of 0.15% with 600 U/kg.

The endogenous phytase in unsupplemented diets was approximately 100 U/kg (73.0 to 128.5 U/kg; Table 3). Scott et al. [15] reported that the corn-based diets they studied contained 46 phytase units/kg and that the wheat-based diets contained 516 phytase units [16] of endogenous phytase. Although corn has low levels of phytase, the diets used in this trial included 8.00 and 10.30% wheat, which likely contributed to greater endogenous phytase levels. The levels of phytase measured in the 300+ and 600+ diets reflected the amounts of enzyme added.

Symptoms of inadequate P for laying hens are high mortality, low feed intake and BW gain, and low egg production [17–20]. Mortality in this trial was 9.8% over the 30-wk experimental period (45 of 458 hens) but was not affected by the diets and was very similar for the 2 strains (24 of 234 LSL White and 21 of 224 Brown Classic). Within each treatment, mortality ranged from 2 hens (of 48) in the LSL control treatment to 8 (of 48) in the LSL 300− treatment.

Feed consumption of these hens (Table 4) was not affected by treatment in the first series of experimental diets (wk 44 to 45), but appeared to be affected by the second series (wk 59 to 60). Feed consumption of hens given the 600− treatment (adjusted for phytase without phytase addition) was greater than feed consumption of those fed the control diet (LSL-Lite) or the 600+ (adjusted for phytase with phytase addition) diet (Brown Classic). Hens are very sensitive to the

Table 3. Phytase activity in laying diets[1,2]

		Phytase activity measured (U/kg)			
		Weeks 31 to 45		Weeks 46 to 60	
Diet	Phytase added	LSL-Lite	Classic Brown	LSL-Lite	Classic Brown
Control	0	102.5	101.5	107.0	99.5
300−	0	128.5	114.5	73.0	109.0
300+	300	375.5	225.5	393.0	356.0
600−	0	113.5	117.5	91.5	103.0
600+	600	494.5	478.5	698.0	538.0

[1]Each measure represents the average of 2 diet mixes. The treatments "300−" and "600−" indicate the modified diets without phytase, and the treatments "300+" and "600+" indicate the modified diets with added phytase.

[2]Measured by Danisco Animal Nutrition [10] following the method of Engelen et al. [12].

Table 4. Feed consumption and FE for hens fed adequate (control) and low-phosphorus diets with or without phytase[1]

	Daily feed consumption (g/d)			FCR (g of feed/g of egg)		
Item	Weeks 29 to 30	Weeks 44 to 45	Weeks 59 to 60	Weeks 29 to 30	Weeks 44 to 45	Weeks 59 to 60
LSL-Lite						
Control	100.0	102.9	105.0[b]	1.881	1.925	2.185
300−	101.7	102.9	108.5[ab]	1.877	1.824	1.966
300+	103.4	104.5	106.7[ab]	1.925	1.903	1.963
600−	100.1	100.8	112.3[a]	1.894	1.808	2.120
600+	99.9	102.6	106.0[ab]	1.843	1.953	1.912
SEM	2.1	2.5	2.0	0.040	0.048	0.108
Brown Classic						
Control	105.6	109.9	115.2[ab]	2.011	1.975	2.331
300−	104.6	109.9	118.6[ab]	1.962	2.018	2.336
300+	106.0	113.0	115.1[ab]	1.944	2.112	2.211
600−	104.2	112.2	138.9[a]	1.984	2.048	2.441
600+	106.0	115.0	113.2[b]	1.887	2.096	2.191
SEM	2.0	3.5	7.8	0.050	0.075	0.146

[a,b]Means within each strain with different letters are different at $P < 0.05$.

[1]Each mean represents 8 two-cage units with 6 birds per unit, reduced by mortality. The treatments "300−" and "600−" indicate the modified diets without phytase, and the treatments "300+" and "600+" indicate the modified diets with added phytase.

dietary energy level [21] and regulate their feed intake according to their requirements [22, 23]. The hens in the 600− treatments may have eaten more to compensate for lower levels of dietary energy, and the addition of phytase should allow the hens to obtain the required energy with less feed. No differences in FE were seen between treatments.

Despite the slightly greater feed consumption of hens in the 600− treatment, the Brown Classic hens in this treatment weighed less (along with those in the 600+ treatment) at 45 and 60 wk than those fed the control diets (Table 5). The Brown Classic hens in the 300− group also weighed less than those in the control group at 45 wk. Body weight gains of Brown Classic hens in the

Table 5. Body weight and BW gain of hens fed adequate (control) and low-phosphorus diets with or without phytase[1]

	BW (g)			BW gain (g)		
Item	Week 30	Week 45	Week 60	Weeks 30 to 45	Weeks 45 to 60	Weeks 30 to 60
LSL-Lite	(44–48)[1]	(42–47)	(38–46)	(42–47)	(38–46)	(38–46)
Control	1,531	1,632	1,661	99	29.6	128.4
300−	1,527	1,599	1,634	67	34.9	102.9
300+	1,536	1,619	1,633	81	21.2	99.1
600−	1,504	1,577	1,617	67	30.0	96.2
600+	1,527	1,615	1,657	79	36.0	116.9
SEM	18	24	25	12.1	14.6	15.5
Brown Classic	(41–47)	(38–44)	(36–43)	(38–44)	(36–43)	(36–43)
Control	1,976	2,150[a]	2,144[a]	168.1[a]	−4.2	169.5
300−	1,922	2,051[bc]	2,072[ab]	129.9[ab]	20.4	149.4
300+	1,989	2,137[ab]	2,115[ab]	168.2[a]	−17.8	140.7
600−	1,921	2,019[c]	2,027[b]	90.4[b]	−8.7	89.0
600+	1,909	2,034[c]	2,012[b]	116.5[ab]	−10.5	103.5
SEM	28	33	36	20.1	17.2	24.6

[a–c]Means within each strain with different letters are different at $P < 0.05$.

[1]The number of hens in each group is shown in brackets. The treatments "300−" and "600−" indicate the modified diets without phytase, and the treatments "300+" and "600+" indicate the modified diets with added phytase.

Table 6. Egg production of hens fed adequate (control) and low-phosphorus diets with or without phytase[1]

Item	Hen-day egg production (%)		
	Weeks 26 to 30	Weeks 31 to 45	Weeks 45 to 60
LSL-Lite			
Control	95.2	94.9	85.2[b]
300−	96.7	95.4	90.3[a]
300+	96.5	95.8	91.7[a]
600−	94.1	93.9	89.0[ab]
600+	97.0	95.2	91.3[a]
SEM	0.6	0.3	0.6
Brown Classic			
Control	91.4	89.5	83.4
300−	94.9	91.6	87.6
300+	93.7	91.3	85.5
600−	92.5	95.0	91.1
600+	97.5	93.6	87.9
SEM	1.0	1.4	1.1

[a,b]Means within each strain with different letters are different at $P < 0.05$.

[1]Each mean represents 8 two-cage units with 6 birds per unit, reduced by mortality. The treatments "300−" and "600−" indicate the modified diets without phytase, and the treatments "300+" and "600+" indicate the modified diets with added phytase.

600− treatment, but not the 600+ treatment, were lower between wk 30 and 45 than those in the control group and the 300+ group. Body weight gain more accurately measures feed use because it removes random variation in BW. There were no differences between treatment groups in BW or BW gains for LSL-Lite hens.

Between wk 45 and 60, egg production (Table 6) of LSL-Lite hens in the control group was lower than that of hens in other groups, except for the 600− group, from which it did not differ. Other differences in egg production were not significant. Egg quality (Table 7) measurements showed that LSL-Lite hens fed the 600+ diet laid smaller eggs at wk 58 than those in other groups and that this was due to a reduction in the weight of the albumen. We have no immediate explanation for the observation of significant differences in egg numbers or egg weights.

Although several significant differences were found that could relate to P deficiency in the 600− diets, with phytase eliminating the difference, it is clear that there were no major effects of the dietary treatments, and a more likely ex-

Table 7. Albumen height and weight of eggs from hens fed adequate (control) and low-phosphorus diets with or without phytase[1]

Item	Week 43					Week 58				
	Albumen height (mm)	Egg weight (g)	Shell weight (g)	Yolk weight (g)	Albumen weight (g)	Albumen height (mm)	Egg weight (g)	Shell weight (g)	Yolk weight (g)	Albumen weight (g)
LSL-Lite	(35–40)	(38–43)[2]				(36–42)	(36–41)			
Control	7.67	59.04	5.68	16.53	36.83	7.55	61.33[a]	5.58	17.23	38.52[a]
300−	7.72	57.83	5.54	16.03	36.25	7.41	60.84[a]	5.66	16.82	38.36[a]
300+	7.55	58.94	5.56	16.30	37.08	7.21	60.77[a]	5.60	17.16	38.01[a]
600−	7.70	58.05	5.66	16.26	36.13	7.41	60.32[a]	5.76	17.21	37.35[ab]
600+	7.60	58.17	5.61	16.51	36.05	7.32	58.57[b]	5.58	16.62	36.38[b]
SEM	0.14	0.64	0.07	0.20	0.50	0.14	0.62	0.08	0.23	0.48
Brown Classic		(35 – 41)		(35–40)	(35–40)	(34–41)	(34–42)			
Control	6.33	61.19	5.94	16.56	38.70	5.96	61.74	5.84	16.79	39.11
300−	6.38	61.96	5.92	16.48	38.59	6.08	62.22	5.99	16.81	39.42
300+	6.37	60.21	5.85	15.98	38.39	6.37	61.12	5.84	16.33	39.05
600−	6.53	59.48	5.76	15.86	37.87	6.28	60.87	5.68	16.46	38.73
600+	6.27	60.87	5.82	16.19	38.86	5.83	60.56	5.81	16.32	38.43
SEM	0.14	0.70	0.09	0.26	0.51	0.15	0.86	0.10	0.25	0.68

[a,b]Means within each strain with different letters are different at $P < 0.05$.

[1]Differences between diets were not significant at wk 29, and these data are not shown. The treatments "300−" and "600−" indicate the modified diets without phytase, and the treatments "300+" and "600+" indicate the modified diets with added phytase.

[2]The sample sizes are shown in brackets above the columns for egg weight. If the sample size differed from that of egg weight, it is provided above the column.

planation for these differences may be random chance. The NRC [9] recommends a level of 0.25% nonphytate P with feed consumption of 100 g/hen per day. This amount has been confirmed in several studies, and several [15, 17–19, 24] have suggested that the true requirements may be even lower. Keshavarz [25] found that nonphytate P levels of 0.25, 0.20, and 0.15% in a phase-feeding program were adequate, although Keshavarz [26] suggested that there were differences between strains. In the poultry industry, however, inclusion of much greater levels of nonphytate P in layer feeds is recommended. Coon [27] suggested including 0.42% nonphytate P, and the management guides that were followed here (Lohmann) recommend 0.34 to 0.39%, depending on the strain and age of the hens.

CONCLUSIONS AND APPLICATIONS

1. The excess of nonphytate P in layer diets was included to ensure that levels were adequate for all strains and to compensate for variation in P in dietary ingredients. As such, the excess provided a margin of safety.
2. Phosphorus levels could be reduced and the safety margin could instead be provided by the inclusion of phytase.
3. Phytase provides the additional benefit of releasing nutrients other than P, especially energy and protein, which allows for a reduction in the amount of high-value ingredients in the complete feed.

REFERENCES AND NOTES

1. Ravindran, V., W. L. Bryden, and E. T. Korngegay. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. Avian Poult. Biol. Rev. 6:125–143.

2. Liu, B. L., A. Rafiq, Y. M. Tzeng, and A. Rob. 1998. The induction and characterization of phytase and beyond. Enzyme Microb. Technol. 22:415–424.

3. Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

4. Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilisation. Nutr. Res. Rev. 13:255–278.

5. Scott, T. A., R. Kampen, and F. G. Silversides. 2001. The effect of phytase in nutrient-reduced corn-and wheat-based diets fed to two strains of laying hen. Can. J. Anim. Sci. 81:393–401.

6. Pacific Pride Hatchery, Abottsford, British Columbia, Canada.

7. Canadian Council of Animal Care (CCAC). 1993. Guide to the Care and Use of Experimental Animals. 2nd ed. Vol. 1. CCAC, Ottawa, Ontario, Canada.

8. Lohmann LSL-Lite Layer Management Guide 05/04, and Lohman Brown Classic Layer Management Guide B103/E, Lohmann Tierzucht, Cuxhaven, Germany.

9. NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

10. Danisco Animal Nutrition, Marlborough, Wiltshire, UK.

11. Celite Corp., Lompar, CA.

12. Engelen, A. J., F. C. Van der Heeft, P. H. G. Ransdorp, W. A. C. Somers, J. Schaefer, and J. C. Van der Vat. 2001. Determination of phytase activity in feed by a colorimetric enzymatic method: Collaborative interlaboratory study. J. AOAC Int. 84:629–633.

13. SAS Institute. 2003. Version 9.1. SAS Inst. Inc., Cary, NC.

14. Zar, J. H. 1999. Biostatistical Analysis. 4th ed. Prentice-Hall, Upper Saddle River, NJ.

15. Scott, T. A., R. Kampen, and F. G. Silversides. 1999. The effect of phosphorus, phytase enzyme, and calcium on the performance of layers fed corn-based diets. Poult. Sci. 78:1742–1749.

16. Scott, T. A., R. Kampen, and F. G. Silversides. 2000. The effect of phosphorus, phytase enzyme, and calcium on the performance of layers fed wheat-based diets. Can. J. Anim. Sci. 80:183–190.

17. Owings, W. J., J. L. Sell, and S. L. Balloun. 1977. Dietary phosphorus needs of laying hens. Poult. Sci. 56:2056–2066.

18. Usayran, N., and D. Balnave. 1995. Phosphorus requirements of laying hens fed on wheat-based diets. Br. Poult. Sci. 36:285–301.

19. Punna, S., and D. A. Roland Sr. 1999. Influence of supplemental microbial phytase on first cycle laying hens fed phosphorus deficient diets from one day of age. Poult. Sci. 78:1407–1411.

20. Francesch, M., J. Broz, and J. Brufau. 2005. Effects of an experimental phytase on performance, egg quality, tibia ash content and phosphorus bioavailability in laying hens fed on maize- or barley-based diets. Br. Poult. Sci. 46:340–348.

21. Summers, J. D., and F. R. Robinson. 1995. Comparative feeding programs for poultry reproduction. Pages 319–358 in Poultry Production. P. Hunton, ed. Elsevier, New York, NY.

22. Scott, T. A., F. G. Silversides, D. Tietge, and M. L. Swift. 1999. Effect of feed form, formulation, and restriction on the performance of laying hens. Can. J. Anim. Sci. 79:171–178.

23. Harms, R. H., G. B. Russell, and D. R. Sloan. 2000. Performance of four strains of commercial layers with major changes in dietary energy. J. Appl. Poult. Res. 9:535–541.

24. Boling, S. D., M. W. Douglas, M. L. Johnston, X. Wang, C. M. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. 1997. Supplemental phytase improves performance of

laying hens consuming diets with low levels of available phosphorus. Poult. Sci. 76(Suppl. 1):5. (Abstr.)

25. Keshavarz, K. 2000. Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. Poult. Sci. 79:748–763.

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

27. Coon, C. N. 2002. Feeding commercial egg-type layers. Pages 267–285 in Commercial Chicken Meat and Egg Production. 5th ed. D. D. Bell and W. D. Weaver Jr., ed. Kluwer Academic Publishers, Norwell, MA.

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