Journal of Animal and Veterinary Advances 8 (7): 1461-1465, 2009
ISSN: 1680-5593

Effect of Phytase Microbial in Laying Hens Diets on Their Performance on Absorption and Use of Phosphorus

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Abstract: The phytase manufactured by microorganism fermentations, have an effects liberating phosphorus of phytic acid of feedstuffs. This study was designed to determine the effect of microbial phytase on feeding laying hens and determine availability and performance. Test diets on a corn-soy base were fed for 30 days with six treatments with three levels of phytase (0, 300 and 600 UF) with and without additional phosphorus, in the first experiment. Same diets were used in the second trial and last 7 days, to determine phosphorus apparent absorption. The result showed a feed intake was increased by phytase contents ($p<0.0004$) and levels of phosphorus ($p<0.05$) and both interaction ($p<0.015$). Daily body weight changes with similar effect where with phytase level ($p<0.03$), phosphorus level ($p<0.0007$) and their interaction. On egg production was showed similar effect of phytase, phosphorus and an interaction ($p<0.005$; 0.0001; 0.0001, respectively). On egg weight produced, no effect was observed by phytase or phosphorus ($p>0.05$). In the trial of apparent phosphorus absorption, P intake (g day^{-1}) was influenced by phytase level ($p<0.0001$), phosphorus level ($p<0.0001$) and their interactions ($p<0.0001$). The same effects were showed on P excretion, by P level ($p<0.0001$) and phytase ($p<0.0001$). The P digestibility quantified as percent, was high on treatments with highest P level ($p<0.002$) and influenced by phytase ($p<0.01$). In conclusion, phytase and phosphorus level on laying hens diets, feed intake and body weight changes, increase. Phosphorus retention and apparent phosphorus digestibility were increased by phytase and phosphorus.

Key words: Laying hens, performance, phytase, phosphorus, phosphorus absorption

INTRODUCTION

Phosphorus in vegetables, is partial digestible. Phosphorus digestible forms represents 30-40% of total phosphorus (NRC, 1994). The rest of phosphorus is present as phytic phosphorus, which has low digestibility. On the other hand, only 10% of the phytic phosphorus in corn and wheat is digested by the bird (Nelson, 1976). It is considered that phosphorus of animal tissues and inorganic supplement, is very utilized for birds and pigs (NRC, 1994). Use of microbial phytase in feeding poultry gives as result of phytate hydrolysis, releasing it, in phosphate form NRC (1994).

Levels of several minerals in soils are in marginal states, some of them are nitrogen and phosphorus (Roland *et al*., 2003). Nitrogen can be taken by micro-organisms of floor and reused to form nitrogenous compounds for its environment (Nelson and Cox, 2000). Phosphorus supply in inorganic form in diet therefore, is also excreted by birds in same way, which represents a hazard for the environment (Roland *et al*., 2003).

Hamilton and Sibbald (1977) were interested to reconsider the effect of reducing the P level when increasing age, with the aim of diminishing the environmental contamination. Consequently, it was necessary to determine if P inferior levels, when the levels of calcium in feed for laying hens are high, it did not affect egg production.

Addition of microbial phytase in diets for pigs (Young *et al*., 1993), broiler chicks (Mitchell and Edwards, 1996) and turkeys (Qian *et al*., 1996) have been investigated in order to see benefit effect of phytic phosphorus liberated of ingredients in diet. In laying hens has been worked with phytase of genetics modified microorganisms, but not with phytase produced commercially by fermentation of a solid state (Roland *et al*., 2003). They found differences in the use of phytic P, when phytase comes from different strains (Punna and Roland, 1999; Edwards, 1983).

Objective of this experiment were to evaluate egg production, intake and feed efficiency of laying hens fed

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with diets with and without phytase, immediately after the posture peak (30 weeks of age) and in a second trial was to calculate phosphorus availability in these diets.

MATERIALS AND METHODS

Diets: The feed was formulated for laying hens on peak, according to NRC (1994), with 2.700 kcal EM kg^{-1}; 17% crude protein, Ca and P, 3.9 and 0.4%, respectively. Feed intake, measured as total quantity of feed, was divided in two rations during the day. The amount feed was 110 g day^{-1}. The feed rejected was weighed every morning to calculated daily intake. Treatments were:

- Without phosphorus and without phytase
- Without phosphorus + 300 U of phytase
- Without phosphorus + 600 U of phytase
- With phosphorus and without phytase
- With phosphorus and 300 U of phytase
- With phosphorus and 600 U of phytase

Feeding trials: Two hundred sixteen laying hens Babcock line at 36 weeks old were assigned in six treatments with three replications of 12 birds each, on 2×3 factorial design for statistical analyses (SAS, 1997). Birds were placed on cages during time of experiment and birds were vaccinated since newborn to laying period. Cages have feeders and water facilities. Time of the study was 5 weeks, 1st week was an adaptation period and the rest of time to record variables and bird parameters.

Body weights change: The hens were weighed on the 1st day of experiment and then every 7 days, until the end of experimental periods. Data determined in g day^{-1}, are the reflex of the energetic balance on birds.

Egg size and production: The eggs produced in each cages, were recollected and weighed. Daily production was determined by the number and weight of the eggs. In each weighting, was used a Sartorius digital scale 12 kg capacity.

Feed efficiency: It was calculated with quantity of diet (kg) required to produced one kilogram of eggs.

Apparent phosphorus availability trial: After production test period, another trial was made with three birds by replicate and with two on each treatment, in order to calculate phosphorus availability. During 4th days, the bird feces were collected from cages that were adapted for the total feces collection. Representative samples were identified, weighed and frozen for later analyzes. The samples were analyzes at the end of 3 days collection period, all feces and feed samples were dried in an air-draft oven at 55°C for 96 h and ground though 1 mm sieve in Wiley mill, feces were pooled and feed samples were dried in a draft oven 105°C to determine DM contents (AOAC, 1997). Phosphorus content of feces and feed were determined by the method modified by Dickman and Bray (1940). Later samples were made ash on muffle. On P determinations concentration was used a spectrophotometer in 800 nm.

Statistical analysis: All data were statistically analyzed using SAS software and at 5% of significance level.

RESULTS AND DISCUSSION

Table 1 and 2 show averages for period, P level and phytase on feed intake, daily body weight changes, egg production and weight eggs produced. Feed intake during 1st week was not increased by phytase contents ($p>0.05$), but not by phosphorus ($p<0.024$). In the 2nd week of study an effect was presented on feed intake due to phosphorus ($p<0.0002$) and phytase level ($p<0.0095$); in 3rd week similarly effects seen by phosphorus ($p<0.00001$) and phytase ($p<0.0165$). At 4th week, feed intake also was similarly: phosphorus ($p<0.0001$) and phytase ($p<0.0061$), where better intake was seen in treatments with phosphorus and 600 U of phytase. The analyses of total period show an effect of levels phosphorus ($p<0.0004$) and phytase ($p<0.05$) and also interaction ($p<0.015$).

Body weight changes were not affected ($p<0.05$) for neither levels of phosphorus or phytase during 1st and 2nd week. In 3rd week, body weigh changes of birds was affected by phosphorus ($p<0.001$) and phytase ($p<0.00001$) and in both interaction ($p<0.029$); similarly effects were observed in 4th week, where phosphorus ($p<0.0078$), phytase ($p<0.0002$) and both interaction ($p<0.04$). In total period P level ($p<0.0007$), phytase ($p<0.03$) and the interaction both ($p<0.042$) showed an effect on weight birds by highest levels of phosphorus and phytase.

Eggs production showed in Table 2, contents the number of eggs produced in average of laying hens in the study. On 1st and 2nd week was not affected by neither, phosphorus or phytase ($p<0.05$). Also, on 3 weeks wasobserved the effect of phytase and phosphorus and both interactions (phytase: $p<0.0008$; phosphorus: $p<0.0001$; interaction, $p<0.03$). Fourth week effect was similarly observed (phytase: $p<0.005$; phosphorus: $p<0.0001$; interaction: $p<0.04$). On total period similar effects was showed on egg production (phytase: $p<0.005$; phosphorus: $p<0.0001$; interaction: $p<0.0001$). Linear

Table 1: Effects of phytase and phosphorus level, on feed intake, daily weight change and feed efficiency of laying hens

	Phosphorus		Phytase (U kg^{-1})				p-value	
Item/week	Low	High	0	300	600	SEM[a]	Phosphorus	Phytase
Feed intake								
1	92.165	102.98	98.300	94.920	99.495	1.7962	0.0237	0.5876
2	92.157	100.95	93.890	95.620	100.160	0.5586	0.0002	0.0095
3	86.880	101.07	91.495	92.102	98.328	0.7218	0.0001	0.0163
4[1]	86.658	100.65	89.005	93.612	98.343	0.7361	0.0001	0.0061
Total	89.465	101.41	93.173	94.064	99.081	0.8245	0.0004	0.0533
Daily gain (g day^{-1})								
1	4.640	4.81	4.760	4.650	4.760	0.1143	0.5018	0.9118
2	5.200	5.76	5.210	5.460	5.770	0.1385	0.0898	0.3238
3[2]	4.550	5.94	4.860	5.140	5.720	0.0485	0.0001	0.0010
4[3]	4.680	5.91	4.950	5.130	5.810	0.0752	0.0002	0.0078
Total[4]	4.760	5.61	4.950	5.100	5.520	0.0660	0.0007	0.0298
Feed efficiency								
1[5]	2.310	2.52	2.380	2.410	2.460	0.0490	0.0739	0.8338
2	2.100	2.03	2.180	2.060	1.950	0.0539	0.5495	0.2862
3	2.120	1.94	2.210	1.950	1.920	0.0352	0.0490	0.0268
4	2.120	1.92	2.050	2.080	1.920	0.0360	0.0321	0.2150
Total	2.260	2.10	2.210	2.280	2.060	0.1122	0.1298	0.2188

[a]SEM: Standard Error of the Mean; [1]P×π (p<0.015); [2]P×π(p<0.03); [3]P×π (p<0.05); [4]P×π (p<0.042); [5]P×π (p<0.01)

Table 2: Effects of phytase and P level on egg production and weight in laying hens

	Phosphorus		Phytase (U kg^{-1})				p-value	
Item/week	Low	High	0	300	600	SEM[a]	Phosphorus	Phytase
Egg weigh (g)								
1	60.81	60.17	60.70	60.32	60.45	0.6031	0.6148	0.9658
2	58.28	60.87	58.37	59.88	61.96	0.3522	0.0652	0.0168
3	62.57	61.33	61.47	61.81	62.58	0.4946	0.2578	0.6634
4	61.85	62.37	62.07	62.43	61.82	0.4905	0.6151	0.8786
Total	61.13	61.19	60.66	61.11	61.70	0.3086	0.9288	0.4331
Egg number/week								
1	4.65	4.80	4.78	4.64	4.76	0.1140	0.5094	0.8631
2	5.19	5.77	5.21	5.47	5.76	0.1385	0.0832	0.3288
3[1]	4.55	5.94	4.85	5.15	5.73	0.0477	0.00001	0.0008
4[2]	4.67	5.91	4.94	5.11	5.82	0.0707	0.0001	0.0052
Total[3]	4.76	5.61	4.94	5.09	5.52	0.26248	0.0001	0.0006

[a]SEM: Standard Error of the Meanl; [1]P×π (p<0.04); [2]P×π (p<0.04); [3]P×π (p<0.0001)

effect was observed on egg production (3 weeks: p<0.0003; 4th week: p<0.002). Figure 1 showing egg production, where 1 and 2 treatments were similar until the experimental ends. Treatments 3-6 were similar in the number of eggs production, but 3 and 4 were better.

No effects were seen on eggs weight by phosphorus or phytase diets (p>0.05). But on 2nd week, phytase influenced, where treatment number 6 (600 UF), was better (p<0.017).

Apparent phosphorus absorption: Main effects of phosphorus and phytase on P absorption showed in Table 3. The P intake (g day^{-1}), was influenced by phosphorus level (p<0.0001) and phytase on diets (p<0.0001), similarly interaction effects (p<0.0001) was observed. Same effects were showed on P excretion, P level (p<0.0001) and phytase (p<0.0001).

Phosphorus retention (g day^{-1}) similarly was influenced by phosphorus (p<0.003) and phytase in diets

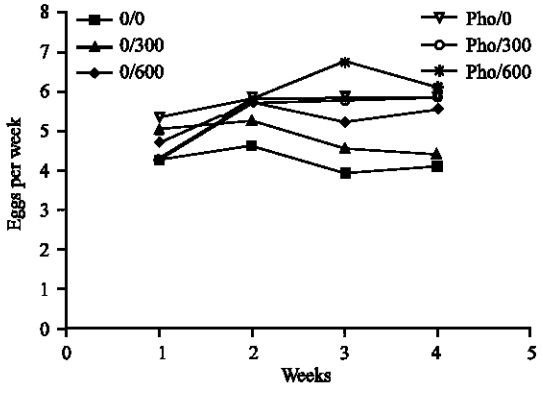

Fig. 1: Effect of the diets with and without phosphorus added with several phytaselevels offered to laying hens in study

(p<0.05) and their interaction (p<0.008). The P retention evaluated in percent, also was affected by phosphorus (p<0.0001) and phytase (p<0.001).

Table 3: Effects of phosphorus and phytase in phosphorus absorptions trials

	Phosphorus		Phytase (U kg^{-1})				p-value	
Items	Low	High	0	300	600	SEM[a]	Phosphorus	Phytase
P intake (g day^{-1})[1]	0.701	0.852	0.760	0.766	0.803	0.0020	0.0001	0.0001
P excreted (g day^{-1})	0.382	0.560	0.425	0.458	0.530	0.0053	0.0001	0.0001
DMI (mg g^{-1})	4.158	0.765	3.427	2.460	1.497	0.3767	0.0040	0.1930
P retention (g day^{-1})[2]	0.259	0.301	0.299	0.281	0.260	0.0060	0.0030	0.0500
Intake (%)	52.59	41.43	51.74	48.24	41.06	0.6850	0.0001	0.0010
P digestibility (%)	12.47	38.75	10.66	28.64	37.52	2.5785	0.0020	0.0140

[a]SEM: Standard Error of the Mean; [1]P×π ($p<0.0001$); [2]P×π ($p<0.008$); DMI: Dry Matter Intake

The P digestibility quantified as percent, was high on treatments with highest P level ($p<0.002$) and influenced by phytase with 600 U kg^{-1} of diet ($p<0.01$).

In present study, laying hens with several phytase levels and P contents on the feed, increasing feed intake and at same time showed interaction and linear effect. The weigh changes in laying hens, also was influenced by phytase, phosphorus and both interactions. These results were similar with experiments of Um and Paik (1999), where feed intake was increased, but the feed efficiencies were not different. Differences between works were the age of the laying hens (21 at 40 weeks) and phytase levels (500 U kg^{-1}) were different.

Eggs production on experimental time also was affected by phytase and phosphorus. Weigh eggs were not modified by any mentioned factors. Although, similar effects were found by Um and Paik (1999). By another way, Scott *et al.* (1999) research, when they used 0, 250 and 500 U phytase kg^{-1} of diet, were found an association between body weigh compensation and eggs production.

The research of Boling *et al.* (2000) agrees with similar conclusion to present research. In another similar experiment reported by Carlos and Edwards (1998), where body weigh changes were increased by supplement of phytase. Reports of Keshvarz and Nakajima (1993) were different from this research on egg production, feed intake and efficiency were not influenced by phytase addition.

Apparent P absorption was similar, such as the reported by Carlos and Edwards (1998), Keshavarz (2000) and Um and Paik (1999). The P excretions were similar as reported by Keshvaraz (2000) i.e., were lowest in the treatments with low phosphorus. However, the retention of P was different, presently work with an increase as long as in g day^{-1} or when it was quantified by percent.

For another hand, some scientific reports say, the supplementation of phytase on diets of laying hens causes the release of phosphorus of phytate and it becomes available to be used in metabolism of bird. Thus, the phosphorus in diet can be reduced and then phosphorus in feces are reduced (Kornegay, 2001).

CONCLUSION

Feed intake and the body weight change of laying hens were increased by phytase and P additions and their interaction. Feed intake and change of corporal weight of laying hens were influenced by increment of phytase and phosphorus quantity.

Egg weight and egg produced were not increased by the phytase and the phosphorus. P intake and their excretion were also affected by both. But the phosphorus intake only had effect on excretion when measuring it in mg g^{-1} of dry matter intake. Retention of P calculated in test was increased by phytase and phosphorus in diet. Apparent P digestibility was influenced by the phytase, phosphorus and the both interaction.

Use of the phosphorus was influenced by phytase addition and phosphorus level in the diets.

REFERENCES

AOAC, 1997. Official Methods of Analysis. 16th Edn. Association of Official Analytical Chemists, Arlington, VA.

Boling, S.D., M.W. Douglas, R.B. Shirley, C.M. Parsonsand and K.W. Koelkebeck, 2000. The effects of various dietary levels of phytase and available phosphorus on performance of laying hens. Poult. Sci., 79: 535-538. PMID: 10780650. http://ps.fass.org/cgi/reprint/79/4/535.

Carlos, A.B. and H.M. Edwards Jr., 1998. The effects of 1.25-Dihydroxycholecalciferol and phytase on the natural phytate phosphorus utilization by laying hens. Poult. Sci., 77: 850-858. PMID: 9628533. http://ps.fass.org/cgi/reprint/77/6/850D.

Dickman, S.R. and R.H. Bray, 1940. Colorimetric determination of phosphate. Ind. Eng. Chem. Anal. Edn., 12 (11): 665-668. DOI: 10.1021/ac50151a013.

Edwards, H.M. Jr., 1983. Phosphorus I. Effects of breed and strain an utilization of suboptimal levels of phosphorus on the ration. Poult. Sci., 62: 77-84. PMID: 6828417.

Hamilton, R.M.G. and I.R. Sibbald, 1977. The effect of dietary phosphorus on productive performance and egg quality of ten strains of white Leghorn. Poult. Sci., 56: 1221-1228. http://www.poultryscience.org/psindexsearch.asp#results:208.

Keshavarz, K., 2000. Non phytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. Poult. Sci. 79: 748-763. PMID: 10824965. http://ps.fass.org/cgi/reprint/79/5/748.pdf.

Keshvarz, K. and S. Nakajima, 1993. Re-evaluation of calcium and phosphorus requirement of laying hens for optimum performance and eggshell quality. Poult. Sci., 72: 144-153. http://www.poultryscience.org/psindexsearch.asp#results:18.

Kornegay, E.T., 2001. Digestions of phosphorus and other nutrients: The role the phytases and factors influencing they activity. Enzimes in farm animal nutrition. CAB international, London, pp: 406. ISBN: 9780851993935.

Mitchell, R.D. and H.M. Edwards Jr., 1996. Effects of phytase and 1.25-Dihydroxycholecalciferol on phytate utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poult. Sci., 75: 95-110. PMID: 8650118. http://www.poultryscience.org/psindexsearch.asp?search=Year&number=1996#results:16.

Nelson, D.L. and M.M. Cox, 2000. Lenhinger, Principles of Biochemistry. 3rd Edn. Worth Edn. Co. USA. ISBN: 1-57259-931-6.

Nelson, T.S., 1976. The hydrolysis of phytate phosphorus by chicks and laying hens. Poult. Sci., 55: 2262-2264. PMID: 1019083. http://www.poultryscience.org/psindexsearch.asp#results:360.

NRC (National Research Council), 1994. Requirements of Poultry. Nutrient Requirements of Domestic Animals. 9th Rev. Edn. National Academy Press. Washington, D.C.NRC.ISBN: 0-309-04892-3.http://books.nap.edu/openbook.php?record_id=2114.

Punna, S. and D.A. Roland Sr., 1999. Variation in the utilization of phytase phosphorus within the same strain of broilers. J. Applied Poult. Sci., 8: 10-16. http://japr.fass.org/cgi/reprint/8/1/10.

Qian, H., E.T. Kornegay and D.M. Denbow, 1996. Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium to phosphorus ratios and phosphorus levels. Poult. Sci., 75: 69-81. http://www.poultryscience.org/psindexsearch.asp?search=Year&number=1996#results:13.

Roland, D.A., Sr., H.A. Ahmad, S.S. Yadalam and T. Sefton, 2003. Effect of no genetically modified phytase supplementation on commercial Leghorn. J. Applied Poult. Res., 12: 257-263. roland1@mail.auburn.edu. http://japr.fass.org/cgi/reprint/12/3/257.

Scott, T.A., R. Kampen and F.G. Silversides, 1999. The effects of phosphorus, phytase enzyme and calcium on the performance of layers fed corn-based diets. Poult. Sci., 78 (12): 1742-1749. PMID: 10626650. http://ps.fass.org/cgi/reprint/78/12/1742.

Statistical Analysis System (SAS), 1997. SAS Institute Inc. Cary, N.C. USA, pp: 445. http://www.sas.com.

Um, J.S. and I.K. Paik, 1999. Effects of microbial phytase supplementation on egg production, eggshell quality and mineral retention of laying hens fed different levels of phosphorus. Poult. Sci., 78: 75-79. PMID: 10023751. http://ps.fass.org/cgi/reprint/78/1/75.

Young, L.G., M. Leunissen and J.L. Atkinsen, 1993. Addition of microbial phytase to diets to young pigs. J. Anim. Sci., 71: 2147-2150. PMID: 8397174.