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The Effects of Microbial Phytase and Dietary Calcium Level on the Performance and Eggshell Quality in Laying Hens Fed Marginal Phosphorus Diets

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ABSTRACT

This experiment was conducted to determine the effect of microbial phytase supplementation accompanied with different levels of dietary calcium on the performance and egg shell quality of laying hens. A factorial design of four levels of calcium (3.8, 3.4, 3.0 and 2.6%) and two levels of microbial phytase (0 and 300 FTU kg^{-1}) was carried out. A total of 72 LOHMAN LSL WHITE laying hens were used. The eight dietary treatments consisted of the corn-SBM basal diet. The metabolizable energy, protein and marginal level of phosphorus were maintained constant in all dietary treatments at 11.56 MJ kg^{-1}, 16.3 and 0.33% TP (0.12% NPP), respectively. Performance and eggshell quality were measured from the age of 22 to 38 weeks. Egg mass, feed efficiency, body weight, eggshell percentage and eggshell density were reduced significantly when hens were fed on 2.6% of dietary calcium level regardless of the presence or absence of phytase. The average eggshell density and shell percentage were significantly increased when hens were fed 3.4% of the Ca level in the presence of phytase. Furthermore, a significant interaction between treatment and age was observed on the eggshell quality. Egg production, egg weight, egg mass, feed intake, feed efficiency and body weight were not affected by phytase enzyme. The information obtained from this experiment indicated that 0.33% TP (0.12% NPP) appears to be sufficient for maintaining production performance and eggshell quality in LOHMAN LSL WHITE laying hens fed the corn-SBM diet containing 3.4% calcium and supplemented with phytase.

Key words: Calcium level, eggshell quality, laying hens, microbial phytase

INTRODUCTION

Phytate phosphorus is the major storage form of phosphorus in cereals, legumes and oilseeds (Erdman, 1979; Diarra *et al*., 2010). Phosphorus in the phytic acid form is poorly available to poultry because they lack phytase, which leads to the use of an inorganic P source to meet the P requirement of poultry (Shol, 1937; Nahm, 2007; Singh, 2008). Calcium is the major dietary divalent cation for laying hens, it can progressively precipitate the phytate by forming the extremely insoluble Ca-phytate complex in the intestine and consequently phytate P, as well as Ca

itself is largely unavailable for absorption (Abdallah *et al*., 1993; Sebastian *et al*., 1996b; Nahm, 2007; Singh, 2008). When, phytic acid is hydrolyzed by microbial phytase, it may release all phytate-bound minerals such as Ca, P, Mg, Cu, Zn, Fe and K (Sebastian *et al*., 1996a). The use of phytase in broiler feed has been an active area of research (Nelson *et al*., 1971; Sebastian *et al*., 1996a; Perney *et al*., 1993), especially in Europe where, P pollution is a limiting factor for animal production (Scott *et al*., 1999; Choct, 2006). Reports of the effect of phytase enzyme in layer diets have not fully investigated the interaction among phytase, P and Ca (Van der Klis *et al*., 1997; Frost and Roland, 1997; Bar *et al*., 2002). Phytase supplementation significantly increased tibia ash and calcium in tibia ash. Microbial phytase in combination with citric acid has no effect on the performance parameters and eggshell quality of laying hens fed a corn-SBM diet containing 2.6% calcium. High dietary calcium (3.8%) decreased phosphorus disappearance of the crop contents compared to 2.6% Ca. The main site of microbial phytase activity in the digestive tract of laying hens is in the crop (Al-Sharafat *et al*., 2009).

Therefore, the objective of the current study was to determine the effect of microbial phytase supplementation at different levels of dietary calcium on the performance production and eggshell quality of laying hens fed marginal P corn-soybean meal diet.

MATERIALS AND METHODS

The experiment was run at the experiments farm in Jarash University between march 2009 to June 2009. Eight experimental diets (Table 1) were arranged in a factorial design as four levels of calcium (2.6, 3.0, 3.4 and 3.8%) accompanied with two levels of microbial phytase (0 and 300 FTU kg^{-1}) fed to 72 LOHMAN LSL WHITE laying hens from 22 to 38 week of age (n = 9 hens $experimental^{-1}$ diet). The hens were housed in a semi open house and kept in ventilated layer cages. The experiment was run at the experiments farm in Jarash University. The daily lighting program was scheduled in which hens were exposed to 14 h light: 10 h dark. The hens were kept in battery cages (40 cm×36 cm× 31 cm), one hen per cage, with 6.9 hens m^{-2} stocking density. Three weeks before starting the experiment, the number of eggs was daily recorded for each hen (Chandramoni *et al*., 1989). Hens were weighed individually at the onset of the experiment and assigned to treatments based on body weight and egg production rate, so that, mean body weight and egg production rate were similar for hens on all treatments. Diets were presented in mash form and feed and water were provided *ad libitum* throughout the experimental period. Composition of the corn-SBM basal diet is shown in Table 2. The basal diets were calculated to contain 0.33% total phosphorus (0.12% NPP). All other dietary nutrients were formulated to meet the nutrient requirements of laying hens according to NRC (1994) and Boling-Frankenbach *et al*. (2001). Limestone was added at the expense of wheat starch to control the calcium levels in the diets (Sifri *et al*., 1977).

Table 1: Design of the experiment

Diet	Ca (%)	Phytase* (FTU kg^{-1})
T1	3.8	0
T2	3.8	300
T3	3.4	0
T4	3.4	300
T5	3.0	0
T6	3.0	300
T7	2.6	0
T8	2.6	300

*RONOZYME-P5000®

Table 2: Ingredients and nutrient composition of the experimental diets

	3.8% Ca	3.4% Ca	3.0% Ca	2.6% Ca
Ingredients (%)				
Corn	57.23	57.23	57.23	57.23
Soybean meal (49)	24.15	24.15	24.15	24.15
Soybean oil	3.10	2.27	1.44	0.59
Wheat starch	2.62	4.60	6.57	8.56
$CaCO_3$	9.90	8.77	7.64	6.52
NaCl	0.05	0.05	0.05	0.05
Cellulose powder	1.85	1.83	1.82	1.80
Premix*	1.00	1.00	1.00	1.00
DL-methionine	0.10	0.10	0.10	0.10
Nutrient content (%)				
Dry matter	91.73	91.22	91.23	91.27
Crude protein	16.40	16.21	16.47	16.57
Crude fat	5.81	4.99	4.18	3.34
Crude fiber	3.34	3.34	3.34	3.34
Crude ash	12.73	11.64	10.56	9.48
Na	0.14	0.14	0.14	0.14
Calcium (total)	3.91	3.54	3.10	2.76
Phosphorus (total)	0.32	0.32	0.30	0.33
Phytate-P	0.22	0.23	0.24	0.24
Metabolizable energy (MJ) (AME)	12.04	11.98	11.98	11.98

*1 kg of premix contains: 600.000 I.U vitamin A, 100.000 I.U vitamin D3, 1.850 mg vitamin E, 160 mg vitamin B1, 480 mg vitamin B2, 500 mg vitamin B6, 2.000 mcg vitamin B12, 200 mg vitamin k3, 2800 mg nicotinic acid, 1000 mg Ca-pantothenat, 60 mg folic acid, 10000 mcg biotin, 80000 mg cholinchlorid, 2500 mg Fe, 1600 mg Cu, 8000 mg Mn, 8000 mg Zn, 120 mg I, 25 mg Se, 55 mg Co, 10000 mg B.H.T, 350 mg canthaxanthin

The microbial phytase RONOZYME-P5000®; Roche Vitamins Ltd., Basel (Switzerland) from *Peniophora lycii*, is a 6-phytase with an activity of 5000 units (FTU) g^{-1}.

Parameters examined: Egg production, egg weight and egg mass were daily recorded. Feed intake was weekly recorded. At the end of the experiment the final body weight and feed efficiency were calculated. The hens were slaughtered and the pH of the crop content was measured. Eggshell percentage and eggshell density were measured every 2 weeks to determine the effect of age on eggshell quality (Table 5 and 6). At the end of the experiment, the average of eggshell percentage and eggshell density for the entire 26th to 38th week period were calculated (Table 4). After breaking the egg, eggshell was cleaned from adhering albumen and dried at room temperature for 24 h. The shell weight for each egg with membranes was determined. Shell percentage was determined by dividing the dried shell weight by egg weight:

$$\text{Percentage shell} = (\text{Dried shell weight}/\text{Egg weight}) \times 100$$

The egg shell density (shell weight $unit^{-1}$ surface area mg cm^{-2}) was calculated by dividing the shell weight (mg) by the egg surface area (cm^2). Egg surface area (S) was calculated from the fresh egg weight (W) in grams by the formula of Mueller and Scott (Tyler and Geake, 1953):

$$S = 4.67 \times W^{2/3}$$

where, 4.67 is a constant.

Statistical analysis: Data were subjected to ANOVA using the general linear model procedure of SPSS program (10.0). Significant differences among treatment means were assessed with the least significant difference test at $p<0.05$ (Carmer and Walker, 1985). Even though significant differences may occur in LSD, if the F-test in the ANOVA has been non-significant, the null hypothesis that treatment means are equal must be accepted.

RESULTS AND DISCUSSION

A summary of Body Weight (BW), Egg Production (EP), Egg Mass (EM), Feed Intake (FI) and Feed Conversion Ratio (FCR) is shown in Table 3. Egg weight, average shell percentage and eggshell density for the entire 22 to 38 weeks period are shown in Table 4. Performance parameters and eggshell quality were differently affected by calcium levels and microbial phytase (Ousterhout, 1980). Egg production and feed intake were not affected by dietary calcium. Lim *et al.* (2003), Frost and Roland (1991) and Rama-Rao *et al.* (2003) reported that the Ca level did not affect the egg production. It is however believed, that if the study had been extended to more than 38 weeks of age, those adverse effects of low calcium diets on the egg production would have been observed (Frost and Roland, 1997). Egg production was also not affected by microbial phytase. Boling *et al.* (2000a) reported that phytase supplementation did show a significant effect on EP. When, diets contained 2.6% calcium, egg mass was significantly reduced compared to 3.4 and 3.8% Ca. Egg mass was not affected by microbial phytase. The average body weight for hens consuming 2.6% calcium decreased ($p<0.04$) to 1821 g hen^{-1}, whereas hens consuming diets containing higher calcium weighed 1952 g or more. Feed efficiency (g feed g^{-1} egg) was significantly improved when hens were fed 3.8% Ca diets compared to the other calcium levels (Simons *et al.*, 1990).

Table 3: The effect of calcium level and microbial phytase on laying hen performance

Diet	Ca (%)	Phytase (FTU kg^{-1})	BW (g)	EP (%)	EM (g d^{-1})	FI (g d^{-1})	FCR (g g^{-1})
T1	3.8	0	1707	96.100	59.00	113.200	1.92
T2	3.8	300	1718	98.200	60.04	115.400	1.86
T3	3.4	0	1688	96.900	60.05	118.300	1.97
T4	3.4	300	1750	99.680	61.60	123.400	2.01
T5	3.0	0	1732	95.080	57.14	112.700	1.99
T6	3.0	300	1695	96.260	58.40	117.100	2.05
T7	2.6	0	1650	92.590	57.03	112.700	2.01
T8	2.6	300	1680	94.700	57.01	115.300	2.07
Main effects							
3.8% Ca			1712^{ab}	97.150	59.51^{ab}	114.730	1.88^{b}
3.4% Ca			1719^{a}	98.290	59.80^{a}	120.860	1.99^{a}
3.0% Ca			1713^{a}	95.670	57.85^{bc}	115.340	2.03^{a}
2.6% Ca			1665^{b}	93.650	57.03^{c}	114.110	2.05^{a}
0 FTU kg^{-1}			1695	94.690	58.33	114.800	1.98
300 FTU kg^{-1}			1710	97.330	58.89	120.220	2.06
Probabilities							
Calcium			0.042	0.106	0.051	0.301	0.023
Phytase			0.232	0.176	0.337	0.170	0.473
Calcium×phytase			0.292	0.980	0.835	0.983	0.710
SE			111.9	2.800	2.430	5.830	0.070

Means within a column with no common superscript letters significantly different ($p<0.05$)

Table 4: The effect of calcium level and microbial phytase on egg weight and average of eggshell quality

Diet	Ca (%)	Phytase (FTU kg^{-1})	Egg weight (g)	Egg shell percentage	Egg shell density (mg cm^{-2})
T1	3.8	0	59.900	9.190^{ab}	77.400^{ab}
T2	3.8	300	61.900	9.100^{ab}	77.010^{ab}
T3	3.4	0	63.400	8.870^{bc}	74.750^{bc}
T4	3.4	300	61.000	9.410^{a}	79.780^{a}
T5	3.0	0	60.200	8.810^{bcd}	73.820^{bcd}
T6	3.0	300	59.200	8.380^{d}	70.080^{d}
T7	2.6	0	59.900	8.600^{cd}	72.370^{cd}
T8	2.6	300	59.900	8.370^{d}	70.150^{d}
Main effects					
3.8% Ca			61.100	9.140^{a}	77.160^{a}
3.4% Ca			62.100	9.140^{a}	77.270^{a}
3.0% Ca			59.700	8.600^{b}	71.960^{b}
2.6% Ca			59.900	8.490^{b}	71.260^{b}
0 FTU kg^{-1}			60.800	8.870	74.580
300 FTU kg^{-1}			60.500	8.810	74.240
Probabilities					
Calcium			0.164	0.000	0.000
Phytase			0.685	0.631	0.724
Calcium×phytase			0.352	0.033	0.012
SE			1.200	0.240	1.900

Means within a column with no common superscript letters significantly different ($p<0.05$)

The average shell percentage and eggshell density were reduced (Table 4) when the hens were fed low calcium diets (2.6 and 3.0% Ca) compared to hens that were fed high calcium levels (3.8 or 3.4% Ca). Present results are in general agreements with previous findings of Clunies *et al.* (1992), Scott *et al.* (1999) and Lim *et al.* (2003), who reported that eggshell weight increased significantly due to increasing the dietary calcium. The egg weight was not affected by dietary treatments. The effect of phytase supplementation on eggshell quality appears to be significantly affected by calcium level. The average shell percentage and eggshell density were significantly higher in hens that were fed 3.4% Ca supplemented with phytase than those who were fed 3.4% Ca without any phytase supplementation. However, adding microbial phytase at 3.0 and 2.6% calcium did not improve the eggshell quality (Table 4). It is believed that the amount of calcium which may be released by microbial phytase from phytate is not enough to support the required eggshell quality when hens are fed low calcium diet. Gordon and Roland (1998) reported that it is unlikely that the phytate molecule would contain enough calcium to improve the eggshell weight. Some investigators observed a beneficial effect of phytase supplementation on eggshell quality (Gordon and Roland, 1998; Punna and Roland, 1999), while others did not find any relation (Van der Klis *et al.*, 1997; Lim *et al.*, 2003).

As shown in Table 5 and 6, the effect of interaction of age×treatment on eggshell quality was observed at 38 weeks; where the shell percentage and eggshell density were reduced in hens fed the low calcium diet (2.6%) without phytase supplementation compared to other dietary treatments. At 38 weeks of age, the eggshell density for hens consuming 2.6% calcium without phytase supplementation was 56.9 mg cm^{-2}; however, for hens consuming the same level of calcium (2.6%) with phytase was 62.9 mg cm^{-2}. Also, at 38 weeks of age, the egg shell percentage for hens consuming 2.6% calcium without phytase supplementation was 6.72%, whereas for hens consuming the same level of calcium (2.6%) with phytase was 7.42%. It seems to be that adding microbial

Table 5: The effect of age on egg shell density of laying hens from 26 to 38 weeks of age

Diet	Ca (%)	Phytase (FTU kg^{-1})	26 weeks	28 weeks	30 weeks	32 weeks	34 weeks	36 weeks	38 weeks	SE
			(mg cm^{-2})							
T1	3.8	0	75.1^{a}	78.9^{a}	80.3^{a}	76.9^{a}	78.0^{a}	78.6^{a}	73.7^{a}	0.86
T2	3.8	300	79.1^{a}	73.6^{ab}	77.6^{ab}	79.2^{a}	79.2^{a}	77.9^{a}	71.9^{b}	0.81
T3	3.4	0	75.7^{ab}	69.6^{b}	77.2^{a}	73.7^{ab}	73.1^{ab}	76.3^{a}	77.7^{a}	0.89
T4	3.4	300	79.4^{a}	78.0^{a}	81.6^{a}	80.7^{a}	81.0^{a}	77.9^{a}	79.8^{a}	0.76
T5	3.0	0	72.4^{a}	72.7^{a}	74.6^{a}	74.7^{a}	72.3^{a}	73.7^{a}	76.5^{a}	0.57
T6	3.0	300	75.5^{a}	69.2^{ab}	70.3^{ab}	69.0^{ab}	70.9^{ab}	64.5^{b}	71.2^{ab}	1.03
T7	2.6	0	76.6^{ab}	71.8^{b}	78.3^{a}	72.8^{b}	75.9^{ab}	74.3^{ab}	56.9^{c}	1.09
T8	2.6	300	69.6^{ab}	68.8^{ab}	72.7^{a}	71.2^{ab}	72.6^{a}	73.3^{a}	62.9^{b}	1.11

Means within a row with no common superscript letters significantly different ($p<0.05$). Age: 0.000; Treatment: 0.000; Age×treatment: 0.000

Table 6: The effect of age on egg shell percentage (%) of laying hens from 26 to 38 weeks of age

Diet	Ca (%)	Phytase (FTU kg^{-1})	26 weeks	28 weeks	30 weeks	32 weeks	34 weeks	36 weeks	38 weeks	SE
			(%)							
T1	3.8	0	9.06^{ab}	9.50^{a}	9.53^{a}	9.08^{ab}	9.27^{ab}	9.32^{ab}	8.62^{b}	0.10
T2	3.8	300	9.46^{a}	8.76^{ab}	9.10^{ab}	9.35^{a}	9.22^{a}	9.07^{ab}	8.46^{b}	0.09
T3	3.4	0	8.96^{ab}	8.24^{b}	9.17^{a}	8.65^{ab}	8.74^{ab}	9.06^{ab}	9.06^{ab}	0.10
T4	3.4	300	9.51^{a}	9.05^{a}	9.65^{a}	9.58^{a}	9.47^{a}	9.21^{a}	9.30^{a}	0.09
T5	3.0	0	8.74^{a}	8.82^{a}	8.98^{a}	8.85^{a}	8.60^{a}	8.73^{a}	8.98^{a}	0.60
T6	3.0	300	9.18^{a}	8.27^{b}	8.41^{ab}	8.20^{b}	8.43^{ab}	7.75^{b}	8.44^{ab}	0.12
T7	2.6	0	9.21^{ab}	8.57^{c}	9.39^{a}	8.64^{bc}	8.91^{abc}	8.76^{abc}	6.72^{d}	0.12
T8	2.6	300	8.50^{a}	8.22^{ab}	8.73^{a}	8.48^{a}	8.58^{a}	8.68^{a}	7.42^{b}	0.13

Means within a row with no common superscript letters significantly different ($p<0.05$). Age: 0.000; Treatment: 0.000; Age×treatment: 0.002

phytase delays but can not prevent the adverse effect of age on the eggshell quality by increasing the calcium availability. However, it is considered that this increase in calcium availability by microbial phytase is not enough to support the eggshell quality until 38 weeks of age compared to hens consuming high dietary calcium (3.0% or more). The egg shell quality (PS and SD) of hens fed 3.4 and 3.0% Ca (with and without phytase), until 38 weeks, was not significantly affected by the age compared to hens fed low calcium diets (2.6%). The reduction in eggshell quality with age at low calcium diets (2.6%) may be due to the increase in egg weight, which in turn demands a high shell weight without a proportional increase of the hen's ability to increase the absorption and utilization of calcium to fulfill the high calcium demand for shell formation (Keshavarz and Nakajima, 1993). Similar results were observed by Lim *et al.* (2003) and Ahmad *et al.* (2003), who reported that eggshell quality tended to decline as the birds got older. Van der Klis *et al.* (1997) also reported that calcium and phosphorus absorption in 36 weeks old hens was significantly lower than in younger hens (24 weeks old). From this study we can observe that at all levels of dietary calcium, adding microbial phytase slightly (but not significantly) increased the egg production, egg mass and feed intake. Present results were in accordance with previous findings of Scott *et al.* (1999), who reported that at low calcium diets, there was a smaller effect of phytase enzyme on egg production, egg weight and feed intake. The pH of the crop contents in this experiment was in the range of 5 to 5.2. However, the microbial phytase which was used in this experiment is active over a narrow pH range, with an optimum pH of 4.5. This difference in the pH may have a negative influence on the phytase activity. The high pH in the crop contents in this study can be attributed to the high

dietary calcium concentration in layer diets. Shafey *et al.* (1991) in their study on growing chickens reported that a high dietary concentration of calcium (2.5 vs. 1.1%) increased the pH of the crop content from 4.8 to 5.3 and the ileum from 6.62 to 7.39, however, the pH of the proventriculus and gizzard contents were not significantly changed.

Present results show that a corn-soybean meal diet containing 3.4% Ca supplemented with phytase (300 FTU kg^{-1}) can be fed as long as 38 weeks with no deleterious effects on production performance and eggshell quality. Moreover, a diet containing 3.4% Ca with no phytase supplementation also supported optimum production performance. An important observation from the current study is that the diets with 0.33% TP (0.12% NPP) without any phytase supplementation have been shown to be sufficient to maintain satisfactory production performance and egg shell quality until 38 weeks of age in laying hens (LOHMAN WHITE-LSL). Boling *et al.* (2000b) reported that a diet containing 0.15% AP without phytase supplementation support optimum egg production performance. However, these results are in contrast to those of Summers (1995), who reported that feeding a diet containing 0.20% AP depressed the performance production of laying hens. The discrepancy between the current study and other studies might have been due to using different strains of laying hens. This conclusion is in consistent with the report of Keshavarz (2003) which indicated that strain differences might have been a factor for a higher NPP requirement. Scheideler and Sell (1986) also found a significant phosphorus level×breed interaction for the eggshell quality, indicating that breeds responded differently to suboptimal levels of dietary phosphorus. Similarly, Rodehutscord *et al.* (2002) used lohamnn-brown laying hens and reported that feeding marginal P level (0.36%) did not reduce the performance parameters and eggshell quality.

The question of difference in the ability of breeds and strains to utilize phytate phosphorus or calcium has not been investigated enough or seriously considered as a factor in explaining the controversy that exists regarding phytate phosphorus requirement. Therefore, more studies should be carried out to differentiate in more details the differences in the ability of various breeds or strains to respond to different dietary calcium and phosphorus levels and to determine the reason for these differences.

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