

Effect of supplemental phytase to laying hen diets of different phosphorus content*

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(Received 6 September 1996; accepted 19 August 1997)

ABSTRACT

The experiment was carried out on 216 Hisex brown hens, aged 48-70 weeks, divided into 12 groups of 18 birds kept in single cages. Layers were fed diets containing 0.45, 0.52 and 0.58% total P (0.25, 0.30, 0.35% available P) supplemented with 0, 150, 300 or 450 units of microbial phytase (FTU) per kg. At 54, 62 and 70 weeks of age, eggs and egg-shell quality were analysed. Level of P and phytase had no effect on feed intake, laying rate, yolk percentage and albumen:yolk ratio. Eggs were heaviest (65.1 g) when no phytase was added or when the diet containing the lowest P level was fed ($P \leq 0.01$). Specific gravity, shell breaking strength, shell thickness, shell percentage and shell index (density) were highest when the diets were supplemented with 150 FTU/kg, regardless of the P level. However, differences were significant ($P \leq 0.05$) only for shell breaking strength and thickness. On the diets supplemented with 450 FTU, some parameters of egg-shell quality were worse than without the addition of microbial phytase. However, a much smaller number of cracked and broken eggs was found when phytase was added.

KEY WORDS: layers, phosphorus, phytase, egg weight, egg-shell quality

INTRODUCTION

In experiments on hens with laying rate under 80%, reduction of available dietary phosphorous (AP) to 0.25% (Daghir et al., 1985), or even to 0.15% of diet (Rodriguez et al., 1984) did not reduce the number of eggs, despite the fact that microbial phytase had not been administered. On the other hand, when

* This study was supported by State Committee for Scientific Research grant No. 5 S305 061 04

Usayran and Balnave (1995) supplemented the diets of hens with similar laying rates, with 500 phytase units (FTU), they found that performance declined by 4% when the diet contained 0.12% AP, and by 6% at AP level of 0.24%.

When laying performance was above 90%, it was found necessary either to supplement diets containing 0.25% AP with 300 FTU/kg, or to give feeds with a 0.30% AP content without adding phytase, or with 150 FTU/kg (Kamińska et al., 1996). A dietary phosphorous level of 0.25% AP/kg without phytase made it possible to achieve a laying rate of up to 85%. These results show that the amount of supplementary phytase, an enzyme increasing the availability of P from phytates, should be adjusted to the amount of available phosphorous in the diet and to the requirement of hens, which depends mainly on laying performance.

The objective of the current study was to examine the reaction of hens with laying rates of about 80% to different dietary phosphorous levels and to various levels of microbial phytase supplementation.

MATERIAL AND METHODS

The experiment was conducted from July 1994 to March 1995 on 216 Hisex Brown hens aged 48-70 weeks assigned to 12 groups of 18 birds kept individually in cages. They were fed *ad libitum* with one of three diets. All of the feed mixtures contained per kilogram (%): ground wheat, 48.95; ground maize, 25; soyabean meal, 14 (46% CP); meat meal (62% CP), 3.5; NaCl, 0.3; vitamin-mineral premix, 0.5; 11.45 MJ AME_N; 168 g of crude protein; 7.4 g Lys; 6.2 g Meth + Cyst; 1.8 g Try. The diets differed in the content of dicalcium phosphate and limestone, which amounted to 0.25 and 7.5, 0.55 and 7.2, and 0.85 and 6.9%, respectively, in diets 1, 2 and 3. It corresponded to 3.0 g Ca; 4.5 and 2.5; 5.2 and 3.0, and 5.8 and 3.5 g total and available P, per kg of diets 1, 2 and 3, respectively. Each of the diets was fed unsupplemented or supplemented with 150, 300 or 450 units of phytase (FTU)/kg. Natuphos[®] 5000, phytase produced by *Aspergillus niger*, was used. One unit of phytase (FTU) is defined as the quantity of enzyme which liberates 1 μ mole of inorganic P per min from 0.0015 mol/l solution of sodium phytate at pH 5.5 and 37°C.

Feed consumption was measured for each hen for three consecutive days in the middle of the experiment. Eggs were collected daily and those laid on 5 consecutive days each month were weighed. At the age of 54, 62 and 70 weeks, 12 eggs were taken from each groups for analysis. Egg weight, specific gravity, breaking strength (measured by applying pressure along the long axis of the egg), shell thickness in three points, yolk, egg white, and dry shell weight were determined percentage share of yolk, egg white and dry shell in total egg mass was calculated, as well as shell surface area (SSA) according to the formula:

$$\text{SSA} = \text{egg weight}^{0.667} \times 4.67.$$

Shell density (shell index) was calculated as a weight of dry shell/SSA (mg/cm^2).

The results were subjected to two-way ANOVA General Linear Models (GLM) analysis, SAS Institute (1985) with phosphorus and phytase levels as the main effects.

RESULTS

Laying rate

The amount of total and available phosphorous in diets did not affect the laying rate of the hens. It averaged in all groups 83% between 48 to 70 weeks of life (Table 1).

Egg weight

Hens fed the diet containing 0.45% TP (0.25% available P) without added phytase laid the heaviest eggs (66.1g). Hens that did not receive phytase laid heavier eggs (65.1 g) than those on the phytase-supplemented diets (62.9-63.8 g) ($P \leq 0.01$; Table 1). The differences in mean egg weight between groups receiving the lowest amount of dietary phosphorous and those receiving the highest ones were also significant ($P \leq 0.01$; Table 1).

Feed consumption

Feed consumption averaged 108 g daily per hen and was independent of the amount of phosphorous in the diet and the supplement of microbial phytase.

Yolk percentage in egg

The yolk constituted an average 28% of total egg mass. Its share in the egg did not change in relation to the amount of available phosphorous in the diet. The egg white: yolk weight ratio averaged 2.24:1 (Table 1).

Specific gravity

No significant differences were found between groups in the specific gravity of eggs.

TABLE 1

Effect of dietary phosphorus level and phytase supplement on performance of laying hens

Dietary P, %		Phytase supplement FTU/kg diet				Mean	SEM	PT x FTU
total P	P available	0	150	300	450			
Laying rate, %								
0.45	0.25	82.3	81.3	82.3	85.1	82.9	0.85	
0.52	0.30	81.1	83.5	81.0	83.0	82.2	0.80	NS
0.58	0.35	81.3	84.8	85.3	84.2	83.9	0.92	
Mean		81.5	83.2	82.9	84.2	83.0		
SEM		0.69	0.82	0.90	0.84	0.76		
Egg weight, g								
0.45	0.25	66.1	64.6	63.4	64.6	64.5 ^A	0.38	
0.52	0.30	65.2	63.7	62.6	63.6	63.8 ^B	0.39	NS
0.58	0.35	64.4	62.8	62.5	63.0	63.2 ^B	0.42	
Mean		65.1 ^A	63.7 ^B	62.9 ^C	63.8 ^B	63.8		
SEM		0.38	0.47	0.52	0.42	0.47		
Feed intake, g/day								
0.45	0.25	107	107	106	110	107	0.99	
0.52	0.30	110	106	103	110	107	1.18	NS
0.58	0.35	111	106	109	107	108	1.11	
Mean		109	106	108	109	108		
SEM		1.12	0.96	1.20	1.08	1.14		
Yolk, %								
0.45	0.25	28.9	27.8	27.1	28.0	27.9	0.19	
0.52	0.30	28.2	27.8	28.4	28.4	28.2	0.18	NS
0.58	0.35	27.9	27.8	28.6	28.9	28.3	0.20	
Mean		28.3	27.8	28.0	28.5	28.1		
SEM		0.24	0.20	0.22	0.20	0.22		
Albumen/yolk ratio								
0.45	0.25	2.17	2.28	2.34	2.26	2.26	0.022	
0.52	0.30	2.23	2.27	2.22	2.20	2.23	0.020	NS
0.58	0.35	2.27	2.26	2.18	2.15	2.22	0.023	
Mean		2.22	2.27	2.25	2.20	2.24		
SEM		0.027	0.025	0.026	0.023	0.026		

A, B, C - $P \leq 0.01$

Shell breaking strength

The differences in shell breaking strength among groups were significant ($P \leq 0.05$; Table 2). The strongest shells (4.01 kg) were from hens receiving 150 FTU/kg of diet, the weakest (3.74 kg) from those receiving the highest (450 FTU/kg) supplementation.

TABLE 2

Egg specific gravity (g/cm³), breaking strength (kg), shell thickness (μ m) shell index (mg/cm²) and shell percent*

Dietary P, %		Phytase supplement FTU/kg diet				Mean	SEM	PT x FTU
total P	P available	0	150	300	450			
Specific gravity								
0.45	0.25	1.08	1.08	1.09	1.08	1.08	0.0006	
0.52	0.30	1.08	1.08	1.08	1.08	1.08	0.0007	NS
0.58	0.35	1.08	1.09	1.08	1.08	1.08	0.0007	
Mean		1.08	1.09	1.08	1.08	1.08		
SEM		0.0008	0.0008	0.0008	0.0007	0.0008		
Breaking strength								
0.45	0.25	3.96	4.00	4.09	3.50	3.88	0.076	
0.52	0.30	3.76	4.08	4.05	3.91	3.95	0.083	NS
0.58	0.35	4.10	3.94	3.64	3.81	3.87	0.073	
Mean		3.94 ^{ab}	4.01 ^a	3.93 ^{ab}	3.74 ^b	3.90		
SEM		0.098	0.085	0.093	0.086	0.087		
Shell thickness								
0.45	0.25	355	367	368	356	362	3.16	
0.52	0.30	364	372	355	363	364	3.35	NS
0.58	0.35	371	377	356	366	368	3.34	
Mean		364 ^{ab}	372 ^a	360 ^b	362 ^{ab}	365		
SEM		4.06	4.00	3.69	3.33	3.86		
Shell index								
0.45	0.25	78.82	81.09	81.70	78.50	80.03	0.72	
0.52	0.30	81.03	82.75	78.89	81.45	81.07	0.77	NS
0.58	0.35	83.09	83.36	79.40	81.32	81.83	0.71	
Mean		81.06	82.45	80.04	80.40	80.99		
SEM		0.90	0.91	0.81	0.76	0.86		
Shell percent								
0.45	0.25	9.12	9.46	9.50	9.07	9.29 ^b	0.087	
0.52	0.30	9.37	9.64	9.24	9.49	9.44 ^{ab}	0.089	NS
0.58	0.35	9.64	9.80	9.34	9.52	9.58 ^a	0.087	
Mean		9.39	9.64	9.36	9.35	9.43		
SEM		0.106	0.113	0.097	0.088	0.103		

* each value is a mean of 36 measurements (3 periods x 12 eggs)
means in a column or row different significant at $P \leq 0.05$

Other egg-shell quality parameters

Shell thickness, shell density (shell index) and dry shell content in the egg were highest in the hens receiving 150 FTU/kg of diet. The shell percentage rose from 9.29 to 9.58% (Table 2) as the phosphorous content in the feed increased.

TABLE 3

Amount of cracked, broken and shell-less egg, % of total amount

Dietary P, %		Phytase supplement, FTU/kg diet				Mean
total P	P available	0	150	300	450	
Cracked eggs						
0.45	0.25	6.78	8.55	1.82	6.09	5.81
0.52	0.30	7.75	2.97	5.85	2.62	4.80
0.58	0.35	8.65	2.97	4.46	3.30	4.84
Mean		7.73	4.83	4.04	4.00	5.15
Broken and without shell						
0.45	0.25	2.38	4.33	0.84	1.38	2.23
0.52	0.30	1.96	1.06	1.74	0.82	1.39
0.58	0.35	1.83	1.40	2.18	0.70	1.53
Mean		2.06	2.26	1.59	0.97	1.72
Cracked, broken and shell-less together						
0.45	0.25	9.16	12.88	2.66	7.47	8.04
0.52	0.30	9.71	4.03	7.59	3.44	6.19
0.58	0.35	10.48	4.37	6.64	4.01	6.37
Mean		9.78	7.09	5.63	4.97	6.87

The hens laid eggs with good quality shells. However, in the groups that did not receive phytase almost 10% of the eggs had cracked shells (Table 3). The lowest number of damaged or broken eggs was found in hens receiving 0.30% AP in their diets (0.52% TP) and phytase, and in the groups receiving 450 FTU/kg of diet.

Health of hens

The health of the laying hens was very good. Only 3 hens died during the experiment.

DISCUSSION

Most of the studies on the effect of microbial phytase supplement in poultry nutrition have been carried out on chicks, because older birds are more capable of utilizing phytate phosphorous than young birds. Among the relatively small number of experiments on laying hens, most did not focus on the practical use of phytase in diets, but only on its effectiveness. Simons and Versteegh (1992) fed hens diets containing 0.30% total P, of which only 0.05% was non-phytate. Daily total phosphorous intake was slightly over 300 mg per chicken, of which the layers utilized only 15%, i.e. 46 mg, while after the inclusion of phytase into the

diet, 33% i.e. 107 mg. The amount of phytate P in excreta decreased from 6.6 to 3.2 g per kg DM. On the basis of these results it was concluded that the addition of 500 FTU per kg of diet increases phosphorous utilization by 100% and decreased by 50% the amount of phytate phosphorous in excreta. Schöner et al. (1993) found that the utilization of phosphorous from a diet containing 0.12% AP ranged from 9-15%, and after the addition of phytase, 30-38%; excretion of P decreased therefore by 45-50%, thus confirming the results of Simon and Vesteegh (1992). Feeding layers on diets made up of vegetable feeds and containing 0.12% non-phytate phosphorus, Peter (1992) found that the laying rate declined to 83% in the 31st week of the hens life. In hens receiving phytase as an additive (the amount was not given) the laying rate was about 90% for ten more weeks. In the above studies, a maize-soyabean diet was used most frequently.

Usayran and Balnave (1995) evaluated the P requirements of laying hens fed on the wheat-based diets with various P levels, unsupplemented or supplemented with 500 FTU/kg of diet. The addition of phytase to a diets containing 0.32% TP (0.12% AP) or 0.45% TP (0.24% AP) significantly lowered the laying rate and egg weight, and increased the number of damaged shells from 5.4 to 12.4% at the lower P level and from 7.5 to 16.4% at the higher P level. On the opposite in our experiment the addition of the phytase decreased the number of damaged eggs, even in the groups of hens receiving phosphorus in amounts according to the Nutrient Requirements of Poultry (1996). Our results are, however, similar to those of Jeroch and Peter (1994) who showed that even when the addition of phytase did not increase the laying rate of hens fed wheat-based diets, it did improve egg shell quality.

Interestingly both, increasing the dietary inorganic P level and the addition of phytase caused a significant decrease in egg weight (Table 1). This supports the results of Budor et al. (1995), although the difference found by them was smaller (62.4 vs. 61.6 g) than in our experiment.

The positive effect of the addition of a small amount of phytase (150 FTU/kg) on egg shell quality parameters can not be explained by the decline in egg size in these groups, since when 300 and 450 FTU/kg were added, the weight of the eggs was similar, but the shell quality worse – shell thickness, shell index, shell percentage and breaking strength deteriorated (Table 2).

The results of the present study, and our earlier papers (Kamińska et al., 1994, 1996), indicate that there is a certain optimum level of phytase supplementation, which depend on the requirements of the birds, diet composition, and that exceeding this amount can have negative consequences on the laying performance and shell quality.

Phytic acid (myo-inositol hexaphosphate), which forms about two thirds of the phosphorus present in seeds, creates indigestible chelates with some cations

like Ca, Mg, Zn, and with proteins, what reduces their availability (Kamińska, 1993). Enzymatic hydrolysis by phytase liberates phosphoric acid and cations from these chelates. Thiel and Weigand (1992) found that the addition of 800 FTU to maize-soyabean diets increased the level of Zn in the serum of broiler chickens, in carcass and their femur, as well as significantly reduced the activity of serum alkaline phosphatase. The considerable differences in the action of phytase observed by some authors may result from differences in the assumed requirements of the birds and the P content of the feed and its availability, which depends on the diet composition and on the amount of microbial and plant phytase. An excess of microbial phytase, which has an activity 74% higher than that of plant phytase (Eeckhout and De Paepe, 1991), is undesirable not only because it increases the cost of the feed, but also because it may unfavourably affect the performance. Moreover, increasing the amount of microbial phytase limits the amount of excreted P only to a certain extent (Kamińska et al., 1996). At a 0.30% dietary AP content (0.55% TP), the addition of phytase in amounts higher than 150 FTU/kg no longer increased the amount of P retained in the body but decreased P in excreta.

Difficulties in determining the appropriate amount of microbial phytase to be added stem, among others, from the fact that, as shown by Eeckhout and De Paepe (1994), the phytase content and activity in wheat grain may differ considerably, e.g. in from 915 to 1580 units/kg. This is not, however, a problem when feeding maize, because of its negligible phytase content (15 FTU/kg on average).

CONCLUSIONS

A 0.45% dietary TP (0.25% AP) content was sufficient for hens with an 80% laying rate. A small supplement of microbial phytase (150 FTU/kg) did, however, favourably affect on egg shell quality, even when the amount of available phosphorus in the diet was in agreement with recommendations (0.35%). This improvement was manifested, among others, in a lower percentage of damaged eggs.

The addition of inorganic phosphorus and phytase in excess of this amount may have adverse effect on egg mass and shell quality parameters.

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STRESZCZENIE

Efektywność mikrobiologicznej fitazy w mieszankach o różnej zawartości fosforu dla kur nieśnych

Doświadczenie przeprowadzono na 216 kurach Hisex Brązowy w wieku 48-70 tygodni, przydzielonych do 12 grup po 18 ptaków trzymanyh w pojedynczych klatkach. Nioski żywiono mieszankami zawierającymi 0,45 0,52 i 0,58% fosforu całkowitego (0,25, 0,30, i 0,35% P przyswajalnego) nieuzupełnionymi lub uzupełnionymi 0, 150, 300 lub 450 jednostek mikrobiologicznej fitazy (FTU)/kg. W 54, 62 i 70 tygodniu życia kur oceniono jakość jaj i skorup. Ani poziom fosforu w paszy ani dodatek fitazy nie wpłynęły na wielkość spożycia paszy, nieśność, procentowy udział masy żółtka w masie całego jaja i stosunek wagowy białka do żółtka. Jaja o największej masie (65,1 g) uzyskano od kur żywionych paszą bez dodatku fitazy, niezależnie od poziomu P, lub mieszanką o najniższym poziomie fosforu ($P \leq 0,01$). Ciężar właściwy jaj oraz ich wytrzymałość na zgniatanie, grubość skorupy, procent skorupy i indeks skorupy (gęstość) były największe, gdy dietę uzupełniono 150 FTU/kg, bez względu na ilość P w paszy, przy czym statystycznie istotne różnice ($P \leq 0,05$) stwierdzono tylko dla wytrzymałości na zgniatanie i grubości skorup. Przy skarmianiu diet uzupełnionych 450 FTU/kg niektóre parametry jakości skorup były nawet gorsze niż bez dodatku mikrobiologicznej fitazy, jednak uzupełnienie diet dodatkiem fitazy spowodowało obniżenie liczby jaj sfluczonych.