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Effects of a low-phosphorus diet and exogenous phytase on performance, egg quality, and bacterial colonisation and digestibility of minerals in the digestive tract of laying hens

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ABSTRACT: The objective of the experiment was to determine the effects of different levels of phytase (0, 150, 250, and 350 phytase units (FTU)/kg; F) produced by *Aspergillus niger* in the diets of hens fed decreased contents of non-phytate phosphorus (1.8 and 2.1 g/kg; NPP) on the hen performance, egg quality, digestibility of calcium and phosphorus in the ileum, and representation of bacterial species in the ileum and caecum. The diet with 2.1 g/kg of NPP and 350 FTU/kg significantly decreased egg weight ($P < 0.001$) and egg mass production ($P < 0.001$). However, this treatment had the highest values for shell percentage ($P = 0.002$), shell thickness ($P = 0.006$), and shell index ($P = 0.003$). The supplementation with F at 350 FTU/kg to the diet with 1.8 g/kg of NPP increased the shell quality to a level that was comparable with the eggs from the hens fed the diet with only 2.1 g/kg of NPP. With the addition of F (350 FTU/kg) to the mixed feed with 1.8 g/kg of NPP, the digestibility of calcium and phosphorus in the ileum increased by 6.1% and 7.4%, respectively, although the increases were not significant. Additionally, the frequency of *Lactobacillus* spp. was higher in the ileum and caecum of hens fed the diet enriched with F than in those fed a diet without F addition. The dietary manipulations with NPP and F improved some performance and shell quality characteristics, and the addition of 3-phytase at a level of 350 FTU per kg to the low-P diet increased the digestibility of minerals and changed the microflora of the digestive tract.

Keywords: layers; eggshell; microbial 3-phytase; ileum; caecum; *Lactobacillus* spp.

INTRODUCTION

The furnished cage systems have a high percentage of egg breakage (more than 10%) directly at the point of lay (Mertens et al. 2006). Because of both the economics and the health risks associated with a cracked shell, the quality of the eggshell remains one of the primary interests of the poultry industry. The eggshell quality is influenced by many factors, of which nutrition being especially important (Englmaierova et al. 2014b, Tumova et al. 2014, Venglovska et al. 2014). Phosphorus is an essential mineral in the formation of eggshell and in the metabolism of hens (Sohail and Roland 2002), and examples of phosphorus-containing compounds include adenosine 5'-triphosphate and phospholipids. These forms of phosphorus in plants can be digested by poultry; however, these forms of digestible P typically account for 30–40% of the total phosphorus (National Research Council

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1994). In plants, most of the phosphorus is complexed with phytic acid (Singh 2008), and these complexes are difficult to be digested at neutral and basic values of pH. The phytate phosphorus is biologically less available to poultry (monogastric animals) because of either insufficient quantity or the lack of secretion of the enzyme phytase, which hydrolyzes the phytic acid in the digestive tract (Simons et al. 1990; Marounek et al. 2008). Therefore, the mixed feeds for poultry are enriched with various commercial sources of phytase to improve the digestibility of the phytate phosphorus. Some components of the diets also show phytase activity to varying degrees. For example, the phytase activity is high in wheat and wheat bran, in contrast to maize and oilseeds. The addition of phytase completely counters the adverse effects associated with the low intake of inorganic phosphorus (P) by animals and also increases the bioavailability of calcium (Ca), which improves the eggshell quality at the marginal level of 3.4% Ca. A phytase supplement decreased the excretion of P in manure and therefore reduced potential environmental problems (Jalal and Scheideler 2001). The exogenous phytases are active primarily in environments with an acidic pH, such as in the crop, proventriculus, and gizzard. Several commercial phytase products are available, such as the 3-phytase Natuphos®, which is produced by a strain of *Aspergillus niger*.

The complete diet for hens in the Czech Republic has unnecessarily high levels of P. The daily recommendation (National Research Council 1994) for the diet of a laying hen is 0.25% non-phytate phosphorus (NPP). Skrivan et al. (2010) showed that 0.27% NPP in a wheat-based and 0.30% NPP in a maize-based diet without added phytase were adequate for hens with an intake of 115 g of feed that contained 3.5% Ca, without negative effects on the performance or egg quality. In a previous study, Englmaierova et al. (2012) found that hens fed a diet that contained 1.3 g/kg NPP achieved a higher performance than those with 4.0 g/kg NPP in the diet. Moreover, 3-phytase supplemented at 150 FTU/kg increased the internal as well as external quality of eggs.

The aim of this study was to compare the effects of different levels of F in low-phosphorus diets for laying hens on the performance, egg quality, ileal digestibility of Ca and P, and bacterial colonisation of the ileum and caecum.

MATERIAL AND METHODS

Hens, husbandry, and diets. A total of one hundred and sixty-eight 38-week-old Lohmann Brown hens were housed individually in three-floor cages (1 hen per cage, 21 cages per treatment) in the same air-conditioned facility. The cages provided 2000 cm^2 of floor area and had a 40 cm feeder and 2 nipple water dispensers. The room temperature was maintained at 20–22°C, and the daily photoperiod was 16 h of light and 8 h of darkness, with a light intensity that was approximately 10 lx in the central storey. The hens were randomly assigned to 8 dietary treatments. The study was a 2 × 4 full factorial design with 2 levels of non-phytate phosphorus (1.8 and 2.1 g/kg; NPP) and 4 levels of phytase (0, 150, 250, and

Table 1. Composition of the hen diets[1]

Ingredient (g/kg)	1.8 g/kg NPP	2.1 g/kg NPP
Wheat	240	240
Maize	360.9	360.5
Soybean meal	280	280
Rapeseed oil	20	20
Monocalcium phosphate	3.6	5.0
Sodium chloride	2	2
Fine-grained limestone	30.1	29.75
Coarse-grained limestone	55.9	55.25
L-Lysine hydrochloride	1	1
DL-Methionine	1.5	1.5
Vitamin-mineral premix[2]	5	5
Analyzed nutrient content (g/kg)		
AME_N (MJ/kg)	11.5	11.5
Crude protein	170	170
Ca	34.4	34.2
TP	4.38	4.71
NPP	1.8	2.1

AME_N = apparent metabolizable energy, TP = total phosphorus, NPP = non-phytate phosphorus

[1]the other experimental diets were supplemented with 150, 250 or 350 FTU/kg of 3-phytase Natuphos®

[2]Vitamin-mineral premix provided per kg of diet: retinyl acetate 3.0 mg, vitamin D_3 3000 IU, vitamin E 30 mg, niacin 25 mg, Ca pantothenate 8 mg, thiamine 2.0 mg, riboflavin 5 mg, pyridoxine 4 mg, folic acid 0.5 mg, biotin 0.075 mg, cobalamin 0.01 mg, choline Cl 250 mg, menadione 2.0 mg, betaine 100 mg, butylated hydroxytoluene 7.5 mg, ethoxyquin 5.6 mg, butylhydroxyanisole 1 mg, DL-methionine 0.7 g, Mn 70 mg, Zn 50 mg, Fe 40 mg, Cu 6 mg, I 1 mg, Co 0.3 mg, Se 0.2 mg

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350 phytase units (FTU)/kg; F). The Natuphos® (BASF, Ludwigshafen, Germany), a preparation of 3-phytase (EC 3.1.3.8) that was produced by *Aspergillus niger*, was used as the source of F. The ingredients and the nutrient compositions of the diets are listed in Table 1. The calculations of the nutrient contents were performed using the standard values (National Research Council 1994). All the diets contained (per kg): 11.5 MJ of apparent metabolizable energy (AME_N), 170 g crude protein, and 34 g Ca. Finely (0.09–0.50 mm) and coarsely-ground limestone (1.00–2.00 mm) were supplied at the ratio of 65 : 35. The feed and fresh water were supplied *ad libitum*. The experiment was conducted for 14 weeks. The study protocol was approved by the Ethical Committee of the Institute of Animal Science.

The number of eggs and the hens and their health status were monitored daily. The hen-day egg production and feed intake were calculated weekly on a per-cage basis. The egg weights were determined once per week, the average values are shown in Table 2.

Egg quality determination. The physical parameters of the eggs were determined three times during the experiment (in weeks 41, 45, and 49 of hens' life) (Skrivan et al. 2015). Once within each collection period, a whole-day egg production was analyzed. A total of 475 eggs were analyzed in the experiment. Egg weight was measured on a laboratory scale, the average values are shown in Table 3. Yolk height was measured using a digital micrometer head IP54 (Swiss Precision Instruments, Inc., Garden Grove, USA). Yolk index (YI) was calculated as

$$YI = (\text{yolk height}/\text{yolk diameter}) \times 100$$

The Haugh units (HU) were calculated according to Haugh (1937). Shell breaking strength was determined on the vertical axis using an Instron 3360 apparatus (Instron, Norwood, USA). Shell thickness (i.e. the average of 3 values from the sharp and blunt ends and the equator) was measured using a micrometre, after removing the shell membranes. The shells with membranes were washed, dried for 2 h at 60°C, and weighed. Shell percentage was determined by considering the individual weight of each egg and the weight of shell. The egg shell index (SI) was calculated as follows (Ahmed et al. 2005):

$$SI = (SW/S) \times 100,\ S = 4.68 \times EW^{2/3}$$

where:
SW = shell weight
S = shell surface
EW = egg weight

The eggshells ash content was determined once during the experiment (in week 45 of hens' life), and 256 eggs were analyzed (n = 8; 4 eggs per sample). The shells were dried at 105°C for 24 h, placed in a desiccator and weighed, and the dried homogenized eggshells were ashed in a muffle furnace at 500°C for 12 h.

Digestibility determination. For the determination of Ca and P digestibility in the ileum, two groups of hens (n = 10) were used, with diets of 1.8 g/kg of NPP either with or without F Natuphos® addition (350 FTU/kg). Titanium dioxide, as a dietary marker, was added to the diet at a rate of 5 g/kg. Hens were fed the diets for seven days; then they were starved for 1 h, given access to the feed for 2 h, and slaughtered. The carcasses were dissected to reveal the lower gastro-intestinal tract between Meckel's diverticulum and the ileo-caecal-colonic junction. Digesta were gently squeezed into a small plastic container, and the

Table 2. Effect of non-phytate phosphorus and phytase on hen performance

NPP	1.8 g/kg				2.1 g/kg				SEM	Probability		
F (FTU/kg)	0	150	250	350	0	150	250	350		NPP	F	NPP × F
Hen-day egg production (%)	96.8	97.3	95.1	96.7	96.3	95.6	95.3	94.7	0.22	0.013	ns	ns
Egg weight (g)	64.7^{c}	65.8^{b}	65.9^{b}	65.8^{b}	64.5^{c}	64.9^{c}	67.0^{a}	63.1^{d}	0.12	0.004	< 0.001	< 0.001
Egg mass (g/day/hen)	62.6^{bc}	64.0^{a}	62.7^{bc}	63.6^{ab}	62.1^{c}	62.1^{c}	63.8^{a}	59.8^{d}	0.16	< 0.001	< 0.001	< 0.001
Feed intake (g/day/hen)	118.8	116.9	115.6	115.2	118.0	119.4	120.4	115.3	0.43	0.046	0.017	ns
Feed intake (g/egg)	122.8	120.3	121.7	119.3	122.6	125.0	126.4	121.9	0.48	< 0.001	0.043	ns
Feed conversion ratio (g/g)	1.90^{ab}	1.82^{c}	1.84^{bc}	1.81^{c}	1.90^{ab}	1.93^{a}	1.89^{ab}	1.93^{a}	0.007	< 0.001	ns	0.009

NPP = non-phytate phosphorus, F = phytase, FTU = phytase units, ns = not significant
$^{a–c}$means within the same row with different superscripts differ significantly

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samples were immediately frozen and then freeze-dried. Digestibility was determined according to the modified methodology of Myers et al. (2004).

Microbial colonization determination. The molecular analyses of microbial colonization was performed using the same two groups of hens as for the digestibility determination. The ileal and caecal samples were collected from 40 animals (20 animals from each treatment group; 80 samples in total). The samples of each group and location were randomly paired, and each pair of samples was pooled to obtain the final number of 40 samples (10 samples for each treatment and location). The samples were collected and immediately stored at –20°C until the molecular analyses of bacteria.

For the molecular analysis (PCR-DGGE), DNA was extracted from the cells with the QIAGEN QIAamp® DNA stool mini kit (Qiagen, Hilden, Germany). After the extraction, the concentration and the A260/A280 ratio were measured using a NanoDrop 1000 instrument (Thermo Fisher Scientific, Waltham, USA) to check the DNA quality.

The fragments of the 16S rRNA genes were amplified from the extracted DNA with PCR using the "DGGE" bacterial primers 5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG–3' and 5''- ATT ACC GCG GCT GCT GG-3'. The PCR conditions were as follows: 1 cycle (94°C for 5 min, 55°C for 1 min, and 72°C for 1 min); 35 cycles (94°C for 1 min, 55°C for 1 min, and 72°C for 1 min); and 1 cycle (94°C for 1 min, 55°C for 1 min, and 72°C for 7 min) (Muyzer et al. 1993). The primers were synthesized by Generi Biotech (Hradec Králové, Czech Republic). The PCR reaction (30 µl) was performed using REDTaq®ReadyMix with $MgCl_2$ (Sigma-Aldrich, St. Louis, USA).

The amplicon resolution was performed using denaturing gradient gel electrophoresis (DGGE) with the BioRad DCode Universal Mutation Detection System (Bio-Rad, Hercules, USA), following the manufacturer's guidelines. The PCR products (25 µl) were loaded onto 9% TAE polyacrylamide gels, which contained a 35–60% denaturant gradient (100% denaturant: 7M urea and 40% deionised formamide). The electrophoresis was performed in 1× TAE (40 mmol/l Tris, 20 mmol/l acetic acid, and 1 mmol/l EDTA) buffer at a constant voltage of 55 V and a temperature of 60°C for 19 h. The gels were stained for 30 min with SYBR®Green I dye at 10 ppm (Mrazek et al. 2008), and the gel image was saved with an EC3 gel documentation system (UVP Bioimaging Systems, Upland, USA). The standard ladder was included with each DGGE gel electrophoresis as a positive control (Michiels et al. 2012).

The DGGE profiles of the samples from the ileum or the caecum were compared using the Ward cluster analysis based on pairwise similarities with the settings as follow: Ochiai band-based similarity coefficient, optimization 1%, tolerance 0.5%, and fuzzy logic on (BioNumerics, Version 7.5, 2014).

The bands of interest (12 bands) were aseptically incised from the gels under UV light, and amplified and identified by the sequencing. For the PCR, the conditions and the primers were similar to the PCR reaction previously described. The only exception was the absence of a CG-clump in the forward primer. The PCR products were further purified with the QiaQuick Purification kit (Qiagen) and sequenced on an ABI 3130 Genetic Analyser (Applied Biosystems, Foster City, USA). The corrected sequences were compared with the blastn (query vs nucleotide database) (Altschul et al. 1997).

Statistical analyses. The results were analyzed using the General Linear Models (GLM) procedure of the SAS software (Statistical Analysis System, Version 9.3, 2003). The data for hens performance and egg quality were analyzed with two-way analysis of variance (ANOVA), and the data from the digestibility determinations were evaluated with the *t*-test. The main effects were the concentration of non-phytate phosphorus (NPP), phytase supplementation (F), and the interaction between these two factors (NPP × F). The differences were considered significant at $P < 0.05$. The results in the tables are presented as the mean and standard error of the mean (SEM).

RESULTS

The results on the hens performance are presented in Table 2. The highest egg weight ($P < 0.001$) was from the hens fed the diet with 2.1 g/kg of P and enriched with phytase at 250 FTU/kg. The phytase supplement (150 FTU/kg) in the diet with the P content of 1.8 g/kg resulted in a consistent increase in egg weight, as did the F doses of 250 and 350 FTU/kg in the diets with the identical P content. The highest dose of F (350 FTU/kg) and 2.1 g/kg of NPP in the mixed feed decreased ($P < 0.001$) egg weight and egg mass production. The lowest feed conversion ratio ($P = 0.009$) was recorded in

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Table 3. Effect of non-phytate phosphorus and phytase on egg quality

NPP	1.8 g/kg				2.1 g/kg				SEM	Probability		
F (FTU/kg)	0	150	250	350	0	150	250	350		NPP	F	NPP × F
Egg weight (g)	65.0	65.2	66.2	65.1	64.6	64.7	66.2	63.2	0.24	ns	0.023	ns
Haugh units	81.7	81.6	81.6	81.6	81.0	79.3	81.7	82.1	0.34	ns	ns	ns
Yolk index (%)	44.0	43.9	43.8	44.0	43.4	44.1	43.0	42.9	0.13	0.029	ns	ns
Shell percentage (%)	9.8^{bcd}	9.6^{cd}	10.0^{ab}	9.9^{bc}	10.0^{ab}	9.8^{bcd}	9.5^{d}	10.2^{a}	0.04	ns	0.029	0.002
Shell thickness (μm)	348^{bc}	343^{c}	357^{ab}	351^{abc}	351^{abc}	348^{bc}	341^{c}	360^{a}	1.4	ns	ns	0.006
Shell breaking strength (N)	41.4	39.8	41.9	43.2	43.4	41.9	39.8	43.5	0.33	ns	0.013	ns
Shell index (g/100 cm^2)	8.4^{bcd}	8.3^{cd}	8.7^{ab}	8.5^{abc}	8.5^{ab}	8.4^{abc}	8.2^{c}	8.7^{a}	0.03	ns	ns	0.003
Ash content of shell (g/kg DM)	954.5	952.7	953.7	952.6	951.9	952.4	953.1	953.6	0.40	ns	ns	ns

NPP = non-phytate phosphorus, F = phytase, FTU = phytase units, DM = dry matter, ns = not significant
$^{a–d}$means within the same row with different superscripts differ significantly

the hens that were fed the mixture with 1.8 g/kg of NPP with either 150 or 350 FTU/kg. The high level of NPP in the mixed feed significantly decreased egg production ($P = 0.013$), egg weight ($P = 0.004$), and egg mass production ($P < 0.001$) and significantly increased feed intake per hen ($P = 0.046$), feed intake per egg ($P < 0.001$), and feed conversion ratio ($P < 0.001$).

As shown in Table 3, the highest values of shell percentage ($P = 0.002$), shell thickness ($P = 0.006$), and shell index ($P = 0.003$) were recorded in the hens that were fed 2.1 g/kg of NPP and 350 FTU/kg. The high content of NPP (2.1 g/kg) in the diet decreased the yolk index ($P = 0.029$). The NPP and the F contents in the diet did not affect the Haugh units or the ash content of the shells. The F increase in the diet increased the shell strength ($P = 0.013$).

The effect of F on the digestibility of Ca and P in the ileum was tested for the diet with 1.8 g/kg of P (Table 4). The phytase addition at 350 FTU/kg increased, although not significantly, the digestibility of these two minerals by 6.1% and 7.4%, respectively.

Identical groups that were used for the digestibility assessment were also used for the microbial colonization determination. Figure 1 shows the pattern from the DGGE analysis on the samples that were collected from the ileum. Among the analyses, a specific pattern in the groups within a particular treatment did not emerge. In the samples collected from the caecum, the results were similar (data not shown). Although no grouping was identified with the DGGE analysis, one group of bands was identified (with the unaided eye) as more frequently pronounced in the group of hens with the F supplementation. After sequencing, the bands ($n = 6$) were identified as *Lactobacillus* spp.

Table 4. Effect of phytase on calcium and phosphorus digestibility in the ileum

NPP	1.8 g/kg	2.1 g/kg	SEM	Probability
F (FTU/kg)	0	350		
Calcium (%)	50.5	53.6	4.24	ns
Phosphorus (%)	43.2	46.4	1.55	ns

NPP = non-phytate phosphorus, F = phytase, FTU = phytase units, ns = not significant

DISCUSSION

Based on previous findings that high doses of NPP had a negative effect on hen performance (Skrivan et al. 2010; Englmaierova et al. 2012, 2014a), low levels of NPP were chosen for this study. An increase in the NPP level from 1.8 to 2.1 g/kg significantly decreased egg production, egg weight, and egg mass production and significantly increased the feed intake per hen, the feed intake per egg, and the feed conversion ratio. In the study of Kozlowski and Jeroch (2011), two very different levels of NPP (2.5 and 1.3 g/kg) were compared, and the reduction in the NPP content resulted in significant decreases in the laying rate and the egg mass of approximately 3.9% and 5.4%, respectively, and a significant increase in the feed conversion ratio of 7.9%. The supplementation of the hen diets with very low levels of NPP (1.0–1.5 g/kg) with F improved the performance parameters (Wu et al. 2006; Liu et al. 2007; Kozlowski and Jeroch 2011; Gao et al. 2013). In the present study, the 3-phytase Natuphos®, which has a bimodal optimum pH of 2.5 and 5.5, was used. The addition of the F supplement

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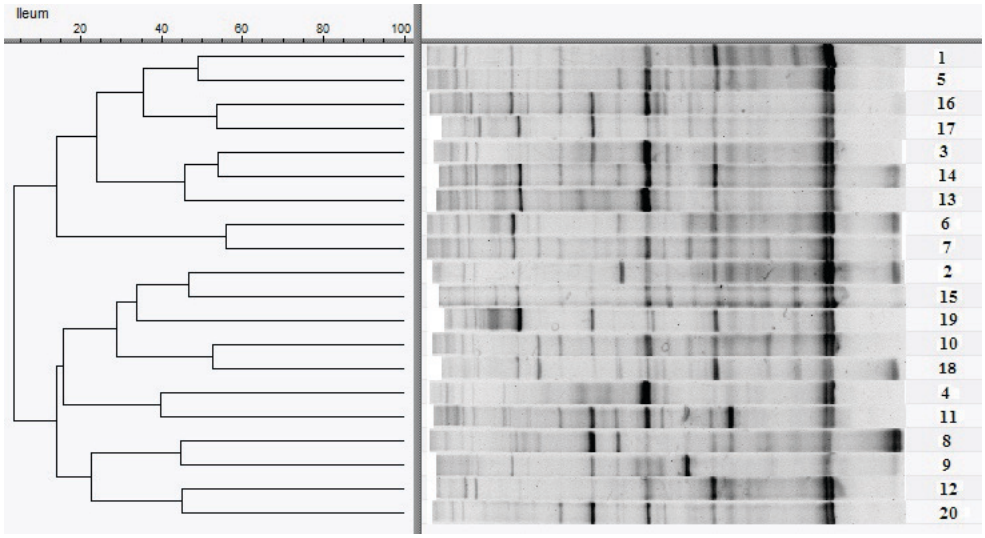

Figure 1. Denaturing gradient gel electrophoresis (DGGE) analysis of the caecum[1], as compared by the Ward cluster analysis[2]

[1]samples 1–10: control group (1.8 g/kg of NPP without phytase), samples 11–20: treatment (1.8 g/kg of NPP with phytase Natuphos®)

[2]Ochiai band-based similarity coefficient, optimisation 1%, tolerance 0.5%, and fuzzy logic on (BioNumerics, Version 7.5, 2014)

(150–350 FTU/kg) to the diet that contained 1.8 g/kg of NPP in the feed decreased the feed conversion ratio and increased the egg weight. These findings were consistent with the results of Ahmadi and Rodehutscord (2012), who calculated the optimal levels of NPP to be 1.8 and 1.5 g/kg with 150 and 300 FTU/kg of feed, respectively. Additionally, Um and Paik (1999) showed that supplementation with 500 FTU of the microbial phytase Natuphos®/kg diet reduced the NPP level to 1.2 g/kg in the diets of layers without affecting the laying performance. The higher levels of NPP and F (2.1 g/kg and 350 FTU/kg) had negative effects on the egg weight and the egg mass production, which was consistent with the findings of Lim et al. (2003) that the levels of NPP and Ca significantly modified the effects of F supplementation.

The albumen quality was not affected by the treatments. Um and Paik (1999) also did not record an effect from the addition of F on the Haugh units. For the shell quality, the results were similar in the diets that contained 1.8 g/kg of NPP with 350 FTU/kg and 2.1 g/kg of NPP without the F supplement. In the study of Yan et al. (2009), the supplementation of F at 500 FTU/kg into a diet with 2.0 g/kg of NPP led to increases in shell breaking strength and shell thickness to levels that were comparable with those of eggs produced by the hens that received the control diet with 4.0 g/kg of NPP; thus, the supplemental F had a beneficial effect on shell quality. The highest percentage, thickness, and index of the shells were detected in the eggs of hens fed the diet with 2.1 g/kg of NPP and the highest dose of F, which were most likely related to the lowest egg weight of the hens from this treatment.

Although the increase was not significant, phytase improved the digestibility of Ca and P in the ileum in the current study. These results were consistent with those of Yan et al. (2009), who reported a significant increase in the digestibility of P in response to supplementation of the diet that contained 2.0 g/kg of NPP with the microbial F Natuphos® at 250 and 500 FTU/kg. Additionally, Boling et al. (2000) reported that F supplementation decreased the P concentration in the excreta by approximately 50%. Bougouin et al. (2014) also showed that F supplementation had a significant positive effect on the retention of P, and the layers that received exogenous F at 371 FTU/kg were associated with a 5.02 percentage unit increase in the retention of P. Because the phytate complexes were, to some extent, cleaved by F (Nair et al. 1991), the retention of P improved in the diet that contained low levels of NPP. The supplementation with phytase greatly reduced the potential environmental pollution problems caused by inorganic P in the feed (Wu et al. 2006). Because Ca is only used efficiently for skeletal growth when P is available simultaneously (Underwood and Suttle 1999), a likely explanation is provided for the increase in the digestibility of P that was accompanied by the increase in the digestibility of Ca.

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In the present study, *Lactobacillus* spp. was more frequent in the ileum and caecum of hens fed a diet enriched with F than of those fed a diet without F. This result was in contrast to that of Lu et al. (2009) in which no enzyme treatment (F or xylanase) affected the lactobacillus or the anaerobic content in the ileal digesta of broilers. However, Aydin et al. (2010) found that in the ileal digesta the viable counts of anaerobic bacteria, *Escherichia coli*, and coliform bacteria were significantly reduced among the broilers fed diets with F. The possible changes in the gastrointestinal tract caused by F may not only modify the pH of the ileum, but also may affect the digestibility of nutrients. Moreover, the addition of dietary F might improve performance, energy, and amino acid availability (Cowieson et al. 2006). Dibner and Buttin (2002) reported that dietary F might change the levels of some bacteria measured in the ileum because of a change in the nutrient supply, which changed the growth of the affected groups of microflora. However, further challenge studies are required to evaluate the effects of dietary F on the gut microflora.

CONCLUSION

The interaction of NPP and F was demonstrated on some characteristics of performance and shell quality. The shell quality of eggs that were laid by hens fed a diet with 1.8 g/kg of NPP and 350 FTU/kg of F Natuphos® was comparable with the shell quality of eggs from hens that received a diet with only 2.1 g/kg of NPP. The supplementation of microbial 3-phytase at a level of 350 FTU per kg in a low-P diet improved the digestibility of minerals and caused a change in the microflora of the digestive tract.

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