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Effect of Suboptimal Levels of Non-Phytate Phosphorus and Exogenous Phytase on Precaecal Digestibility of Phosphorus and Calcium in Laying Hens

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ABSTRACT

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The precaecal digestibility of phosphorus and calcium in laying hens was evaluated at two dietary levels of non-phytate phosphorus (NPP; 1.58 and 2.03 g/kg) and two levels of exogenous 3-phytase (F; 0 and 300 phytase units (FTU)/kg). A total of 192 ISA Brown hens were used for the study, and were housed in enriched cages (8 per cage). At the end of the experiment, which was the peak of the egg production, the content of the posterior half of the ileum from each hen was collected. The lower level of NPP significantly ($P < 0.05$) improved the precaecal phosphorus digestibility coefficient. There was no significant difference in the amount of absorbed phosphorus between the lower and the higher level of NPP. The level of NPP with supplementation of exogenous phytase had a significant effect ($P < 0.001$) on the concentration of phosphorus in ileum, while the higher level of NPP increased phosphorus concentration in the ileum and phytase decreased phosphorus content in the ileal digesta. The phytase had no significant effect on phosphorus precaecal digestibility. There was no significant effect of the both factors, level of NPP, and exogenous phytase on the daily calcium intake, the amount of absorbed calcium, the content of calcium in ileal digesta, and on the precaecal digestibility of calcium. However a significant effect ($P < 0.05$) of the interaction between NPP and phytase was observed on the amount of absorbed calcium and on the precaecal digestibility coefficient. It can be concluded that the estimation of phosphorus ileal digestibility depends on the level of dietary phosphorus.

Keywords: layers; ileum; calcium and phosphorus absorption

The recommended levels of available phosphorus per kg of the diets for laying hens vary from 2.0 to 3.5 g (Boorman and Gunaratne 2001). The recommendation of the National Research Council (1994) for laying hens is 2.5 g of non-phytate phosphorus (NPP). The results of many short- and long-term experiments indicate that the NPP requirements of laying hens may be even lower than the National Research Council (1994) recommendation (Keshavarz and Nakajima 1993; Van der Klis and Versteegh 1996; Gordon and Roland 1997; Parsons 1999). 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 of NPP in the diet.

According to Zobac et al. (1997), phosphorus contained in plants is bioavailable from only 20–30% in monogastric animals. A large proportion of

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phosphorus in cereals, oilseed, and grain legumes is in the form of phytate (myo-inositol hexakisphosphate) (Marounek et al. 2008), however phytase (phosphohydrolase, EC 3.1.3.8) catalyzes the release of phosphorus from phytate. Notwithstanding, plant ingredients in diets for poultry contain native phytase activity, in common feed practices, phytase is added to poultry diets as exogenous phytase (Yao et al. 2007). It is a well-documented fact that in broilers phytase increases not only the bioavailability of phosphorus, but also the bioavailability of calcium and amino acids (Singh 2008). Gordon and Roland (1997) reported that laying hens consuming a low NPP diet with supplementary phytase performed as well as laying hens fed diets containing higher levels of NPP without supplementary phytase. In recent study Englmaierova et al. (2015) confirmed that 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 phytase Natuphos® was comparable with the shell quality of eggs from hens that received a diet with only 2.1 g/kg of NPP. On the other hand, there is a lot of factors, both external and internal, affecting egg shell quality (Tumova et al. 2014; Ketta and Tumova 2016; Skrivan et al. 2016).

Over the last 70 years various approaches have been developed to determine and estimate phosphorus availability from different feeds. The content of ash and phosphorus in the tibia of growing poultry (Gillis et al. 1954; Hurwitz 1964), breaking strength of the tibia (Morrison et al. 1956; Rowland et al. 1967; Hemme et al. 2005), bone mineral density (Onyango et al. 2003; Angel et al. 2006), blood criteria (Gardiner 1962; Hurwitz 1964; Lima et al. 1997) or growth and feed conversion (Summers et al. 1959) have all been used as qualitative measurements of phosphorus availability. Data obtained using these methods are more qualitative than quantitative and they provide only relative availability values for tested phosphorus sources.

Conversely, quantitative approaches are based on phosphorus retention and precaecal digestibility. Quantitative values for feed raw materials are needed in order to allow meat and egg producers to make feed formulation decisions about feed phosphorus and commercial phytase based on the economic value of products compared to the disadvantages of phosphorus in poultry excreta. The retention of phosphorus may be measured either by complete excreta collection (Rodehutscord and Dieckmann 2005) or by calculating the retention value with indigestible markers (Leske and Coon 2002). Different definitions and several approaches for the determination of phosphorus availability have caused confusion in communication making it almost impossible to compare obtained data. As a result, Working Group No 2, from the nutrition branch of The European Federation of Branches of WPSA, formulated a standardization protocol regarding the evaluation and dietary requirement of phosphorus, helping harmonize communication within the field.

The aim of the study was to determine the effect of suboptimal levels of non-phytate phosphorus (1.58 and 2.03 g/kg) and exogenous phytase on the availability of phosphorus and calcium in laying hens while the level of calcium in the diets remained identical (34.8 g/kg). The hypothesis was following: when there is no negative effect of suboptimal NPP on both egg production and eggshell quality, there should be difference in phosphorus availability. The second hypothesis was following: when a suboptimal level of NPP is used, phytase increases phosphorus and calcium availability. In this study the availability of both calcium and phosphorus was expressed by coefficients of precaecal digestibility (WPSA 2013).

MATERIAL AND METHODS

The research on live hens met the guidelines approved by the institutional animal care and use committee and the research was authorized by the Ministry of Education, Youth and Sports of the Czech Republic (MSMT-14470/2016-2).

Birds and management. A total of 192 ISA Brown pullets were used in this experiment. Pullets, aged 16 weeks, were housed in three batteries with three-floor enriched cages with 8 birds per cage unit (in total 24 cages) in the same air-conditioned facility. The cages were equipped with a nest, perch, dust bath area, and equipment for the abrasion of claws, conforming to the European Union Council Directive 1999/74/EC (1999). Rearing, including feeding and lighting schemes, was carried out according to the technological instructions for hybrid ISA Brown. A diet for rearing was used in weeks 16–17 of age and a pre-lay diet was used in weeks 18–19 of age. From the beginning of laying to the start of the

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experiment, the hens were fed a commercial diet for laying hens. The room temperature was kept at 21°C. Light intensity was approximately 10 lx in the central floor. The experiment started when laying hens were at the peak of egg production (97%) in the 26th week of age and lasted 9 days. The average weight of laying hens was 1.85 kg. All hens were allowed *ad libitum* access to the feed and water. Feed intake was checked daily, and average daily intake was calculated. A 15-h photoperiod from 03.00 to 18.00 h was used throughout the experiment. There was no any mortality during the experiment.

Experimental diets. At the beginning of the experiment, the cages with laying hens were divided into 4 groups according to their respective diet treatments, with 6 repetitions per treatment (each replication had 8 hens). The number of hens and replications was chosen according to the protocol of the Working Group No 2 (WPSA 2013).

The composition of the diets and content of the nutrients are shown in Tables 1 and 2. Diets were for-

Table 1. Composition of the diets

Component	Treatments[1]			
	T1	T2	T3	T4
Maize (g/kg)	386.1	386.1	386.1	386.1
Wheat (g/kg)	296.4	296.4	294.1	294.1
Rapeseed oil (g/kg)	13.1	13.1	13.8	13.8
Soybean meal (g/kg)	197.5	197.5	198.0	198.0
DL-Methionine (g/kg)	1.1	1.1	1.1	1.1
L-Lysine HCl (g/kg)	0.4	0.4	0.4	0.4
Salt (g/kg)	3.7	3.7	3.7	3.7
Monocalcium phosphate (g/kg)	2.0	2.0	4.0	4.0
Limestone, powder (g/kg)	43.5	43.5	43.5	43.5
Limestone, grit (g/kg)	48.2	48.2	47.3	47.3
Aminovitan[2] (g/kg)	3.0	3.0	3.0	3.0
Cr_2O_3 (g/kg)	5.0	5.0	5.0	5.0
Level of added phytase (FTU/kg)	0	300	0	300
Activity of natural phytase (FTU/kg)	86			
Calculated nutrient composition (feed matter basis)				
Crude protein (g/kg)	166.9			
Dry matter (g/kg)	886.3			
Fat (g/kg)	36.2			
Total dietary fibre (g/kg)	23.9			
Metabolizable energy (MJ)	11.5			
Arginine (g/kg)	10.2			
Isoleucine (g/kg)	6.71			
Methionine (g/kg)	3.72			
Methionine + cysteine (g/kg)	6.77			
Threonine (g/kg)	5.96			
Triptophan (g/kg)	1.91			

[1]T1 = 1.58 g/kg non-phytate phosphorus (NPP) without phytase, T2 = 1.58 g/kg NPP + phytase 300 FTU/kg, T3 = 2.03 g/kg NPP without phytase, T4 = 2.03 g/kg NPP + phytase 300 FTU/kg

[2]premix provided per kg diet: retinol 3 340 000 IU, cyanocobalamin 3300 μg, cholecalciferol 1 000 000 IU, α-tocopheryl acetate 11 000 IU, menadione 670 mg, niacinamid 8350 mg, folic acid 170 mg, thiamine 670 mg, biotin 25 000 μg, riboflavine 1700 mg, cholinchlorid 80 000 mg, pyridoxine 1350 mg, Cu 2000 mg, Mn 23 350 mg, Fe 13 350 mg, Zn 16 670 mg, I 340 mg, Se 67 mg, butylhydroxytoluen 400 mg, butylhydroxyanisol 80 mg

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mulated to contain the same levels of metabolizable energy (ME) 11.8 MJ/kg and crude protein 16.6%.

Monocalcium phosphate (MCP) was the main source of inorganic phosphorus in the diets. The experimental design with an arrangement of treatments was employed, including two NPP levels (1.58 and 2.03 g/kg) and 2 phytase levels (0 and 300 phytase units (FTU)/kg). The source of the phytase was a preparation of 3-phytase (EC 3.1.3.8) produced by *Aspergillus niger*. The content of phosphorus and calcium in the diets is shown in Table 2. The activity of natural phytase in the diets without exogenous phytase was 86 FTU/kg.

On day 9, after feeding the experimental diets, all laying hens were slaughtered. The digestive tract of each hen was immediately removed. The digesta of the posterior half of the ileum, except the 2 cm prior to the ileo-caeco-colonic-junction, were flushed out with distilled water and dried immediately at 65°C and were subsequently ground for analysis. Chromium oxide was used as an indigestible marker within the diets.

Analyses and calculations. The dry matter was determined by drying the samples (the contents of the ileum and diets) at 103 ± 2°C for 2 h. The ash was determined at 550 ± 20°C for 6 h. Within the feed and the ileal digesta, the content of calcium was determined by the atomic absorption spectrometry (ContrAA; Analytik Jena AG, Germany) (ISO 6869:2000) and phosphorus was determined by the spectrometric method (ISO 6491:1998). The phytase activity of the diets was determined in the laboratory according to ISO 30024:2009. The content of chromium oxide was determined by titration after oxidation to dichromate.

Coefficients of phosphorus and calcium digestibility were calculated according to the formula:

$$\text{Coefficient of nutrient digestibility} = 1 - (I_d \times N_i)/(I_i \times N_d)$$

where:

I_d = content of marker in diet
I_i = content of marker in ileal digesta
N_d = content of nutrient in diet
N_i = content of nutrient in ileal digesta

The amount of feed intake was recorded in each cage. Consequently the amount of calcium and phosphorus intake was calculated, too. On the basis of precaecal digestibility coefficients and the amount of phosphorus and calcium intakes the amount of absorbed phosphorus and calcium was evaluated.

Data were analyzed using a two-way ANOVA statistical test and an LSD-test using the software package Unistat (Version 5.1). Cage served as the experimental unit for all statistical analyses, and differences were considered significant at $\alpha = 0.05$. Data from the two levels of NPP and two levels of phytase were analyzed as a completely randomized design. The analysis was in accordance with the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + e_{ijk}$$

where:

y_{ijk} = the parameter
μ = overall mean
α_i = non-phytate phosphorus level
β_j = phytase level
γ_{ij} = interaction of non-phytate phosphorus and phytase
e_{ijk} = error term

Table 2. Calculated and analyzed contents of phosphorus, calcium, and phytase in the diets

Calculated contents	T1	T2	T3	T4
Non-phytate phosphorus level (g/kg)	1.58		2.03	
Phytase (FTU/kg)	0	300	0	300
Calcium (g/kg)	34.8	34.8	34.8	34.8
Total Ca/total P	9.38	9.38	8.37	8.37
Phytate P (g/kg)	2.13	2.13	2.12	2.12
Total P (g/kg)	3.71	3.71	4.16	4.16
Available P (g/kg)	2.55	2.55	2.95	2.95
Analyzed contents				
Calcium (g/kg)	33.5	35.0	33.9	35.2
Total P (g/kg)	3.82	3.77	4.12	4.24
Phytase (FTU/kg)	85.5	364.5	86.3	336.5

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Table 3. Daily feed intake (g/day) during the 9-day experiment, at peak egg production

Treatments[1]	Daily feed intake (g/day)	SE	v_x
T1	109	3.80	0.09
T2	113	1.93	0.04
T3	110	1.01	0.02
T4	110	2.24	0.05
P-value	> 0.05		

SE = standard error, v_x = coefficient of variability

[1]T1 = 1.58 g/kg non-phytate phosphorus (NPP) without phytase, T2 = 1.58 g/kg NPP + phytase 300 FTU/kg, T3 = 2.03 g/kg NPP without phytase, T4 = 2.03 g/kg NPP + phytase 300 FTU/kg

RESULTS

As shown in Table 3, there was no significant difference in the average daily feed intake between the treatments. Table 4 summarizes the results of the analysis of daily intake, absorption, and precaecal digestibility of P. The level of the NPP in the diets had a significant positive effect ($P < 0.001$) on daily phosphorus intake, which ranged from 415.1 mg/day (T1) to 467.3 mg/day (T4). Neither the diet supplementation of exogenous phytase 300 FTU/kg, nor the interaction of NPP × phytase had a significant effect on daily intake of P. Although, while there was a significant effect of the level of NPP on daily phosphorus intake, there was no significant difference between the treatments in the daily amount of phosphorus absorbed. In addition, the amount was slightly higher in T1 and T2 treatments, with a lower NPP level, compared to T3 and T4. Additionally, there was no significant effect of the supplementation of phytase on the amount of absorbed phosphorus; however, the amount of absorbed phosphorus was slightly higher in laying hens fed the diets with exogenous phytase (T2 and T4) than in hens fed the diets without phytase (T1 and T3).

The results reveal a significant ($P < 0.05$) effect of the interaction NPP × phytase on the concentration of phosphorus in the ileal digesta. In the treatments with phytase (T2 and T4), the concentration of phosphorus in the ileal digesta was lower than in treatments T1 and T3. However, a significant effect of phytase was observed only in the treatments with a lower NPP content.

Table 4. Effect of exogenous phytase and the level of non-phytate phosphorus (NPP) on the intake, absorption, and precaecal digestibility of phosphorus

Treatments[1]	Intake of P (mg/day)	Amount of absorbed P (mg/day)	Concentration of P in digesta (g/kg DM)	Precaecal digestibility coefficient
T1	415.1	141.5	8.62[b]	0.341
T2	425.9	154.4	6.86[c]	0.363
T3	454.4	139.3	9.59[a]	0.306
T4	467.3	150.1	9.05[ba]	0.322
NPP effect				
1.58 g/kg	420.5[b]	148.0	7.74[b]	0.352[a]
2.03 g/kg	460.9[a]	144.7	9.32[a]	0.314[b]
Added phytase effect				
0 FTU/kg	434.8	140.4	9.10[a]	0.324
300 FTU/kg	446.6	152.3	7.95[b]	0.342
SEM	6.28	3.75	0.25	0.009
***P*-values**				
NPP	$P < 0.001$	ns	$P < 0.001$	$P < 0.05$
Phytase	ns	ns	$P < 0.001$	ns
NPP × phytase	ns	ns	$P < 0.05$	ns

DM = dry matter, SEM = standard error of the means, ns = not significant

[1]T1 = 1.58 g/kg NPP without phytase, T2 = 1.58 g/kg NPP + phytase 300 FTU/kg, T3 = 2.03 g/kg NPP without phytase, T4 = 2.03 g/kg NPP + phytase 300 FTU/kg

[a–c]statistically significant difference between treatments ($P < 0.05$) for the same characteristic

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The coefficient of precaecal digestibility of phosphorus ranged from 0.306 (T3) to 0.363 (T2). The level of NPP had a significant negative effect ($P < 0.05$) on the precaecal digestibility of phosphorus. Both supplements of exogenous phytase and the interaction NPP × phytase had no significant effect on the precaecal digestibility of phosphorus. Notwithstanding, the coefficients of precaecal digestibility of phosphorus in the treatments with phytase (T2 and T4) were slightly higher than in T1 and T3 treatments.

The results obtained from the analysis of calcium are displayed in Table 5. There was no significant effect found in both of the levels of NPP and exogenous phytase on the daily calcium intake, the amount of absorbed calcium, the concentration of calcium in the ileal digesta, and the precaecal digestibility of calcium. Nevertheless, the interaction between NPP and phytase had a significant effect on the amount of absorbed calcium and the precaecal digestibility. The highest coefficients of precaecal digestibility of calcium were found in the treatment T4 (0.452) and T1 (0.441).

DISCUSSION

Significantly higher coefficients of phosphorus precaecal digestibility were found in treatments with lower NPP in the diets (0.341 (T1) and 0.363 (T2) in comparison with 0.306 (T3) and 0.322 (T4)). And the amount of absorbed phosphorus was not affected by the levels of NPP. Thus, the lower dietary levels of NPP do not mean lower amount of digested phosphorus. At lower levels of NPP, phosphorus is more effectively utilized than from diets with higher levels of NPP. This can explain why the performance of layers is the same or higher when using the lower levels of NPP in the diets (Englmaierova et al. 2012).

Gao et al. (2013) used an even lower NPP level (1.0 g/kg, total phosphorus 3.4 g/kg) than in this study, and they also reported a higher coefficient of apparent ileal digestibility of phosphorus from this diet (0.506) compared with phosphorus digestibility from diet with total phosphorus 5.7 g/kg and 3.2 NPP g/kg (0.474). Thus, it can be inferred that the digestibility of phytate phosphorus in

Table 5. Effect of exogenous phytase and the level of non-phytate phosphorus (NPP) on the intake, absorption, and precaecal digestibility of calcium

Treatments[1]	Intake of Ca (g/day)	Amount of absorbed Ca (g/day)	Concentration of Ca in digesta (g/kg DM)	Precaecal digestibility coefficient
T1	3.86	1.70[a]	62.6	0.441[a]
T2	3.81	1.14[b]	69.4	0.298[b]
T3	3.81	1.40[ab]	69.7	0.368[ab]
T4	3.82	1.72[a]	61.5	0.452[a]
NPP effect				
1.58 g/kg	3.84	1.45	66.0	0.378
2.03 g/kg	3.82	1.56	65.6	0.410
Added phytase effect				
0 FTU/kg	3.84	1.54	66.2	0.401
300 FTU/kg	3.82	1.49	65.5	0.390
SEM	0.03	0.09	2.16	0.023
***P*-values**				
NPP	ns	ns	ns	ns
Phytase	ns	ns	ns	ns
NPP × phytase	ns	$P < 0.05$	ns	$P < 0.05$

DM = dry matter, SEM = standard error of the means, ns = not significant

[1]T1 = 1.58 g/kg NPP without phytase, T2 = 1.58 g/kg NPP + phytase 300 FTU/kg, T3 = 2.03 g/kg NPP without phytase, T4 = 2.03 g/kg NPP + phytase 300 FTU/kg

[a,b]statistically significant difference between treatments ($P < 0.05$) for the same characteristic

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their study was also higher. This idea can be supported by both Zeller et al. (2015) and Shastak et al. (2014) who published that mineral phosphorus supplementation reduces the degradation of *myo*-inositol phosphates. On the other hand, Hafeez et al. (2016) also published very similar coefficients of apparent ileal phosphorus digestibility in layer diets (from 0.311 to 0.409). Conversely, they used 7.8 g/kg of monocalcium phosphate (MCP), while in this study 2.0 g/kg (or 4.0 g/kg) of MCP were used. It seems that lower total phosphorus in the diets increases phosphorus digestibility.

If phytase can improve the utilization of phytate phosphorus and thereby increases the utilization of phosphorus from the diets, the supplementation of inorganic phosphorus through mineral supplements could be reduced. The beneficial effect of phytase supplementation was clearly evident from the findings of Gordon and Roland (1997) who demonstrated that when hens consume 0.1% NPP diet with phytase (containing only 3.7 mg of phosphorus from supplemental phosphate), they are bound to perform as well as hens fed on diets containing higher levels of NPP without phytase. In this study, the results show a significant effect ($P < 0.001$) of exogenous phytase (300 FTU/kg) on the concentration of phosphorus in the ileal digesta. Although the concentration of phosphorus in the ileal digesta was significantly affected by phytase, there was no significant effect on either the coefficient of precaecal digestibility or the amount of absorbed phosphorus. However, in groups with supplemented phytase (T2 and T4), the results demonstrate a slightly higher digestibility coefficient and an increase in the amount of absorbed phosphorus in comparison with treatments without phytase (T2 0.363 vs T1 0.341 and T4 0.322 vs T3 0.306). Additionally, the positive influence of phytase has been shown in other studies as well. Kozlowski and Jeroch (2011a) reported that the supplementation of phytase in diets based on corn and soybean meal improved the ileal digestibility of calcium and phosphorus in laying hens. Similarly, Liu et al. (2007) found that adding phytase to the diet, in which NPP level was reduced by 0.13%, the total phosphorus was reduced by 0.14%, and calcium level was reduced by 0.12% improved the digestibility of phosphorus and calcium ($P < 0.05$). Gao et al. (2013) also reported a significant positive ($P < 0.01$) effect of phytase on apparent ileal digestibility of phosphorus at an NPP level of 1.0 g/kg, however they used the phytase activity 500 and 5000 FTU/kg.

The coefficients of precaecal calcium digestibility in this study ranged from 0.298 to 0.452. Studies by Hafeez et al. (2016) demonstrated low coefficient ranges from 0.232 to 0.251 in laying hens at 23 weeks of age. Hafeez et al. (2016) used almost the same content of calcium in the diet (35.7 g/kg) in comparison with our study (34.8 g/kg).

Phytase significantly decreased calcium digestibility, when a lower level of NPP was used in the diets. However Kozlowski and Jeroch (2011b) did not observe any effect of three different levels of phytase (125, 250, or 500 FTU/kg) on both calcium or phosphorus ileal digestibility using even lower content of available phosphorus in the diets – 1.3 g/kg. Lim et al. (2003) reported a significant effect of NPP on calcium availability ($P < 0.05$), a higher level of NPP (0.25% vs 0.15) decreased calcium retention. On the other hand, they did not observe any effect of phytase or the NPP × phytase interaction on calcium availability. Anyway, the calcium absorption and consequently both retention and ileal digestibility are affected by eggshell formation (Bar 2009). To obtain digesta, it is necessary to slaughter the hens, but it is impossible to do it in one time, in the same stage of shell formation.

This study was conducted to determine the effect of suboptimal levels of non-phytate phosphorus (1.58 and 2.03 g/kg) and exogenous phytase (300 FTU/kg) on precaecal digestibility of phosphorus and calcium in laying hens at the peak of production. The results demonstrated that the lower level of NPP significantly ($P < 0.05$) improved the precaecal phosphorus digestibility coefficient. However, there was no significant difference in the amount of absorbed phosphorus between the lower and the higher level of NPP. The phytase had no significant effect on both phosphorus and calcium precaecal digestibility, however calcium digestibility was significantly depressed after addition of the phytase to the low NPP diets ($P < 0.05$). It can be concluded that the estimation of phosphorus ileal digestibility depends on the level of dietary phosphorus.

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