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Efficacy of Phytase Preparations to Improve P Availability in Young Turkeys

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ABSTRACT-Phytate phosphorus of plant ingredients is not substantially available to poultry because of the lack of endogenous phytase enzymes in their gastrointestinal tract. There are substantial differences among broilers, turkeys, and ducks in terms of plant P utilization. We studied whether the availability of P is different for two Phytase enzymes in turkeys. Finase and a New Phytase product developed recently were tested in 3 to 5 week old turkeys. The efficacy of Finase and the New Phytase were determined on the basis of P balance and tibia data. A low P maize soybean meal based diet was supplemented at 0, 250 and 500 FTU/kg levels of Finase and 0, 250, 500 and 750 FTU/kg of the New Phytase. Excretions were collected in a balance trial and tibia parameters were obtained after the turkeys had received their respective diets for 17 days. Utilization of P from the basal diet was 35.5% which significantly improved by Finase to a maximum of 47.3% and by the New Phytase to a maximum of 48.7% at the highest level of supplementation. The utilization of Ca did not improve. Tibia ash concentration improved by Finase and the New Phytase supplementation. It is concluded that the Finase and the New Phytase efficiently improve the utilization of P in turkeys and may help protect the environment by causing a reduction in excretion P.

Keywords: Finase, Phosphorus, Phytase, Turkey

INTRODUCTION

Nitrogen and phosphorus from poultry excretion have been reported to have potential negative effects on air, soil, and water quality (Loehr, 1972; O'Connor et al., 1988; Smith et al., 2001). Measures taken to reduce nutrient excretion include dietary modifications (Smith et al., 2004; Wu-Haan et al., 2007) comprising of the mass reduction of the dietary nutrients fed over the lifetime of the animal (Elwinger and Svensson, 1996) and the use of phytase, an enzyme that works by releasing phosphate groups tightly bound to the phytate molecule, thereby making phosphorus available for absorption and a reduction in dietary CP concentration (Ferguson et al., 1998).

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Ravindran *et al*. (1994) reported that phytate phosphorus constituted 56–77% of the total P of the studied plants ingredients. In plant-based diets, the use of inorganic phosphates can be reduced in cases where microbial phytase is supplemented. This replacement depends on the product specific efficiency in releasing P under *in vivo* conditions. This is one reason why the efficiency of phytase needs to be determined in balance experiments. P utilization at marginal P supply, generally considered as the most sensitive parameter for the efficiency of phytase, was determined based on quantitative excreta collection.

Extensive research has been conducted on the use of phytase in broilers and layers (Cowieson et al., 2008; Hughes et al., 2008; Leytem et al., 2008; Liu et al., 2008; Manangi and Coon, 2008; Nyannor and Adeola, 2008; Peebles et al., 2008; Powell et al., 2008; Powers et al., 2008; Ravindran et al., 2008; Francesch and Geraert, 2009 and Liem et al., 2009), but research on its use in turkey diets is less inclusive. Turkeys are, like other poultry species, restricted in utilizing phytate P in cases when diets do not contain the enzyme phytase. Additionally, it is known that various phytase preparations may affect the utilization of phytate P to a different extent, triggered by differences in their *in vitro* characteristics such as pH optimum or their resistance against proteolytic enzyme activity. Due to the scarcity of P utilization studies on turkeys and the substantial differences among broilers, turkeys, and ducks regarding plant P utilization (Rodehutscord and Dieckmann, 2005), the present work aimed at finding out the efficacy of Finase and a New Phytase product for improving P utilization in turkeys.

MATERIALS AND METHODS

Diets

A basal diet was calculated according to the recommendations of GfE (2004), adequate in ME and all nutrients with the exception of P and Ca. Ingredients were selected to achieve a combination of a low total P content, a high proportion of phytate P in total P, and a low intrinsic phytase activity. The composition of the diet was (in g/kg): maize 500.7, solvent extracted soybean meal (44% crude protein) 450, soybean oil 20, vitamins and trace element premix[†] (P-free) 12, calcium carbonate 16 and DL-methionine 1.3. The calculated concentration of ME was 13.8 MJ/kg DM, and the analyzed concentrations of ash, P and Ca were 55.2, 5.45 and 6.72 g/kg DM, respectively.

The total amount of feed needed for the experiment was mixed in one lot and subsequently divided into 6 equal portions. Enzyme premixes were then supplemented to 5 portions to achieve activities as detailed in Table 1, and the diets were mixed again. Diet preparation was done in the feed mill facilities of the University Research Centre for Animal Sciences in Merbitz, Germany. Feed was pelleted at 70°C. Enzymes were supplied as premixes by AB Enzymes. Lot numbers were Limes 2007-1396-1, 2 and 3 for the new product, and Limes 2007-1396-4 and 5 for Finase. Results of chemical analysis confirmed the intended concentrations for proximate nutrients. The calculated concentration of phytate P was 3.0 g/kg in the basal diet. Analyzed phytase activities were higher than intended, but the differences among treatments were confirmed (Table 1).

Table 1. Intended and analyzed phytase activity in the pelleted diets

Sample	Phytase activity (FTU/kg)	
	Intended	Analysed
Basal diet	**0**	**51**
FINASE P	**250**	**340**
FINASE P	**500**	**501**
New Phytase	**250**	**374**
New Phytase	**500**	**561**
New Phytase	**750**	**754**

Animals, Housing and Sampling

One hundred and twenty, 20-day-old male turkeys were kept in Martin Luther University Research Centre for Animal Sciences in Merbitz, Germany, in group pens. During days 1-15 post-hatch a commercial starter diet was fed (P 6.0 g/kg, Ca 9.0 g/kg). On day 15, 60 out of 120 turkeys were selected for the experiment based on body weight (BW). Each bird was individually kept in a balance cage that was 37cm high, 36cm wide, and 55cm deep. After allowing three days for adaptation to the cages all each turkey was randomly allocated to one of the 6 diets (n=10 turkeys per diet).

Diets were given for a total period of 17 days with quantitative collection of excreta for 5 days starting from day 6. During the collection period and the three preceding days, feed allowance was slightly restricted (80 g/d) in order to avoid feed refusals. Feed was offered twice daily (at about 07:00 and 15:00 h). After the collection period, feed was offered for another 7 days *ad libitum*. Tap water was continuously available from nipple drinkers with attached cups.

During the collection period, excreta were sampled every day before the morning feeding and stored for each individual at -18°C until further handling. Turkeys were weighed at the beginning of pre-feeding time, the end of the collection period, and before slaughter. Intake and excretion were measured quantitatively for each bird. 'Utilization' was calculated as the difference between measured intake and measured excretion relative to intake.

At the end of the experiment turkeys were killed by carbon dioxide exposure. The left leg was removed and stored at -18°C until further preparation. Later the tibia was carefully removed from the thawed legs. Adhesive tissues were removed by incubating the bones for 2 days at 55°C in a solution that mainly consisted of water, fatty acid alcohol, protease and alpha-amylase (Biozym SE, COSMEDA, 47506 Neukirchen-Vluyn, Germany). Bones were then cleaned in distilled water and remaining soft tissues removed. Bones were dried for 24 h and weighed. Cleaned air-dry bones were later used for analysis.

Analyses and data evaluation

Except for the phytase activity, all analyses were carried out in the University Nutrition Institute's laboratory. Concentrations of dry matter, proximate nutrients, Ca and P were determined in the feed according to VDLUFA standard methods (Naumann and Bassler, 1976). Feed and excreta were analyzed for dry matter, ash, P and Ca. For P and Ca analyses, samples of diets and excreta, as well as the entire tibia were incinerated at 550°C, and the remaining ash was treated with 6 N HCl. P and Ca were determined from filtered ash solutions using an Inductively Coupled

Plasma spectrometer (ICP-OES). Phytase activity in the feed was determined with the internal AB Enzymes method B-021 D by AB Enzymes Company.

Data were subjected to ANOVA procedures using the software package STATISTICA for Windows 7.1. In case of any significant treatment effect, means were compared using HSD Tukey test. Non-linear regression analysis was performed with the program GraphPad Prism 4.02. An exponential model of the following type was fitted to the data:

$y = a \times (1 - e^{(-b \times (x-c))})$

where a: upper y asymptote (estimated maximum)
b: parameter describing the steepness of the curve
c: estimated x intercept
y: response criterion (P utilization or utilized P concentration)
x: supplemented phytase (FTU/kg).

RESULTS AND DISCUSSIONS

Turkeys weighed 554 g (SD 24.2) when feeding with the experimental diets started. Feed intake was 71.1 g/d (SD 3.13) during the collection period, without any significant effect of phytase supplementation (Table 2). Table 2 also shows the data for excretion and utilization of organic matters, P and Ca. The excretion of P was significantly reduced ($P \leq 0.05$) by phytase supplementation. Correspondingly, the effect of phytase on the utilization of P was significant as well ($P \leq 0.05$). A distinct plateau in P utilization could not be achieved within the level of supplementation studied (Figure 1).

The unexcreted organic matter proportion, expressed in relation to organic matter intake, was affected significantly by the treatment. This can be taken as an indication that phytase affects digestion of organic fractions of the diet. The concentration of ash in the tibia was affected significantly only by Finase 250. This may be the result of different ratios between Ca and available P in different diets with different phytase sources, because the ratio between Ca and available P can change the digestibility and retention of minerals in bones.

As for the other treatments, the concentration of ash in the tibia as well as the total amounts of ash, P and Ca in the tibia were not significantly improved by phytase supplementation (Table 3). It should be remembered that all phytase products are not the same. Phytase enzymes differ in the source from which they are derived. They may differ in characteristics such as pH optimum, thermostability, and the ability to resist hydrolysis within the digestive tract. Any difference in these characteristics will affect the ability of the phytase enzyme to function effectively and consistently within the digestive tract (Onyango et al., 2005). Therefore, all phytase enzymes produced must be tested *in vivo* to ensure efficacy before they are introduced to the monogastric feed market.

Table 2. Intake, excretion and utilization of P and Ca in young turkeys fed with diets consisting of different levels of phytase (mean and standard deviation, N = birds number per treatment)

	Supplemented phytase (FTU/kg)						*P*
	0	**F 250**	**F500**	**NP250**	**NP500**	**NP750**	***ANOVA***
N	**10**	**8**	**9**	**10**	**10**	**10**	
Final BW (g)	**554**	**553**	**557**	**553**	**555**	**551**	**0.998**
	±22.8	***±20.9***	***±22.5***	***±25.6***	***±27.7***	***±29.7***	
BW gain (g/d)	**41.5**	**37.9**	**40.4**	**42.4**	**41.7**	**42.9**	**0.509**
	±2.47	***±7.73***	***±9.97***	***±3.86***	***±4.96***	***±2.27***	
Feed intake (g DM/day)	**71.4**	**71.5**	**71.7**	**71.2**	**69.3**	**71.7**	**0.527**
	±0.16	***±0.67***	***±0.27***	***±1.65***	***±7.26***	***±0.72***	
Excretion							
OM (g/day)	**21.2**	**19.9**	**18.5***	**18.3***	**17.4***	**18.6***	**<0.001**
	±0.57	***±0.55***	***±1.35***	***±1.44***	***±2.14***	***±0.56***	
P (mg/day)	**233**	**201***	**191***	**204***	**183***	**186***	**<0.001**
	±11.8	***±12.4***	***±11.3***	***±11.8***	***±24.3***	***±10.5***	
Ca (mg/day)	**314**	**386***	**423***	**430***	**402***	**411***	**<0.001**
	±21.5	***±46.0***	***±48.4***	***±28.3***	***±47.9***	***±23.2***	
Utilization = (intake – excretion)/intake × 100							
UOM[1] (%)	**68.6**	**70.6*a**	**72.6***	**72.9*b**	**73.5***	**72.6***	**<0.001**
	±0.68	***±0.96***	***±1.96***	***±1.90***	***±1.34***	***±0.75***	
P utilization (%)	**35.4**	**44.3***	**47.3***	**43.3***	**47.6***	**48.7***	**<0.001**
	±3.31	***±3.37***	***±3.09***	***±2.93***	***±4.46***	***±2.86***	
Ca utilization (%)	**34.5**	**29.1**	**31.4**	**28.3**	**30.5**	**30.5**	**0.256**
	±4.58	***±8.59***	***±7.82***	***±4.13***	***±5.12***	***±3.94***	
Utilzed P (g/kg diet)	**1.8**	**2.2***	**2.4***	**2.2***	**2.4***	**2.5***	**<0.001**
	±0.17	***±0.17***	***±0.16***	***±0.15***	***±0.22***	***±0.14***	
Utilized Ca (g/kg diet)	**2.3**	**2.2**	**2.7**	**2.4**	**2.6**	**2.5**	**0.312**
	±0.31	***±0.65***	***±0.67***	***±0.35***	***±0.43***	***±0.32***	

1 UOM – unexcreted organic matter
ab-Values without common superscripts are significantly different within the same phytase levels according to HSD Tukey test (p<0.05)
*** Marked values are significantly different from the basal group diet on Dunnett's test (p<0.05)**

Our result was consistent with Leim et al. (2009) and Francesch and Geraert (2009) in which they showed that Phytase supplementation increased the bone ash of chicken. Kornegay and Qian (1996) also showed that Phytase increased the retention of Ca and P in the body. Our results indicated that Finase supplementation and the New Phytase improved tibia ash concentration.

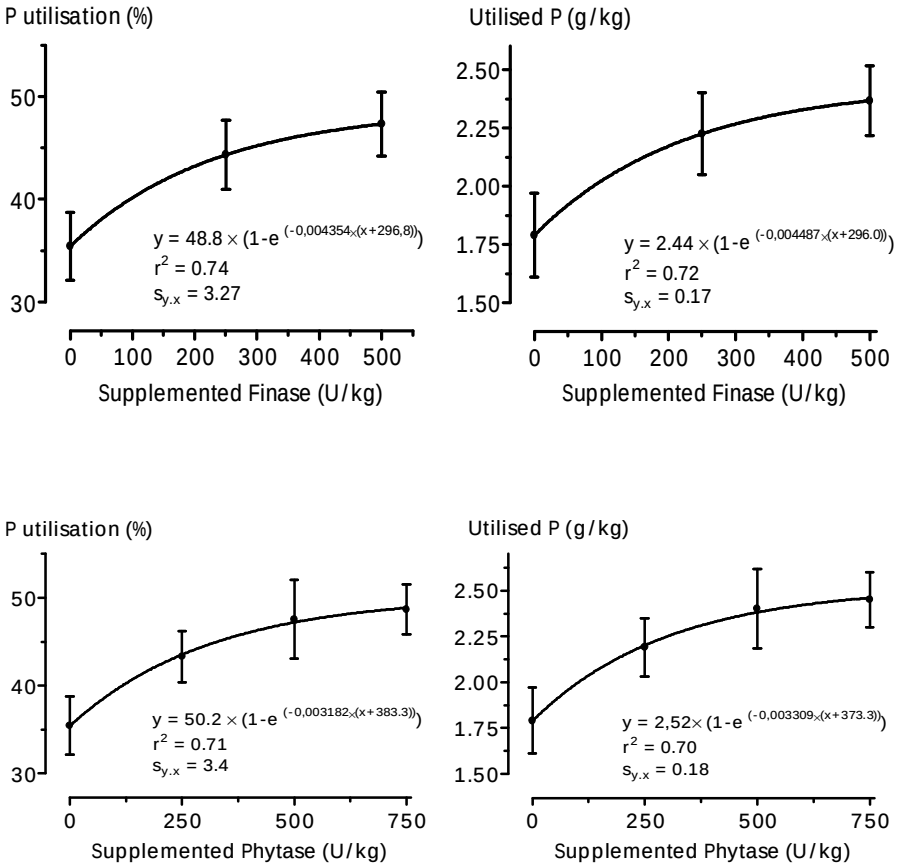

Fig. 1. Effect of Finase and Phytase supplementation on P utilization (left) and content of utilized P in the diet (right) (values are means and standard deviations, n=10 birds per treatment)

Table 3. Content of crude ash, P and Ca of the tibia (mean and standard deviation)

	supplemented phytase (FTU/kg)						*P*
	0	**F 250**	**F500**	**NP250**	**NP500**	**NP750**	***ANOVA***
N	**10**	**8**	**9**	**10**	**10**	**10**	
BW on d 35 (g)	**1669**	**1653**	**1695**	**1715**	**1680**	**1748**	**0.762**
	±91.0	***±141.2***	***±148.7***	***±71.5***	***±249.5***	***±92.5***	
Tibia DM (g)	**2.4**	**2.2**	**2.4**	**2.2**	**2.4**	**2.5**	**0.095**
	±0.18	***±0.28***	***±0.24***	***±0.16***	***±0.43***	***±0.16***	
Tibia ash (g/kg DM)	**386**	**414***	**395**	**397**	**405**	**404**	**0.137**
	±23.3	***±14.9***	***±22.9***	***±21.8***	***±28.2***	***±16.5***	
Tibia P (g/kg ash)	**193**	**194**	**193**	**192**	**192**	**193**	**0.787**
	±1.7	***±2.2***	***±2.2***	***±1.3***	***±4.2***	***±2.0***	
Tibia Ca (g/kg ash)	**388**	**389**	**389**	**388**	**387**	**389**	**0.913**
	±2.5	***±3.2***	***±4.1***	***±2.9***	***±4.8***	***±3.1***	
Tibia Ca : Tibia P	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**0.879**
	±0.02	***±0.03***	***±0.02***	***±0.02***	***±0.04***	***±0.02***	

***Marked values are significantly different from the basal group based on Dunnett's test ($p<0.05$)**

CONCLUSION

It is concluded that, the Finase and the New Phytase improved the turkeys' utilization of P, did not affect their performance and can protect the environment by decreasing excretion P. The interaction between phytate and phytase and the digestive physiology, cellular and humoral immunity and microbiology warrant further investigation, particularly the role of phytase in poultry nutrition and during disease challenge.

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سطح مطلوب استفاده از آنزیم فیتاز برای افزایش قابلیت استفاده از فسفر جیره در بوقلمون های جوان

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چکیده -فسفر فیتاتی موجود در نمونه های مواد خوراکی گیاهی به صورت اسید فایتیک است که برای طیور قابل استفاده نیست، زیرا دستگاه گوارش طیور فاقد آنزیم فیتاز برای هضم این اسید است. به همین دلیل برای افزایش بهره وری منابع فسفر باید از آنزیم های سنتز شده استفاده کرد. فیناز و فیتاز جدید تولید شده که اخیرا توسعه یافته، در سن ۳-۵ هفتگی بوقلمون ها، آزمایش شد. درجه اثرگذاری فیناز و فیتاز جدید بر پایه تعادل فسفر و داده های درشت نی تعیین شد. جیره ی پایه در این آزمایش با سطح پایین فسفر بر مبنای کنجاله سویا– ذرت تهیه شد و با سطوح ۰، ۲۵۰ و ۵۰۰ FTU/kg آنزیم فیناز و ۰، ۲۵۰، ۵۰۰ و ۷۵۰ FTU/kg فیتاز جدید مکمل سازی گردید. فضولات پرندگان در یک آزمایش تعادلی جمع آوری شد و پارامترهای درشت نی بعد از اینکه بوقلمون ها جیره های مربوط به خود را برای ۱۷ روز دریافت کردند، اندازه گیری شد. استفاده واقعی فسفر جیره های پایه ۳۵/۵% بود. استفاده واقعی از فسفر به طور معنی دار با فیناز به یک حد بیشینه ۴۷/۳% و با فیتاز جدید به حد بیشینه ۴۸/۷ % در سطح بالاترین سطح مکمل سازی، بهبود یافته بود. در این آزمایش استفاده واقعی از کلسیم بهبود نیافت. یافته‌های این آزمایش نشان داد که غلظت خاکستر در درشت‌نی با مکمل فیناز و فیتاز جدید افزایش یافت. آنزیم فیناز و فیتاز جدید در بهبود استفاده از فسفر در بوقلمون ها موثر است و می‌تواند با کاهش دفع فسفر از طرق مواد دفعی به حفظ محیط زیست کمک کند.

واژه های کلیدی: **بوقلمون، فسفر، فیتاز، فیناز**

*به ترتیب استادیار، استاد و دانشجوی کارشناسی ارشد
** مکاتبه کننده