Digestibility and performance responses of broiler chickens fed a pea-based diet with different levels of dietary microbial phytase

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Abstract

Although the benefits of phytase are well documented, the purpose of this study was to determine the effect of supplemented phytase on pea-based diets in poultry, as research is lacking on pea diets. A 21-day feeding trial using male broiler chicks on the day of hatch was conducted to assess the effects of adding four levels of dietary microbial phytase (0.0, 0.1, 0.3 and 0.9 g/kg diet; with respective activity levels of 0, 500, 1500 and 4500 FTU or phytase units) to a pea-based diet on bird productivity and digestibility. The responses were evaluated in terms of broiler performance, nutrient digestibility and apparent metabolizable energy (AME). This research demonstrated that digestibility of ash and starch increased with phytase supplementation. Protein and fat digestibility remained relatively constant, but an increase in apparent metabolizable energy (AME) was seen. The performance of broilers also improved as increased weekly gains attributable to phytase were seen through improved feed conversion. The results of this research are indicative that phytase supplementation in poultry pea-based diets has a positive impact.

Keywords: broilers, phytase, growth, digestibility, apparent metabolizable energy, peas

Introduction

Including phytase in poultry diets has become of interest due to concerns of phosphorus (P) pollution to the environment (Sebastian et al. 1998; Selle and Ravindran 2007), in addition to the need to satisfy the animal's physiological requirement for phosphorus (Sebastian et al. 1998). Phytate, or phytic acid, is a naturally occurring organic complex found in plants and is the primary storage form of phosphorus within plant seeds. This phytate phosphorus is unavailable to poultry due to their insufficient endogenous phytase, which hydrolyses the phytate into organic phosphorus, and therefore this phytate phosphorus is excreted in the excreta (Sebastian et al. 1998). In addition to reducing phosphorus availability phytate can reduce the availability of minerals by forming complexes with them (Erdman 1979; Cheryan 1980), as well as reducing protein and starch digestibilities (Cheryan 1980).

To overcome the inefficiencies associated with phytate, exogenous microbial phytase has been supplemented to aid low endogenous phytase levels in breaking down phytate. Microbial phytase has been well documented to hydrolyze phytate, release nutrients, and thereby improve performance and increase apparent metabolizable energy (AME) (Ravindran et al. 2000; Camden et al. 2001). As well, it has been documented that phytase can improve calcium, phosphorus and nitrogen (Ravindran et al. 2000; Walk et al. 2012), amino acid

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(Rutherford et al. 2002; Pirgozliev et al. 2011), fat, and starch digestibilities (Camden et al. 2011). But while the many positive effects of supplemental phytase are well documented for poultry, there is a lack of research on diets based on peas. In a review of phytase in poultry nutrition by Selle and Ravindran (2007), most of the diets in the summarized research were based on maize, soybean meal, and wheat. Phytase is effective in breaking down phytate in peas, but little research has been attempted to examine total digestion in a diet based exclusively on peas.

This study was carried out to examine the response of exogenous phytase added at increasing levels into pea-based diets on performance and digestibility parameters of broilers. It is hypothesized that phytase will positively affect nutrient digestibility and apparent metabolizable energy in broiler chickens fed a pea-based diet.

Materials and Methods

The experimental procedures were approved by the Animal Care Committee of the University of Saskatchewan and complied with the recommendations of the Canadian Council on Animal Care in the Guide to the Care and Use of Experimental Animals (1993).

Diets

The experiment was conducted using a completely randomized design to evaluate the effects of three concentrations of dietary microbial phytase (0.0, 0.1, 0.3 and 0.9g/kg diet; with respective activity levels of 0, 500, 1500 and 4500FTU or phytase units) on productivity and nutrient digestibility. There were six replicates per treatment. The phytase product (Quantum Blue) used was an *Escherichia coli* derived phytase (AB Vista: Woodstock Court, Marlborough, Wiltshire, SN8 4AN, UK).
The diets were in mash form with pea as the main ingredient at approximately 87% (Table 1) and formulated based on National Research Council (1994) recommendations for broiler chickens. The diets were equalized for gross energy, protein, fat, and phytate phosphorus. Celite was included in the diets (1.5%) as an inert marker used to calculate apparent metabolizable energy and nutrient digestibilities. The birds were allocated a treatment for 21 days, and feed and water were provided *ad libitum*.

Birds

Male broiler (Ross 308) chicks were obtained on the day of hatch from a commercial hatchery (Lilydale, Wynyard, SK) and were randomly distributed to 24 cages of five birds each, thereby resulting in six replications per treatment. The chicks were housed in Jamesway battery cages with dimension of 44cm wide, 85cm long, and 25cm high (floor space/bird). The lighting was 23h of fluorescent lighting with 1h of dark and a light intensity of 40lux. The temperature was initially 32°C and was decreased by 1°C every 3 days.

Excreta Collection, Processing, and Chemical Analysis

On days 0, 7, 14, and 21 body weight and feed intake data were collected to assess performance. On days 20 and 21 clean excreta was collected twice daily from the liners under the cages, and the excreta was pooled from the four collections by treatment. Feed samples from each treatment were also taken. Both the feed and excreta samples were stored at -20°C until they were dried in a forced air oven (at 55°C for 72 hours) and then ground (0.5mm screen; Retsch ZM 100, Retsch GmbH, Germany) for analysis.

Feed and excreta samples were pooled together according to phytase treatment level and were analyzed for moisture, ash, and crude protein. Due to the pooling of samples by treatment, there were not sufficient replicates for statistical analysis of digestibilities and AME within the experiment. Neutral detergent fiber was analyzed using a filter bag technique described by ANKOM Technology. Gross energy was determined using an oxygen bomb calorimeter (Parr Instrument Company, Moline, IL), and ether extract was determined by extraction using anhydrous diethyl ether by a Goldfisch Extraction Apparatus (Labcono model 35001). Analyses were performed according to the Official Methods of Analysis from the AOAC (1990).

Calculations

Average weekly gain (g gain/bird/week), feed intake (g feed/bird/week), and feed conversion (feed:gain) were calculated using the body weight and feed intake measurements taken during the 21d experiment.

Digestibilities of nutrients and AME were determined using the feed and excreta chemical analyses. The equation: %-nutrient digestibility=100-(%indicator in feed/%indicator in excreta)*(%nutrient in excreta/%nutrient in feed)*100, was used to calculate digestibility for dry matter, ash, starch, protein, fat, and NDF. The equation to calculate AME was AME = (Gross $Energy_{Diet}$-(($Indicator_{Diet}$/$Indicator_{excreta}$)*Gross $Energy_{Excreta}$)), where the indicator was the amount of Celite.

Statistical Analysis

Data were analyzed as completely randomized design using PROC REG of SAS (SAS Institute 2002) with pen as an experimental unit. The level of significance was fixed at $P \leq 0.05$ unless otherwise stated.

Table 1. Composition (%) of experimental diets

Ingredient (%)	**Diet**
Pea	87.02
Phytase (Four treatment levels)	0.0, 0.01, 0.03 and 0.09
Canola oil	7.20
NaCl	0.44
Calcium phosphate[1]	0.65
Limestone	2.11
Vit./Min. premix[2]	0.50
$FeSo_4$	0.03
Choline chloride	0.10
Celite	1.50
DL Methionine	0.48
Calculated Nutrients (%)	
AME (kcal/kg)	2900
Crude protein	20.73
Crude fat	8.26
Calcium	1.00
Non-phytate P	0.30
Phytate	0.52
Phytate P	0.15
Total P	0.45
Sodium	0.20
Chloride	0.32
Linoleic acid	2.14
Arginine	1.22
Lysine	1.39
Methionine	0.71
Methionine and cysteine	0.89
Threonine	0.82
Tryptophan	0.21

[1] Calcium phosphate: 26.47% P, 17.12% Ca (Sigma C8017 min 95%)

[2] Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11000 IU; vitamin D, 2200 IU; vitamin E (dl-α-topheryl acetate), 300 IU; menadione, 2.0mg; thiamine, 1.5mg; riboflavin, 6.0mg; niacin 60mg; pyridoxine, 4mg; vitamin B_{12}, 0.02mg; pantothenic acid, 10.0mg; folic acid, 0.6mg; biotin, 0.15mg; iron, 80mg; zinc, 80mg; manganese, 80mg; copper, 10mg; iodine, 0.8mg; selenium, 0.3mg; and $CaCO_3$, 500mg.

Results

The average gain, feed intake, and feed efficiency of the broilers from 0 to 21 days is shown in Table 2. Nutrient digestibilities and apparent metabolizable energy data are shown in Table 3.

Broiler performance

The average weekly gain of the broilers was improved by increasing levels of dietary microbial phytase content at 7-14 days (Table 2). Overall 0 to 21 day body weight tended to increase linearly with increasing phytase level (P=0.08). There was no significant difference in the average weekly feed intake during any time period during the experiment. Average feed conversion was improved with increasing phytase level at 0 to 7 days and 7-14 days (P< 0.05).

Table 2. Impact of feeding increasing levels of phytase on 0-21 days performance of broilers

		Treatment by g/kg of phytase in diet				*P*	SEM
		0	**0.1**	**0.3**	**0.9**		
Average gain (g gain/bird)	**0-7d**	14.5	14.9	15.1	14.4	0.97	3.24
	7-14d	34.3	36	40.3	39.9	0.005	7.63
	14-21d	58.6	61	65.1	63.3	0.25	18.4
	0-21d	107.3	111.9	120.5	117.6	0.08	24.6
Average feed intake (g feed/bird)	**0-7d**	21.3	20.9	21.4	19.9	0.29	4.07
	7-14d	55.4	61	59.4	59.8	0.26	11.2
	14-21d	101.3	104.9	108.6	107.3	0.29	23.3
	0-21d	178	188	187.3	189.9	0.17	29.5
Average feed conversion (gain:feed)	**0-7d**	0.682	0.709	0.708	0.722	0.05	0.01
	7-14d	0.62	0.632	0.663	0.656	0.02	0.01
	14-21d	0.579	0.582	0.632	0.588	0.57	0.0301
	0-21d	0.603	0.613	0.646	0.625	0.43	0.0192

Data analyzed by regression analysis; SEM - standard error of means

Table 3. Impact of increasing dietary phytase on digestibility of nutrients and AME in broiler chicks from 0 to 21 days

	Treatment by g/kg of phytase in diet			
	0.0	**0.1**	**0.3**	**0.9**
% dry matter digestibility	62.3	62.0	62.9	63.6
% ash digestibility	32.7	33.5	37.6	44.8
% starch digestibility	68.8	66.7	70.4	74.7
% protein digestibility	62.1	61.6	63.9	62.1
% fat digestibility	90.1	85.8	86.6	87.7
% NDF digestibility	48.3	52.9	52.6	55.6
AME (kcal/kg as is)	2698.1	2690.6	2729.5	2799.6

Digestibility and AME

The influence of increasing amounts of microbial phytase supplementation in the diet affected some nutrient digestibilities. Dry matter, protein, and fat digestibilities remained relatively constant regardless of level of phytase. Alternatively, although not statistically analyzed, digestibility of ash and starch improved with increasing levels of phytase (Table 3). Similarly, AME increased with increasing levels of phytase supplementation (Table 3).

Discussion

There has been considerable interest in the use of microbial phytase to enhance performance and nutrient digestibility in poultry diets; however, research including pea-based diets is lacking. The present results indicate that exogenous phytase in pea-based diets for broilers is effective in improving digestibility of ash and starch, and performance of broilers through better feed conversion.

The increased daily gains attributable to phytase were seen through better feed conversion (gain:feed). This result with a pea-based diet is in agreement with the previous report by Cabahug et al. (2010) where they found that phytase was beneficial and overcame the anti-nutritional factors present in a wheat-sorghum-soyabean meal diet, allowing for increased growth and feed efficiency.

In the present results, there was a decrease in the rate of improvement between the supplemented phytase levels of 1500 FTU and 4500 FTU on performance. This may be indicative of a maximal response of the phytase in the pea based diet below or close to 4500 FTU. But this maximum activity is different compared to results found by Ravindran et al. (2000) in research based on a wheat-sorghum-soybean diet. In their experiment, no difference in phytase response was seen between 400 – 800 FTU, suggesting a maximal activity level requirement of around 400 FTU. This may indicate that pea-based diets are still positively influenced by phytase, but may require a higher phytase activity level than other feed ingredients. Higher phytase activity may be required since most diets do not contain such high levels of peas, so there may be higher levels of phytate compared to normal diets. Further research may be conducted to define the maximum phytase activity that can be utilized by poultry fed a pea-based diet.

The results of the current research also demonstrated an increase of AME with phytase, and this may be due to phytase releasing molecules bound to phytate to be utilized for energy. An increase in AME with phytase was also seen by Ravindran et al. (2000), as they found phytase increased AME values by 1.3% in low non-phytate phosphorus diets. AME improvement was maximized by 400 FTU/kg added phytase. Camden et al. (2001) provided agreement as they found addition of graded levels of phytase to linearly increase AME values. Though Pirgozliev et al. (2011) found AME to be unaffected by phytase, they saw large increases in net energy for production and suggest that even though AME did not increase, dietary phytase still improves energy utilization but through mechanisms not accounted for by the AME procedure.

Addition of phytase caused improvements in the digestibility of some factors in our results, and this has been previously demonstrated by many reports. Walk et al. (2012) found apparent ileal P and Ca digestibilities to increase with phytase. Similarly, Ravindran et al. (2000) saw supplemental phytase increased ileal digestibilities of P and nitrogen while also improving retention of dry matter, P and N. The hydrolysis of phytate can release the nutrients that it binds, allowing for better digestibility of many nutrients within the ash component.

Protein digestibility did not change much with increased phytase supplementation in our results. This is contrary to many previous experiments. Ravindran et al. (2000) found that ileal digestibility of essential amino acids was negatively influenced by the presence of phytate but the effects could be overcome with supplemental phytase. Pirgozliev et al. (2011) found amino acid utilization particularly of threonine at high doses of phytase. Rutherford et al. (2002) found amino acid digestibility to be greater for all amino acids in wheat, several in maize and rapeseed meal, and for one amino acid in rice bran and soyabean meal. They found increases in amino acid digestibility in the range of 6-13% for the amino acids affected. These findings indicate that amino acid and protein digestibility effects due to phytase may be specific to feedstuffs. Thus, additional research on pea-based diets may indicate whether microbial phytase supplementation can positively influence protein digestibility.

In our experiment there was no apparent change in fat digestibility with the various levels of phytase, and this differs compared to previous results by Camden et al. (2001) who found phytase increased fat digestibility. They suggest that phytate-calcium complex binds fatty acids to form metallic soaps in the lumen of the gut, reducing the utilization of fats. Phytase releases the fatty acids, allowing for improved fat digestibility. More research is needed to clarify whether the results proposed by Camden et al. (2001) may be applicable to pea-based diets for fat digestibility.

Also, Ca-phytate affects starch digestibility. Starch digestibility increased with phytase in our experiment, and this may be explained by phytate complexing with Ca (Cawley and Mitchell 1968). Ca is necessary for α-amylase function and stability and therefore will be suppressed with reduced Ca due to phytate complexing (Sebastian et al. 1998). Increased phytase presence will breakdown the phytate, releasing Ca, allowing for a more optimal α-amylase function and therefore increasing starch

digestibility. In agreement with this, Camden et al. (2001) suggest that phytase may also bind starch directly, and they also saw enhanced starch digestibility with phytase supplementation. These mechanisms may account for our results of increased starch digestibility.

In conclusion, supplemented phytase improved both performance and digestibility in broiler chicks on a pea-based diet similar to the well-documented effects of phytase on other diets.

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