THE EFFECT OF PHYTASE SUPPLEMENTATION ON BROILER AND INTERACTION BETWEEN PHYTASE AND INTERMITTENT FEEDING AND STRUCTURAL COMPONENTS

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Master Thesis 30 credits 2013

Abstract

An experiment was conducted to investigate the effect of exogenous phytase supplementation and the interaction between phytase and feeding regime and between phytase and component structures on the performance, bone ash content, jejunal P digestibility, jejunal phytic acid (IP6) degradation and AME of broiler chickens. 308 broiler chickens (Ross 308) either were intermittently or ad libitum fed a diet with or without oat hulls and with or without phytase (2x2x2) from 7 to 21 days of age. Ad libitum fed birds were fed continuously, while intermittently fed birds had access to feed four times a day with three 1 hour and one 2 hour feeding bout a day from 7 to 14 days of age and four 1 hour feeding bout a day from 14 days of age until the end of experiment. At 21 days of age, two birds per cage were killed, and contents from crop, gizzard, duodenum+ jejunum and ileum were quantitatively collected and frozen in liquid nitrogen. Exogenous phytase supplementation increased feed intake ($P< 0.001$), weight gain ($P< 0.001$) and feed/gain ratio ($P=0.0162$). There was an interaction between phytase and structural components on weight gain ($P= 0.031$) and feed/gain ratio ($P< 0.001$). Both of toe ash ($P= 0.0012$) and tibia ash ($P< 0.001$) were increased with phytase addition, while no interaction between phytase and intermittent feeding and structure components was found on bone ash content. Phytase addition also improved jejunal P digestibility ($P= 0.003$), jejunal phytic acid degradation ($P= 0.003$) and AME ($P= 0.0188$). Moreover, an interaction between phytase and intermittent feeding was found on phytic acid degradation.

Acknowledgements

I would like to thank my family who have offered me unconditional endless love and continuous support in my life.

I am deeply grateful to my supervisor, Prof. Dr. Birger Svihus, who devoted me his precious time, assistance and support. He provided lots of good advice, idea and guidance.

I would like to thank Dejan Miladinovic and Ismet Nikqi at the FôrTek for assistance with feed processing. I am grateful to Frank Sundby and Stephanie Collins who aided me in the laboratories, and I acknowledge them for their skillful technical assistance.

My thanks also go to my friends Qi Ji, Adiya, Sigrun Schumpa that worked together with me and helped me a lot during the experiment. I am also grateful to my teacher Li Ming, who encouraged me to study overseas and helped me with learning English.

I express my deepest appreciation to each of the above.

Ås, August 2013
Zhamuer

Table of Contents

1. Introduction 4

2. Literature 5

2.1 Intermittent feeding in poultry production 5

2.2 Fibre addition in broiler diet 6

2.3 Phytase 7

3. Material and method 10

3.1 Diet composition and processing 10

3.2 Experimental animals and feeding 13

3.3 Sample analysis 15

3.3.1. Phytic acid analysis 15

3.3.2. Ash content analysis 15

3.3.3 Excreta analysis 16

3.4 Calculations 16

3.5 Data analysis 16

4. Results 17

4.1 Effect of phytase on performance 17

4.2 Effect of phytase on bone mineralization 17

4.3 Effect of phytase on AME, jejunal IP6 digestibility and jenunal P digestibility 18

5. Discussion 20

6. Conclusion 23

1. Introduction

The nutrient requirement of animal is met by the diet, which includes requirement for phosphorus (P). Phosphorus is an essential nutrient for animals and is playing many important biological roles. In addition P deficiency can also cause feed intake reduction (Francesch and Geraert, 2009).

It is common to add inorganic P in the poultry feed to meet the requirement, because the hydrolysis of phytate and utilization of phytate-P is quite limited for poultry (Cowieson et al., 2004). However using inorganic P in production is expensive and contributes to environmental pollution. High quantity of P is released into the environment with poultry litter, which is a hazard to water quality (Powell et al., 2008).

In recent years, phytase has been widely used in poultry diet to solve these problems. Supplementation of exogenous phytase results in reduced P excretion of birds into the environment by increasing phytate utilization, which causes less supplementation of inorganic Phosphorus in diet as well (Yu et al., 2004). Moreover, because of optimal pH range of phytase, it indicates that fore-stomach is the main site for phytase activity (Selle and Ravindran, 2007).

The present study focused on the effect of exogenous phytase on the performance of broiler chickens, the effect on the bone mineralization, phytic acid degradation in fore-stomach, jejunal phosphorus digestibility and the influence of phytase on AME. In addition, the interactions between phytase and intermittent feeding regime and between phytase and structural components were studied as well. The hypothesis for current study was that intermittent feeding and structure components would boost response of phytase.

2. Literature

2.1 Intermittent feeding in poultry production

A fast growth rate and efficient feed conversion are required in the broiler production; therefore, ad libitum feeding has been a common approach, which is easy to handle and can ensure a high feed intake. However, this feeding method might cause some adverse effects, such as overconsumption and leg weakness.

The intermittent feeding program can be a solution and reduce these problems, which can be performed by application of either intermittent lighting or feed removal strategy. Intermittent feeding may increase feed efficiency compared with ad libitum feeding, because fewer activities of birds in darkness cause decreased energy utilization. Mahmud et al. (2011) observed that birds obtained increasing weight gain under intermittent lighting regime, while the feed consumption had no significant difference with the birds under continuous lighting regime.

Under the intermittent feeding program, birds eat large amount of diet in a short time and use their anterior digestive tracts as storage organs (Svihus et al., 2010). Moreover, Barash et al. (1993) reported that intermittent feeding regime increased crop and gizzard content weight, and improved the feed storage capability of the gastrointestinal tract.

In addition, using upper part of digestive tract as a storage organ improves the retention time of feed in this section of digestive tract. Prolonged retention time may have a positive effect on efficiency of exogenous enzyme. Svihus et al. (2010) observed that the percentage of phytic acid in crop content was decreasing with time passing after feeding the diet added phytase to broilers (Figure 1).

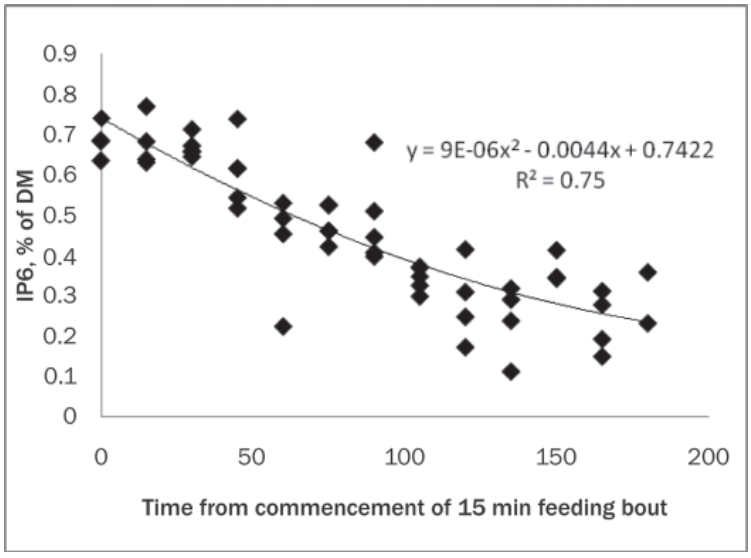

Figure 1. Percentage of inositol 6-phosphate (IP6) in the crop contents of broiler chickens that were killed at different time points after 15 min of being fed diet with phytase. (Svihus et al., 2010).

2.2 Fibre addition in broiler diet

Increasing structural components in poultry diets, like whole grains, oat hulls, wood shavings or large cereal particles, has been shown to have many effects.

Gizzard will be influenced by inclusion of structural components in the diet. The heavier gizzards have been observed in previous studies, when birds have been fed diet with larger particles or insoluble fibres. Hetland and Svihus (2001) reported that the weight of empty gizzard was significantly increased with oat hulls addition. An increased gizzard weight of broiler was also found by Hetland et al. (2003) when whole cereals, as well as oat hulls were used, whereas wood shavings increased gizzard weight of layers by 50%. Svihus et al. (2010) showed similar result, that addition of whole wheat caused a large increase in gizzard weight. The hulls addition to the diet caused larger and fuller gizzards and stimulated gizzard development (Sacranie et al., 2012). The explanation for this is that the need for particle size reduction was increased (Svihus, 2011).

However, not only the gizzard weight is affected by structural components, it also increases the volume of gizzard, which may cause a longer retention time. Hetland et al.

(2003) reported that the amount of gizzard content was increased with both wood shavings and whole-wheat utilization. Similarly, the digesta contents in the gizzard were found to be greater for the birds fed whole-wheat diet than those fed ground wheat feed (Amerah and Ravindran, 2008). Moreover, Sacranie et al. (2012) observed that when hulls were added to the diets, the pH of the gizzard content was decreased.

It has been shown that addition of structural components has effects on performance as well. Hetland and Svihus (2001) observed that when increasing oat hulls inclusion, the feed intake and feed:gain ratio increased. However, Sacranie et al. (2012) also showed that hulls addition increased feed intake and improved gain:feed ratio. Moreover, a reduced feed intake and an increased gain:feed ratio was found by Svihus et al. (2010) when birds received whole-wheat diets. In addition, some articles also reported that structural components addition may increase the apparent metabolic energy (AME) (Biggs and Parsons, 2009; Hetland and Svihus, 2001).

2.3 Phytase

For monogastric animals, cereal, beans and oilseed crops are the main sources of feed, and contain sufficient phosphorus (P) (Elkhalil et al., 2007). However, most of P in these plants is present as phytate (Pirgozliev et al., 2008), which is salt of phytic acid. Due to low efficiency of endogenous phytase, the hydrolysis of phytate and utilization of phytate-P is quite limited for poultry (Cowieson et al., 2004; Elkhalil et al., 2007; Pirgozliev et al., 2008). Therefore, the inorganic phosphorus is added to diet to meet the P requirement of poultry; nevertheless, it costs and increases the P pollution to the environment. Moreover, phytate may be considered as anti-nutritional factor reducing the digestibility of energy-yielding nutrients (Woyengo et al., 2010) by forming insoluble complexes in the stomach and intestine (Santos et al., 2008).

One way to solve these problems is adding exogenous phytase to poultry diet, which is the enzyme that hydrolyses the ester bonds between the phosphate groups and inositol ring of phytate and increases availability of phytate-P (Pirgozliev et al., 2008). Since 90s, the phytase is widely produced and used in poultry industry. It has been found that phytase can be produced from plants, fungi and bacteria. The fungal and bacterial

origin phytase is most commonly used in poultry diets (Rutherfurd et al., 2012), because phytase from plant is heat labile and relatively ineffective (Pirgozliev et al., 2012).

Unit of phytase (FTU) is used to express phytase activity and defined as the amount of phytase that release 1 μmol of inorganic phosphorus per minute from 0.00015 mol/L sodium phytate at pH 5.5 and temperature of 37℃ (Lu et al., 2009). Elkhalil et al. (2007) observed that optimal pH range for phytase activity is between 4.5 to 5.5, indicating that crop is the main site where phytate is hydrolysed by phytase because of pH in fore-stomach (Yu et al., 2004; Selle and Ravindran, 2007; Elkhalil et al., 2007). Denstadli et al. (2006) also reported that under the similar condition with the crop, the phytate was degraded quickly.

In addition to hydrolysis of phytate and improving P utilization, many other beneficial effects of phytase supplementation are demonstrated. There are plenty of studies that have shown that using phytase in low-P diet can improve live performance of broiler chickens. Yu et al. (2004) observed that the broilers fed phytase diet gained weight significantly faster than the birds fed low non-phytate phosphorus diet, and the same result was obtained by Prigozliev et al. (2008). Moreover, improved feed intake (Amerah and Ravindran 2009; Prigozliev et al., 2008; Lu et al., 2009) and feed efficiency (Lu et al., 2009) was observed when chicken got access to the diet with phytase supplementation.

However, the advantages of phytase are not limited to these parts. Phytase also has positive effect on P retention (Figure 2), bone mineralization, and P excretion. Simons et al. (1990) showed that phytase supplementation resulted in decreased amount of P in the droppings. An increased tibia ash content was observed when diet with phytase was used in broiler production (Woyengo et al., 2008; Francesch and Geraert, 2009; Lu et al., 2009). Also, Powell et al. (2011) found improved bone breaking strength, ash weight and percentage tibia ash while using phytase in the diet. In addition, Amerah and Ravindran (2009) reported that P retention was higher for the birds fed phytase diet than the ones fed diet without phytase. Besides, the beneficial effect of phytase on energy utilization was reported in some studies. Selle and Ravindran (2007) reported that phytase supplementation increased AME by an average of 0.36 MJ kg^{-1}

DM (or 2.8%) compared with non-supplemented controls.

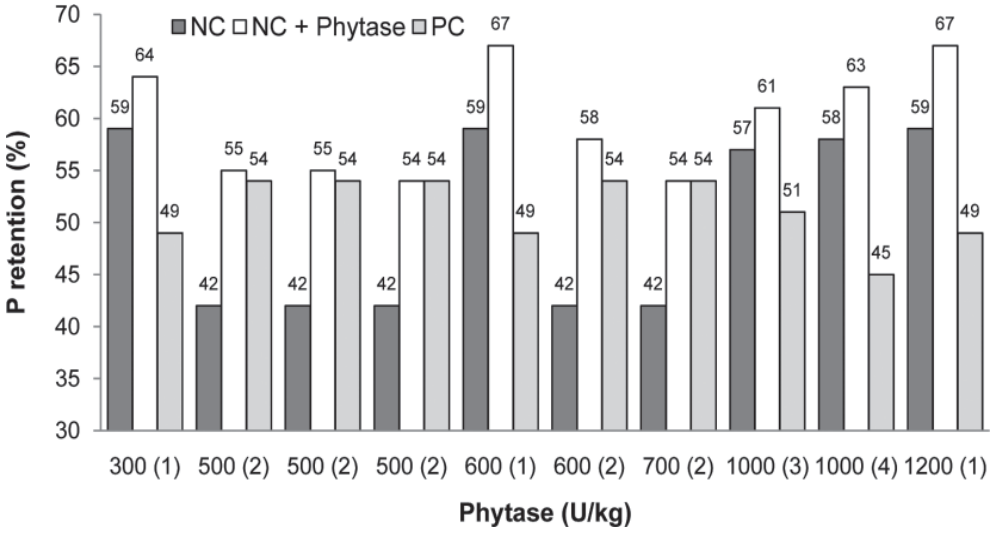

Figure 2. The effect of phytase supplementation on P retention in broiler chickens. The numbers in parentheses refer to data reported by (1) Cowieson et al. (2006), (2) Elkhalil et al. (2007), (3) Leytem et al. (2008), and (4) Olukosi et al. (2008). PC= P-adequate positive control diet. NC= P-deficient negative control diet. (Slominski, 2011)

The trial was performed to study the influences of phytase on broilers and if there is an interaction effect between phytase, and structural components/intermittent feeding. The following hypotheses were tested:

i) Does phytase supplementation improve broiler performance, bone mineralization, AME and phosphorus and phytic acid digestibility?

ii) Does intermittent feeding regime and structural components boost phytase activity through influencing the crop and gizzard?

3. Material and method

3.1 Diet composition and processing

The diets were produced in the Centre of Feed technology (FôrTek), at the University of Life science (UMB) in Ås, Norway. The diets were based on wheat with high protein and high fall number, grown and harvested in Drammen area in Norway, in 2012.

Four wheat-based diets were processed. Table 1 shows the different diets, diet 1 and 3 were feed contained oat hulls that were without or with phytase, and diet 2 and 4 were feeds without oat hulls that were without or with phytase. Titanium dioxide was the marker.

The diets were made to meet nutritional requirements of experimental birds according to Ross 308 Broiler Management Manuel (2007), except that phosphorus was provided to a large extent in the form of phytic acid, and with a somewhat lower total provision.

Wheat, soybean and rapeseed were ground separately by hammer mill on a 3 mm sieve (mill model: E-22115 TF, Muench-Wuppertal, Germany, under Bliss-USA, 18.5 kW and 2870 rpm). The oat hulls were sieved by using a 1.4 mm sieve to avoid fine particles.

Four batches were produced continuously, each weighing 230 kg mixed in a 400 l mixer conditioner (Twin shaft paddle, Tatham of England, Forberg, Norway, 7.5 kW). The 2 diets without phytase were processed first to avoid contamination. The duration time of mixing for each batch were 2 minutes when micro ingredients and oat hulls or cellulose were added. Then soy oil was sprayed on the mash with a pressure of 4 bars for 4 minutes and 45 seconds. The spray nozzle had capacity size of 6505 (angle 65, size 05, Unijet, spraying systems Co, Wheaton, Illinois, USA) and spraying capacity of 2.3 l/min (based on water viscosity). The mixing time after oil addition was 2 minutes.

Table 1. Feed composition, Diet 1 – 4:

Ingredients	Diet 1 g/kg	Diet 2 g/kg	Diet 3 g/kg	Diet 4 g/kg
Wheat	529.5	529.5	529.5	529.5
Soybean meal	200	200	200	200
Rapeseed meal	80	80	80	80
Rice bran	60	60	60	60
Oat hulls	50		50	
90 cellulose + 10 flour		50		50
Soya oil	40	40	40	40
Limestone	14	14	14	14
Salt	1.8	1.8	1.8	1.8
Sodium bicarbonate	2.6	2.6	2.6	2.6
Mineral premix[1]	1.3	1.3	1.3	1.3
Vitamin A	0.7	0.7	0.7	0.7
Vitamin D3	0.7	0.7	0.7	0.7
Vitamin E	0.4	0.4	0.4	0.4
Vitamin ADKB[2]	0.8	0.8	0.8	0.8
DL-methionine	2	2	2	2
L-lysine	3	3	3	3
Titanium	5	5	5	5
L-threonine	2	2	2	2
Xylanase, Econase® XT 25	5	5	5	5
Choline chloride	1.2	1.2	1.2	1.2
Phytase, Quantum Blue®			0.028	0.028

[1]Mineral premix supplied the following per kg of diet: Fe 50 mg; Mn 40 mg; Zn 70 mg; Cu 10 mg; I 0.5 mg; Se 0.2 mg.

[2]Vitamin premix supplied the following per kg of diet: retinol 3.4 mg; cholecalciferol 0.062 mg; tocopherol 55 mg; menadione 6.6 mg; pyridoxine 4.4 mg; riboflavin 17.6 mg; pantothenic acid 18.25 mg; biotin 0.286 mg; thiamine 2.75 mg; niacin 55 mg; cobalamine 0.022 mg; folic acid 2.75 mg.

Table 2. Calculated diet composition (as-fed basis)

Metabolizable energy (MJ/kg)	11.76
Crude protein (g/kg)	197.3
Calcium (g/kg)	7.1

Total Phosphorus (g/kg)	5.0

Three samples from each diet were taken after mixing process from the "waiting hopper" (before conditioning, after mixing). The mixed samples were taken directly from different places – representative samples – and then mixed together in a bucket and distributed into plastic bags.

The feed mash was sent through the twin pass/double conditioner (Twin Pass, Muench, Germany, 1.2 t/h, 2 x 1.8m x 30cm). There was 4% steam added at 75℃ in 20-30 seconds (retention time) before it was processed in a pellet mill (Muench, Germany, 1.2 t/h max. capacity, 2 x 18.5 kW). During the pelleting progress, processing parameters were recorded, shown in table 3. Immediately after the pelleting, the temperatures of feed were measured manually with a thermometer in an insolating box.

Table 3. Processing parameters:

Die Specification		
Conditioner temp	ºC	74.8
Production capacity	kg/h	700.0
Die diameter	mm	3.0
Die length	mm	36/42
Knife distance	mm	6.6
Motor load	%	22.8
Amperes Motor 1	amp	13.6
Amperes Motor 2	amp	12.9
Average amperes motor	amp	13.3
Energy Consumption	kW	8.1
Specific Energy Cons.	kWh/kg	0.0116
Steam	kg / h	51.0
ISO - Box	ºC	79.2

The pellets were cooled in a counter-flow cooling system for 30 minutes, which used ambient air to reduce temperature of the products (Miltenz, New Zealand, capacity 1.2

t/h). Then each cooled pellet product was packed in 1000 l bags containing the final product 200 ± 6 kg. Then 3 representative pellet samples from each diet were taken directly from the filled bags with grain sampler. Before each diet was processed, the system was cleaned by 30 kg of ground wheat to avoid contamination.

3.2 Experimental animals and feeding

The experiment was performed from 12th of October to the 14th of November 2012 at the Animal Production Experimental Centre (Senter for Husdyrforsøket), UMB. There were 380 day-old female Ross 308 broiler chickens placed in brooder cages in a room with 23 h of light and a temperature of 32℃, and were fed on commercial starter diet and water ad libitum till 7 days of age. Feed consumption and weight gain was recorded weekly/every Friday for all birds used in the experimental trials.

Excreta collection and dissection

At 7 days of age, 4 randomly selected birds were weighed and placed in each of 48 mesh floor cages (50cm x 35cm x 20 cm). Two racks of cage were placed such that the intermittently fed birds did not have visual contact by placing the ad libitum birds facing the wall and intermittent facing each other. The four diets were given in rows of four and sequentially. A bucket was assigned to each cage and contained with 5kg of feed. The gross weight of the buckets was recorded.

The ad libitum chickens had a 2x4 hours dark period (23.00 – 03.00 and 04.00 – 08.00). The intermittent feeding regime last from 7 to 14 days of age, while birds had access to feed consumption (ad libitum) from 08.00 – 09.00, 12.00 – 13.00, 16.30 – 17.30 and 21.00 until light went off at 23.00. From 14 days of age until termination of the experiment at 21 days of age, feed for the intermittently fed group were available for ad libitum consumption from 08.00 – 09.00, 13.00 – 14.00, 17.30 – 18.30 and 22.00 – 23.00. Temperature was reduced to 29 ℃ when chickens reached 7 days of age, and further reduced to 26 ℃ at 21 days of age.

On day 17, in preparation of excreta collection the birds and feed were weighed at 08.00, and the trays under cages were removed and cleaned. After 6 hours at 14.00, the clean trays were placed back under the cages.

Excreta collection was carried out at 18, 19 and 20 days of age. The trial excreta were collected from the trays after it was cleaned for feed and feathers and then put in white boxes. At day 20 there has been made a mistake, the feed and birds were not weighed at 08.00 but 12.00. The excreta were collected at 18.00 instead of 14.00.

At the age of 21days, the lights were switched on at 04.00 and feed was removed from intermittently fed birds. At 06.30 all birds and feed were weighed.

The half of the intermittently fed birds was given access to feed every 20 minutes starting at 07.40. After 40 min from time of feeding the feed were removed. Another half of the intermittently fed birds was all given access to feed at 07.00. After 1 hour the feed were removed. And at 11.40, 12.00, 12.20, 12.40 every three cages of the birds were giving access to feed respectively. After 40 min from time of feeding the feed were removed. All the intermittently fed birds were killed exactly 3 hours after commencement of the feeding.

The ad libitum fed birds got access to feed when the light was switched on at 04.00 until dissection. The half of them got killed 08:20h and another half was killed 12.40 respectively.

Two birds from each cage were killed in mentioned order and a strap was wrapped around the bird's neck immediately to hinder crop content regurgitation, before the birds were weighed. For the sampling, the contents of crop, proventriculus + gizzard, duodenum + jejunum and ileum were collected. All samples were frozen in liquid nitrogen. The gizzard pH and empty gizzard weight were taken for all the birds, while the crop pH were measured only for intermittently fed birds. The 2 middle toes and the left thigh from each bird were collected and put in freezer.

3.3 Sample analysis

3.3.1. Phytic acid analysis

Contents collected from duodenum + jejunum were freeze dried in a freeze dryer (Beta 1-6, LMC-2, Christ, Osterode, Germany) at -56˚C and 25 mbars for 72 hours to obtain the dry matter without any possible biochemical changes in the samples.

The method of Newkirk and Classen (1998) was used to extract Phytic acid (inositol hexakisphosphate; IP6) from diets and freeze dried duodenum + jejunum contents and analysed via HPLC.

3.3.2. Ash content analysis

2 thighs and 4 toes from two 21 days of age birds in each 48 cages were thawed out at room temperature.

- Tibia

Most of meat on the tibia was removed carefully by using a scalpel, while making sure no any part of bone was removed. The few remaining meat particles still stuck on the tibia was removed by cleaning with a paper. Then tibia (with fibula) was taken and weighed to get the crude weight. 48 crucibles were weighed and marked. Two tibias from 2 birds in the same cage (for 10 cages, only one thigh were used, because another ones were not taken intact from birds) were put in same crucible. The tibia was dried at 80 ℃ for 24 hours in oven. The weight of tibias together with crucibles was taken after cooling in room temperature. Then tibias were ashed at 480 ℃ for 24 hours and weighed after cooling.

- Toes

Four toes from 2 birds (for 1 cage, only 2 toes were used, because the other two toes were lost) in same cage were put in same crucible that was weighed and marked. The same procedure as the one used on tibia was used to dry and ash toes. The weight per thigh and per toe was calculated for statistical analysis.

3.3.3 Excreta analysis

The collected excreta was sent to Nutreco and analysed according to a standard method.

3.4 Calculations

Apparent metabolizable energy (AME) was calculated according to the following equation:

$$\text{AME}=\frac{(\text{feed intake}\times\text{GEdiet})-(\text{excreta output}\times\text{GEexcreta})}{\text{feed intake}}$$

GE denotes Gross energy.

P digestibility in the jejunum was calculated using the following equation:

$$\text{P digestibility}=1-\left(\frac{\text{P concentration in jejunum}}{\text{P concentration in feed}}\times\frac{\text{marker concentration in feed}}{\text{marker concentration in jujunum}}\right)$$

IP6 digestibility in the jejunum was calculated using the following equation:

$$\text{IP6 digestibility}=1-\left(\frac{\text{IP6 concentration in jejunum}}{\text{IP6 concentration in feed}}\times\frac{\text{marker concentration in feed}}{\text{marker concentration in jujunum}}\right)$$

3.5 Data analysis

Data from experiment were subjected to a three-way ANOVA (feeding regime ×diet structure × enzyme addition) and, followed by pair-wise comparisons using the Ryan-Einot-Gabriel-Welsh procedure when relevant, with $P < 0.05$ as the significance level (SAS Institute, 2006). The square root of mean square error in the analysis of variance (residual standard deviation, RSD) was used as a measure of random variation.

4. Results

4.1 Effect of phytase on performance

There was a significant increase ($P<0.001$) on feed intake when the birds were fed the diet with phytase supplementation, compared with the diet without phytase. However, there was no interaction of the combination of exogenous phytase and feeding regime, phytase and structural component and the combination of three factors (phytase, coarseness and feeding regime) on feed intake (Table 4).

The weight gain was significantly higher ($P<0.001$) for the birds fed diet added phytase than the diet without phytase. However, the weight gain was higher ($P=0.031$) with phytase when no oat hulls were added than when there were oat hulls used. Moreover, no interaction was observed between enzyme and feeding regime and between enzyme, feeding regime and structural components (Table 4).

The exogenous enzyme supplementation reduced feed/gain ratio ($P=0.016$). Also, there was an interaction between enzyme and structure in that the feed/gain ratio was decreased more ($P<0.001$) when the diet contained phytase and no oat hulls, compared with the diet with both of them. However no interaction of the combination of enzyme and feeding regime on feed/gain ratio was observed, as well as the combination of these three factors (Table 4).

4.2 Effect of phytase on bone mineralization

For the birds fed diet with phytase, the ash contents in toes were significantly higher ($P=0.0012$) than in toes from the birds fed diet without phytase, and same improvement ($P<0.001$) was found for tibia ash. Also, an increase ($P<0.001$) was found for the ash percentage in dry matter for both of toe and tibia, when phytase were added in the diet. However, there was no important effect from interaction between enzyme and feeding regime, and between enzyme and coarseness, and combination of three factors (enzyme,

feeding regime and coarseness) as well (Table 4).

Table 4. Results of performance and ash content from birds in 4-bird cages from 7 to 21 days of age[1]

Feeding Regime	Oat hulls Structure	Enzyme addition	Feed intake, g	Weight gain, g	Feed/gain	Toe ash, % of DM	Toe ash, g	Thigh ash, % of DM	Thigh ash, g	Crop pH	Gizzard pH
Ad libitum	Coarse	No	1003	713[bc]	1.41	10.3	0.026	31.9	0.75	-	1.9
Ad libitum	Fine	No	1021	689[c]	1.48	10.0	0.025	31.3	0.75	-	2.8
Ad libitum	Coarse	Yes	1094	773[ab]	1.42	11.1	0.030	35.5	0.96	-	2.2
Ad libitum	Fine	Yes	1125	793[a]	1.42	11.4	0.029	34.7	0.95	-	3.3
Intermittent	Coarse	No	906	645[c]	1.40	10.2	0.022	32.4	0.78	5.2	2.1
Intermittent	Fine	No	977	672[c]	1.45	10.0	0.023	32.2	0.75	5.3	2.5
Intermittent	Coarse	Yes	982	688[c]	1.43	11.7	0.025	34.1	0.88	5.6	1.9
Intermittent	Fine	Yes	1094	775[ab]	1.41	11.2	0.027	35.7	0.98	5.3	2.7
√MSE			54.2	39.6	0.025	0.54	0.0037	1.69	0.097	0.48	0.69
Feeding regime											
Ad libitum			1061	742	1.43	10.7	0.027	33.3	0.85	-	2.5
Intermittent			990	695	1.42	10.8	0.024	33.6	0.85	-	2.3
Structure											
Fine			1054	732	1.44	10.7	0.026	33.5	0.86	5.4	2.8
Coarse			996	705	1.41	10.8	0.026	33.5	0.84	5.3	2.0
Enzyme											
No			977	680	1.44	10.1	0.024	31.9	0.76	5.2	2.3
Yes			1074	757	1.42	11.4	0.028	35.0	0.94	5.4	2.5
Main effects											
Feeding regime		NS	<0.001	<0.001	NS	NS	0.0095	NS	NS	-	0.086
Structure		NS	<0.001	0.022	<0.001	NS	NS	NS	NS	NS	<0.001
Enzyme		NS	<0.001	<0.001	0.0162	<0.001	0.0012	<0.001	<0.001	NS	NS
Feeding*Structure		NS	0.038	0.014	NS	NS	NS	NS	NS	-	NS
Feeding*Enzyme		NS	NS	NS	NS	NS	NS	NS	NS	-	NS
Structure*Enzyme		NS	NS	0.031	<0.001	NS	NS	NS	NS	NS	NS
Feed*Structure*Enz		NS	NS	NS	NS	NS	NS	NS	NS	-	NS

[ab]Means within a column not sharing a common superscript differ at P<0.05.
[1]Each treatment combination had either 6 or 12 replicates

4.3 Effect of phytase on AME, jejunal IP6 digestibility and jenunal P digestibility

Phytase addition to the diet increased (P= 0.0188) apparent metabolizable energy (AME) of the birds, while there was no interaction between phytase supplementation and intermittent feeding and between enzyme addition and structural components (Table 5). The phytic acid degradation in jejunum for the birds fed diet with phytase was significantly higher (P=0.003) than the birds fed diet without phytase (Table 5). Moreover, there was a significant interaction effect between feeding regime and enzyme for IP6 degradation (P= 0.03). For the phosphorus digestibility in jejunum, phytase also had an improving effect (P= 0.03), while there was no interaction between phytase and

feeding regime and between phytase and structural components (Table 5).

Table 5. Results of AME, P digestibility and IP6 digestibility from birds in 4-bird cages from 7 to 21 days of age[1]

Feeding Regime	Oat hulls Structure	Enzyme addition	AME, kcal/kg	Jejunal P dig.	Jejunal IP6 dig.
Ad libitum	Coarse	No	3390^{abc}	0.05^{c}	0.36^{b}
Ad libitum	Fine	No	3300^{d}	0.13^{b}	0.34^{b}
Ad libitum	Coarse	Yes	3460^{a}	0.15^{abc}	0.37^{b}
Ad libitum	Fine	Yes	3324^{cd}	0.26^{ab}	0.40^{b}
Intermittent	Coarse	No	3410^{abc}	0.19^{abc}	0.43^{b}
Intermittent	Fine	No	3359^{bcd}	0.22^{abc}	0.37^{b}
Intermittent	Coarse	Yes	3436^{ab}	0.26^{ab}	0.60^{a}
Intermittent	Fine	Yes	3377^{abcd}	0.33^{a}	0.53^{ab}
√MSE			48.7	0.108	0.106
Feeding regime					
Ad libitum			3369	0.15	0.37
Intermittent			3396	0.25	0.48
Structure					
Fine			3340	0.24	0.41
Coarse			3424	0.16	0.44
Enzyme					
No			3364	0.15	0.38
Yes			3399	0.25	0.48
Main effects					
Feeding regime		NS	0.0632	0.003	0.001
Structure		NS	<0.001	0.024	NS
Enzyme		NS	0.0188	0.003	0.003
Feeding*Structure		NS	0.0448	NS	NS
Feeding*Enzyme		NS	NS	NS	0.030
Structure*Enzyme		NS	NS	NS	NS
Feed*Structure*Enz		NS	NS	NS	NS

[ab]Means within a column not sharing a common superscript differ at P<0.05.
[1]Each treatment combination had either 6 or 12 replicates.

5. Discussion

The response of the phytase was because there was no P level in the diet, and phytase released P that the birds needed to grow and therefore perform better. At the same time, when look at the bone ash data, it shows there is more mineral composition in the bones, which fits with the hypothesis that it is mineral-release issue. This shows that the phytase works by at least releasing P.

The weight gain, feed intake and feed efficiency were increased by the phytase supplementation, indicating the efficacy of phytase on releasing phytate-bound P and improving P digestibility. There were many studies reporting similar results. Yan et al. (2001) reported that body weight gain and feed conversion were improved by the phytase supplementation for 3-6 weeks age of chicks. Same results were found by Viveros et al. (2002), where the weight gain was improved by 6.7% and 6,1% for the 3 and 6 weeks of age birds, respectively, while the feed consumption was only increased (5.3%) at 3 weeks of age. Dilger et al. (2004) also illustrated that feed efficiency was improved by 1.0% and 7.7% with phytase supplementation of 500 and 1000 FTU/kg for the 22-day of age broilers, respectively. They explained this result by the fact that the improvements of weight gain were greater than those of feed intake, indicating that phytase addition could increase the utilization of P. Adedokun et al. (2004) showed that phytase supplementation of 656 and 1081 FTU/kg diet to the low-nPP diet increased that body weight gain by 31% and 34%, and feed intake by 19% and 24%, respectively. There were also some recent studies showing the same results that phytase addition in the low-nPP diet improved broiler performance (Amerah and Ranvindran, 2009; Pirgozliev et al., 2011; Rutherfurd et al., 2012; Milica et al., 2012).

In current study, for the results of weight gain and feed/gain ratio, enzyme had a larger effect when diets without oat hulls were used than with oat hulls. This interaction might be explained that coarse ingredients reduced pH in the gizzard to 2.0 (Table 4), which may be too low for the phytase activity, if gizzard is an important site for phytase activity (Selle, 2007). It also could be explained that this interaction was due to the feed

intake. An increased feed intake when using finely ground diet, which is negative for feed/gain ratio, but positive for weight gain, is more pronounced with enzyme than without (Table 4). It is surprising that intermittent feeding did not improve phytase effect on performance, particularly since in the present experiment, the phytic acid degradation increased when birds were fed diet with phytase under intermittent feeding. This indicates that retention time in the crop may be not a limited factor for phytase activity. It is also possible that anterior digestive tract is not the main site for *E. coli* phytase activity (Onyango et al., 2005b).

The phytase supplementation to the diet resulted in increased tibia ash content, ash percentage in tibia, and as well as improved toe ash content and ash proportion in toe. These results were simply caused by the efficacy of phytase on releasing phytate-bound P (Selle and Ravindran, 2007) and improving P digestibility (Woyengo et al., 2010), which was also found in current study. Several authors have reported similar improvements. Yan et al. (2001) found that significantly increased tibia ash was caused by the phytase supplementation to the lower-nPP diet. Viveros et al. (2002) reported that the tibia ash was increased with phytase addition to low-nPP diets by 5.1%, and claimed the improvement might be caused by the increased mineral retention due to phytase action. In 2004, Dilger et al. reported that the both toe and tibia ash from 22-day of age birds were increased by 10% to 12% for toe and 42% to 47% for tibia, respectively, when adding phytase to the diet with low nPP. Adedokun et al. (2004) found that the tibia and toe ash from the broilers fed diet with 1081 FTU phytase kg^{-1} increased by 25% and 20%, respectively, compared with the birds fed lower-nPP diet. And they also reported that the data of both tibia and toe ash were similar, when comparing 0.5g P with 656 FTU phytase kg^{-1} diet, as well as 1.0 P compared with 1081 FTU phytase kg^{-1} diet. Moreover, Amerah and Ravindran (2009) illustrated that phytase supplementation increased the toe ash contents, when birds were fed the medium particle size diet, but no effect was found on the bird fed the coarse particle size diet. In addition, phytase supplementation may have no benefit when the higher level of nPP was used in the diet (Yan et al., 2001).

However, there was no interaction between phytase and structure and between phytase and feeding regime found in the current study. The bone ash being affected was

probably due to the P releasing effect of phytase, since bone is 30% of phosphorus. So for the P release, it appears that phytase was working independent of structure. In the current study, only for the weight gain and feed/gain ratio, there was an interaction between enzyme and structure found, however, this was probably because of the effect of structural components on feed intake.

The phytase supplementation improved AME value, which was reported in several previous studies. Woyengo et al. (2010) also found that adding phytase to negative control diet increased the AME value. Cowieson et al. (2006) reported that the phytase-supplemented diet increased AMEn by the average of approximately 120 kcal/kg, compared with the negative control diet. As well, Onyango et al. (2004) showed that AME was improved with the phytase addition for the birds from 8 to 22 d of age. However, Amerah and Ravindran (2009) did not find influence of phytase on AME. Overall, Selle and Ravindran (2007) summarized that phytase supplementation improved AME by an average of 0.36 MJ/kg dry matter, comparing with non-supplemented diets.

The phytase effect on AME may be able to be explained by the increased energy-yielding nutrients digestibility (Woyengo et al., 2010). The phytase substrate may reduce digestibility of energy yielding nutrient by following ways: binding to protein in digestive tract, binding to carbohydrates and fat in small intestine and binding to endogenous enzymes (summarized by Wonyengo et al., 2010).

Phytase supplementation increased phosphorus digestibility in jejunum. An improved P digestibility in ileum with phytase addition was reported in several studies. Santos et al. (2008) observed an increased digestibility coefficient of P with phytase addition, compared with the negative control diet without enzyme, at 21 day. Woyengo et al. (2010) also found increased ileal P digestibility when phytase was added to the diet. However, Amerah and Ravindran (2009) reported that phytase supplementation increased the ileal P digestiblity for the birds fed the medium particle size diet, but not for those fed coarse particle size diet.

In the present study, there was no interaction between enzyme and intermittent feeding

found on jejunal P digestibility. It appears that the percentage increase in P digestibility is higher as a consequence of intermittent feeding alone for P digestibility (Table 5). Therefore, one possibility explanation could be that the effect of intermittent feeding itself had a strong beneficial effect on P digestibility, and there was no more room for extra improvement due to the phytase.

The phytase supplementation improved jejunal phytic acid degradation, which was simply because of catalyzing effect of phytase on phytic acid degradation. Moreover, there was an interaction between phytase and intermittent feeding. This indicates that there was a response that intermittent feeding helped phytase release P, however time may be not a limited factor for the phytase in this experiment. There was also one alternative explanation that maybe intermittent feeding was also facilitating P absorption in a better way, for example through better moisturization of the feed. Since there was no strong response between ad libitum without enzyme and intermittent without enzyme, it might be not the degradation that was affected by the intermittent feeding without enzyme.

Svihus et al. (2010) also found a significant degradation of phytic acid in the crop after a long retention time, when diet contained phytase. Denstadli et al. (2006) reported that 68% of phytic acid was degraded within incubation for 2 min and 86% of the phytic acid was degraded within 45 min of incubation, by phytase under a similar condition as in the crop with 45°C, 45% moisture and pH 4.7 (Svihus et al., 2010). In the current studies, these are data from one specific time for intermittent feeding, 3 hours after commencement of the feeding, so if the bird were killed after 4 or 5 hours after, a larger effect may be observed.

6. Conclusion

The result of current study indicates that exogenous phytase has positive effect on broiler performance and bone mineralization, as well as on the P digestibility, IP6 degradation and AME. An interaction between phytase and intermittent feeding was

observed only on the IP6 degradation, indicating that intermittent feeding may be helpful to phytase activity, but it is not a limited factor. Structural component had no positive effect on phytase, which may be because it affects pH and feed intake.

References

Adedokun, S. A., J. S. Sands, and O. Adeola. (2004). Determining the equivalent phosphorus released by an Escherichia coli-derived phytase in broilers. Canadian Journal of Animal Science 84: 437–444.

Amerah, A. M. and V. Ravindran. (2008). Influence of method of whole-wheat feeding on the performance, digestive tract development and carcass traits of broiler chickens. Animal Feed Science and Technology 147: 326–339.

Amerah, A. M. and V. Ravindran. (2009). Influence of particle size and microbial phytase supplementation on the performance, nutrient utilisation and digestive tract parameters of broiler starters. Animal Production Science 49(8): 704-710.

Biggs, P. and C. M. Parsons. (2009). The effects of whole grains on nutrient digestibilities, growth performance, and cecal short-chain fatty acid concentrations in young chicks fed ground corn-soybean meal diets. Poultry Science 88: 1893-1905.

Cowieson, A. J., T. Acamovic, et al. (2004). The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. British Poultry Science 45(1): 101-108.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. (2006). Supplementation of corn-soy-based diets with an Eschericia coli-derived phytase: Effects on broiler chick performance and the digestibility of amino acids and metabolizability of minerals and energy. Poultry Science 85: 1389–1397.

Denstadli, V., R, Vestre., B, Svihus., A, Skrede. and T, Storebakken. (2006) Phytate degradation in a mixture of ground wheat and ground defatted soybeans during feed processing: Effects of temperature, moisture level, and retention time in small and medium-scale incubation systems. Journal of Agriculture and Food Chemistry

54: 5887-5893.

Dilger, R. N., E. M. Onyango, J. S. Sands, and O. Adeola. (2004). Evaluation of microbial phytase in broiler diets. Poultry Science 83: 962–970.

Elkhalil, E. A. I., K. Manner, et al. (2007). In vitro and in vivo characteristics of bacterial phytases and their efficacy in broiler chickens. British Poultry Science 48(1): 64-70.

Francesch, M. and P. A. Geraert. (2009). Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralization of broilers fed reduced nutrient corn-soybean-based diets. Poultry Science 88(9): 1915-1924.

Hetland, H. and B. Svihus. (2001) Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. British Poultry Science 42: 354 – 361.

Hetland, H., B. Svihus, & V. Olaisen. (2002). Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. British poultry science 43 (3): 416-423.

Hetland, H., B. Svihus, and Å. Krogdahl. (2003) Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. British Poultry Science 44: 275 – 282.

Leytem, A. B., B. P. Willing, et al. (2008). Phytate utilization and phosphorus excretion by broiler chickens fed diets containing cereal grains varying in phytate and phytase content. Animal Feed Science and Technology 146(1-2): 160-168.

Lu, M. B., D. F. Li, et al. (2009). Effects of Supplemental Microbial Phytase and Xylanase on the Performance of Broilers Fed Diets Based on Corn and Wheat. Journal of Poultry Science 46(3): 217-223.

Mahmud, A., Saima, Rafiullah, and I. Ali, (2011). Effect of different light regimens on performance of broilers. The Journal of Animal & Plant Sciences 21(1): 104-106.

Milica, Z. B., K. Mira, et al. (2012). The Effectiveness of Phytase in Broiler Diets in Improving Production Performances and Bone Features. Acta Veterinaria-Beograd 62(2-3): 297-311.

Nahm, K. H. (2004). Additives to reduce phosphorus excretion and phosphorus solubility in poultry and swine manure. Australian Journal of Experimental Agriculture 44(8): 717-728.

Onyango, E. M., M. R. Bedford, and O. Adeola. (2004). The yeast production system in which Escherichia coli phytase is expressed may affect growth performance, bone ash, and nutrient use in broiler chicks. Poultry Science 83: 421–427.

Onyango, E.M., Bedford, M.R., and O. Adeola. 2005b. Phtase activity along the digestive tract of the broiler chick: a comparative study of an Escherichia coli-derived and Peniophora lycii phytase. Canadian Journal of Animal Science 85: 61–68.

Pirgozliev, V., O. Oduguwa, et al. (2008). Effects of dietary phytase on performance and nutrient metabolism in chickens. British Poultry Science 49(2): 144-154.

Pirgozliev,V., M.R. Bedford , T. Acamovic , P. Mares & M. Allymehr. (2011) The effects of supplementary bacterial phytase on dietary energy and total tract amino acid digestibility when fed to young chickens. British Poultry Science 52: 245-254.

Pirgozliev, V., M. R. Bedford, et al. (2012). The effect of supplementary bacterial phytase on dietary metabolisable energy, nutrient retention and endogenous losses in precision fed broiler chickens. Journal of Animal Physiology and Animal Nutrition 96(1): 52-57.

Powell, S., S. Johnston, et al. (2008). The effect of dietary phosphorus level and

phytase supplementation on growth performance, bone-breaking strength, and litter phosphorus concentration in broilers. Poultry Science 87(5): 949-957.

Powell, S., T. D. Bidner, et al. (2011). Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. Poultry Science 90(3): 604-608.

Rousseau, X., M. P. Letourneau-Montminy, et al. (2012). Phosphorus utilization in finishing broiler chickens: Effects of dietary calcium and microbial phytase. Poultry Science 91(11): 2829-2837.

Rutherfurd, S. M., T. K. Chung, et al. (2012). Effect of a novel phytase on growth performance, apparent metabolizable energy, and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers. Poultry Science 91(5): 1118-1127.

Sacranie, A., B. Svihus, et al. (2012). The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. Poultry Science 91(3): 693-700.

Santos, F. R., M. Hruby, et al. (2008). Effect of Phytase Supplementation in Diets on Nutrient Digestibility and Performance in Broiler Chicks. Journal of Applied Poultry Research 17(2): 191-201.

Selle, P. H. and V. Ravindran (2007). Microbial phytase in poultry nutrition. Animal Feed Science and Technology 135(1-2): 1-41.

Selle, P. H., V. Ravindran, et al. (2009). Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral retention and growth performance in wheat-based broiler diets. Animal Feed Science and Technology 153(3-4): 303-313.

Shaw, A. L., J. P. Blake, et al. (2010). Evaluation of commercial phytase enzymes on

performance and tibia-breaking strength of male broiler chicks. Journal of Applied Poultry Research 19(4): 415-421.

Shaw, A. L., J. B. Hess, et al. (2011). Assessment of an experimental phytase enzyme product on live performance, bone mineralization, and phosphorus excretion in broiler chickens. Journal of Applied Poultry Research 20(4): 561-566.

Slominski, B. A. (2011). Recent advances in research on enzymes for poultry diets. Poultry Science 90(9): 2013-2023.

Shaw, A. L., J. P. Blake, et al. (2011). Evaluation of Commercial Phytases on Performance and Skeletal Strength of Two Broiler Strains. Journal of Poultry Science 48(1): 47-50.

Simons P. C. M., H. A. J. Versteegh, et al. (1990). Improvement of phosphorus availability by microbial phytase in broilers and pigs. British Journal of Nutrition 64: 525–540.

Slominski, B. A. (2011). Recent advances in research on enzymes for poultry diets. Poultry Science 90(9): 2013-2023.

Svihus, B., A. Sacranie, et al. (2010). Nutrient utilization and functionality of the anterior digestive tract caused by intermittent feeding and inclusion of whole wheat in diets for broiler chickens. Poultry Science 89(12): 2617-2625.

Svihus, B. (2011). The gizzard: function, influence of diet structure and effects on nutrient availability. Worlds Poultry Science Journal 67(2): 207-223.

Viveros, A., A. Brenes, I. Arija, and C. Centeno. (2002). Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poultry Science 81: 1172–1183.

Woyengo, T. A., W. Guenter, et al. (2008). Nutrient utilisation and performance

responses of broilers fed a wheat-based diet supplemented with phytase and xylanase alone or in combination. Animal Feed Science and Technology 146(1-2): 113-123.

Woyengo, T. A., B. A. Slominski, et al. (2010). Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multicarbohydrase. Poultry Science 89(10): 2221-2229.

Yan, F., J. H. Kersey, and P. W. Waldroup. (2001). Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poultry Science 80: 455–459

Yu, B., Y. C. Jan, et al. (2004). Exogenous phytase activity in the gastrointestinal tract of broiler chickens. Animal Feed Science and Technology 117(3-4): 295-303.