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Effects of KDN phytase on the performance and Ca and P metabolism of broilers fed low phosphorus diets

Peng Ying [1,2#], Wence Wang [1#*], Weigang Duan [3*], Francois Blachier [6], Shu Xu-gang [4], Yunchao Wang [3], Mingzhe Fan [5] and Jie Pan [3]

*[1] Key Laboratory for Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, 410125 Hunan, China. [2] Hunan Radio & TV University, Hunan, Changsha 410004, China. [3] Animal Metabolism Laboratory of KDN Biotech Group, 266061 Qingdao, China. [4] Zhongkai University of Agriculture and Engineering, 510225 Guangzhou, China. [5] Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario N1 G 2W15, Canada. [6] INRA, CNRH-IdF, AgroParisTech, UMR 914 Nutrition Physiology and Ingestive Behavior, 16 rue Claude Bernard, 75005, Paris, France.*e-mail: weigang@kdnbiotech.com, lanser1022@163.com.*

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Abstract

An experiment was carried out to study the effects of phytase on the performance and calcium (Ca) and phosphorus (P) metabolism of AA broilers by supplementing phytase in low P diets. The basal diet (A) contained 0.41% of available P and diets B, C and D contained 0.26, 0.34 and 0.34, respectively. Phytase (U/kg) was supplemented to Diets B, C and D at 500, 500 and 250, respectively. Each diet was allocated to 6 replicates of 24 birds each. Two phases of feeding program were applied and the broilers were fed with different treatment diets at 0-21 d and 22-42 d. No significant difference was observed on average daily gain (ADG), feed/gain ratio (F/G) and daily feed intake (DFI) ($P>0.05$) of the treatment groups and on the occurrence of leg diseases. Phosphate excretion of broilers in groups B, C and D decreased by 26.7% ($P<0.05$), 24.0% ($P<0.05$) and 20.6% ($P>0.05$), respectively. No significant difference ($P>0.05$) was observed on length of tibia, ash content and Ca and P content in ash, but there was a significant reduction in tibia weight and strength in group B. There was no significant difference ($P>0.05$) in serum Ca, P and alkaline phosphatase among all the treatment groups.

Key words: Phytase, broiler, performance, Ca and P metabolism.

Introduction

Phosphorus is one of the essential macroelements for livestock and poultry. It is a component of animal bones and plays significant roles in energy metabolism, protein synthesis and reproduction [1-4]. Lack of phosphorus will cause slow growth, productivity decline and decrease in feed utilization efficiency. It also causes osteomalacia or rickets if in acute deficiency [5]. Corn-soy based diets are rich in phytic acid, which is largely not available to poultry and other non-ruminant animals [6-9]. Lack of phytase in gastric tract of monogastric animals results in low efficiency of phosphorus utilization [10]. Therefore, usually large quantity of inorganic phosphorus is supplemented to meet the nutrition demand for phosphorus. However, feed cost is usually increased with the price flow up of inorganic phosphorus. Meanwhile, the accompanying phosphorus excretions also cause severe pollution to the environment.

KDN achieved large-scale industrialized production of phytase enzyme via bio-fermentation technology, taking advantage of advanced biotechnology and production facilities. The addition of KDN phytase not only can improve the bioavailability of phosphorus but also the utilization of inorganic nutrition ions and protein, reduce the supplement of inorganic phosphorus and excretion of phosphorus, thus being environmentally friendly

The current experiment was designed to assess the effects of KDN phytase on the performance and Ca and P metabolism of broilers.

Materials and Methods

Materials: Phytase (5000U/g) was offered by Weifang KDN Biotech Co.,ltd. Day-old AA broilers were supplied by the breeder farm of Shandong Liuhe Group Co., Ltd.

Bird management: The protocol was approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, The Chinese Academy of Sciences [11]. A total of 576 day-old broilers were randomly distributed on weight basis to 4 dietary treatment groups based on the techniques of Huang [12], Kong *et al*. [13] and Li *et al*. [14]. Each treatment consisted of 6 replicates of 24 birds each. The experiment was carried out in Metabolism Lab of KDN Biotech, Qingdao, China. Broilers were fed according to 2 feeding stages of starter (0-21d) and finisher (22-42 d), respectively. Broilers were fed with 4 diets tagged A, B, C and D, respectively. The levels of Ca and P and other nutrients in the basal diets were fixed using NRC [15] requirement for poultry

[#]Peng Ying and Wence Wang are joint first authors.

as a guide. Limestone and dicalcium phosphate were used to supply Ca and P in all diets [16, 17]. In order to achieve the intended levels of P in treatments B, C and D, the levels of dicalcium phosphate in these diets were reduced and replaced with sand. Total phosphorus in the starter diets (%) was 0.68, 0.53, 0.61 and 0.61, while available P was 0.41, 0.26, 0.34 and 0.34 in diets A, B, C and D, respectively. Available P (%) was progressively reduced by 37, 17 and 17 in diets B, C and D compared to the control group. Treatment A served as the control and was without phytase supplementation. Diets B, C and D contained 500, 500 and 250 U/kg, respectively. The P content of the finisher diets was similar and followed the same pattern as in the starter diets and were supplemented with the same amount of phytase. The gross composition and mineral composition of the experimental diets are shown in Tables 1 and 2, respectively.

Birds were raised in enclosed 3-layered cages with windows for ventilation. Feed and water were offered to birds on *ad libitum* basis and the cages were cleaned regularly. Each treatment was equally represented in the 3-layered cages and consisted of 2 replicates each in the upper, middle and lower compartments, respectively. Immunization program was strictly followed by eye drip Newcastle and Lentogen vaccine for 7d broilers, eye and nose drip of Bursa of Fabricius vaccine for 14 d broiler; enforce immune Newcastle by water line for 21d broiler. BW was measured at day 21 and 42. The record of weight, feed consumption, mortality, and number of birds with leg diseases, were kept and analyzed.

Analysis of P excretion: At stages of 18~20 d and 39~41 d, about 500 g of fresh feces was collected and mixed from each replicate. Fecal samples were kept in zip-loc bags and stored in refrigerator for P excretion test [18-21].

Analysis of biochemical indicator: At the end of experiment, 5 ml vein blood was randomly collected from selected birds in each duplicate [22-24]. Blood with pre-added anticoagulant was centrifuged at 3000 r/min, plasma was transferred to another vial and stored in refrigerator [25-27]. Plasma was used for assay of alkaline phosphatase, serum Ca and P.

Analysis of tibia: By the end of experiment, one bird was randomly selected and killed to collect the left shinbone in each duplicate. After peeling flesh from shin bone, the length and strength were measured; then shin bone samples were identified and stored for assay of Ca, P and ash content.

Table 1. Composition of basal diets fed to test broilers (%).

Ingredient	0~21d	22~42d
Corn	49.60	51.54
Wheat	15.50	18.10
Soybean Meal	28.50	24.50
Fish Meal	3.00	2.00
Lard	—	0.50
Salt	0.20	0.20
Additive[1]	0.60	0.60
Others[2]	2.60	2.50
Total	100.0	100.0
Calculated values		
ME (MJ/kg)	11.80	12.03
Crude Protein	20.80	19.00
Methionine	0.49	0.42
Lysine	1.02	0.96

[1]Additive: [2]Ca and P sources. See Table 2 for details

Table 2. Composition of treatment in each group.

Category	0~21d	22~42d
Group A		
Limestone (%)	1.30	1.20
Dicalcium phosphate (%)	1.30	1.30
Total Ca (%)	0.98	0.90
Total P (%)	0.68	0.64
Available P (%)	0.41	0.38
Group B		
Limestone (%)	1.85	1.75
Dicalcium phosphate (%)	0.43	0.43
Sand (%)	0.32	0.32
Total Ca (%)	0.98	0.90
Total P (%)	0.53	0.49
Available P (%)	0.26	0.23
Phytase (U/kg)	500	500
Group C		
Limestone (%)	1.57	1.47
Dicalcium phosphate (%)	0.87	0.87
Sand (%)	0.16	0.16
Total Ca (%)	0.98	0.90
Total P (%)	0.61	0.54
Available P (%)	0.34	0.31
Phytase (U/kg)	500	500
Group D		
Limestone (%)	1.57	1.47
Dicalcium phosphate (%)	0.87	0.87
Sand (%)	0.16	0.16
Total Ca (%)	0.98	0.90
Total P (%)	0.61	0.54
Available P (%)	0.34	0.31
Phytase (U/kg)	250	250

The unlisted items in Group D are the same with items in Group B.

Data and chemical analyses: Ash content of bones was determined using a muffle furnace at 600°C, and total phosphorus content was determined spectrophotometerically using the molybdovanadate reagent after mineralization of the sample with HCl [28]. Serum levels of Ca, P and alkaline phosphatase were determined using the method of Ruan *et al*. [16]. Data were subjected to one-way analysis of variance (ANOVA) and means were separated by Duncan's multiple range test using SPSS 13.0 for Windows.

Results

Effects of phytase on the performance of broilers: There was no significant difference between the control group and the low P microbial phytase supplemented groups in terms of performance indices in the starter and the finisher stages (Table 3).

Effects of phytase on P excretion of broilers: Compared with the control group, P excretion of low P diet supplemented with phytase was decreased by 26.7% ($P<0.05$), 24.4% ($P<0.05$), 20.6% ($P>0.05$) in groups B, C and D, respectively (Table 4).

Tibia status after treatments: Results showed no significant differences ($P>0.05$) between groups on the length of tibia, ash content, Ca and P in ash (Table 5). However, mass of tibia showed significant difference between Group B and control group ($P<0.05$). It implies that phosphorus reduction up to 37% of the requirement may reduce the mass of tibia although there is no significant change of length or ash content. Since tibia mass is evidently low in group B, the shear force of this group is significantly lower than in the control, C and D groups.

Table 3. Effects of KDN phytase on broiler performance.

Stage	Parameters	Group A	Group B	Group C	Group D
0~21d	Initial Weight (g)	42.1±0.7	42.3±0.6	42.8±0.5	42.7±0.5
	Final Weight (g)	612.2±35.2	565.5±28.2	629.6±25.4	607.9±37.8
	Daily Gain (g)	27.2±1.6	24.9±1.9	27.9±1.1	26.9±1.4
	Daily Feed Intake (g/bird)	41.5±0.9	40.1±1.2	41.9±1.4	41.7±1.1
	Feed/Gain Ratio	1.53±0.07	1.61±0.08	1.50±0.06	1.55±0.10
22~42d	Initial Weight (g)	612.2±35.2	565.5±28.2	629.6±25.4	607.9±37.8
	Final Weight (g)	1835.6±62.5	1709.3±52.3	1913.4±71.2	1857.4±64.3
	Daily Gain (g)	58.3±3.5	54.5±2.9	61.1±2.6	59.5±2.7
	Daily Feed Intake (g/bird)	125.3±8.4	119.8±9.2	129.6±10.2	126.7±9.0
	Feed/Gain Ratio	2.15±0.09	2.20±0.11	2.12±0.08	2.13±0.12
0~42d	Daily Gain (g)	42.8±1.7	39.7±2.1	44.5±2.7	43.2±2.4
	Feed/Gain Ratio	1.95±0.07	2.01±0.09	1.93±0.06	1.95±0.10
	Incidence of leg diseases (%)	2.2±1.9	4.4±3.9	3.3±1.1	4.4±3.9

Table 4. Effects of KDN phytase on P excretion content.

	Group A	Group B	Group C	Group D
P Excretion (g/kg)	3.5±0.4^{a}	2.5±0.2^{b}	2.6±0.2^{b}	2.7±0.4^{ab}

Different superscript means significant differenceP<0.05).

Table 5. Effects of KDN phytase on broilers tibia.

	Group A	Group B	Group C	Group D
Mass (g)	7.95±0.37^{a}	6.81±0.38^{b}	7.59±0.63^{ab}	7.42±0.76^{ab}
Length (cm)	10.34±0.32	10.27±0.43	10.34±0.21	10.32±0.15
Ash Content (%)	38.3±2.2	36.6±1.6	37.8±2.8	37.2±1.4
Ca content in Ash (%)	18.5±0.4	18.2±0.5	18.5±0.2	18.3±0.2
Ca Content in P (%)	36.3±0.5	36.2±0.2	36.5±0.5	36.4±0.4
Shinbone Strength (kg)	26.9±2.5^{a}	19.6±1.1^{b}	27.7±3.3^{a}	27.3±4.9^{a}

Different superscript means significant difference (P<0.05).

Biochemical indicator after treatments: Assay of biochemical indicator from blood serum is shown in Table 6. No significant difference (P>0.05) of serum Ca, P or alkaline phosphatase was observed among treatment groups. Serum Ca was quite stable while fluctuation of value was observed in serum P and no pattern could be found. Compared with control group, alkaline phosphatase increased in all treatment groups.

Table 6. The effects of KDN phytase on blood serum biochemical indicator.

	Group A	Group B	Group C	Group D
Serum Ca (mmol/L)	2.53±0.08	2.52±0.04	2.55±0.06	2.53±0.06
Serum Phosphate (mmol/L)	2.73±1.28	2.08±1.49	2.82±0.87	2.51±0.65
Serum Alkaline Phosphatase (IU/L)	537.3±30.8	612.4±79.2	589.1±58.7	573.8±65.3

Different superscript means significant difference (P<0.05).

Analysis of economic benefit: Economic benefit was analyzed with the addition of phytase (Table 7). Compared with the control group, the gross profit from groups C and D increased by ¥0.47 and ¥0.27; however, that of group B decreased by ¥0.35. To consider the performance and economic benefit for each group, C is the optimum.

Discussion

The current experiment supplemented 250 and 500 U/kg KDN phytase, respectively, to the diets containing 0.34% and 0.26% of available phosphorus (AP) and 0.53% and 0.61% of total phosphorus (TP).The results showed ADG and F/G of broilers from treatment groups in both feeding KDN phases are better than the control group containing 0.68% TP and 0.41% AP. Moreover, broiler performance was improved with the supplementation of KDN phytase. This is mainly due to different enzyme levels leading to the change of hydrolysis of phytate phosphorus. Denbow *et al.*[29] reported the release of P from phytate phosphorus went up from 31% to 58% by supplementing phytase from 250 U/kg to 1000 U/kg to the diets when tested with broilers [29]. With the supplement of phytase to broiler's corn-soybean meal diets, Sebastian *et al.* [30] found indicator of chicks like BW approached the group of normal P level.

Although birds in group B (0.53% TP and 0.26% AP) were supplemented with 500 U/kg phytase, their ADG was lower than in the control group whereas F/G was higher. This is mainly due to the low AP content in the diets. Supplementation of phytase improves the utilization of phytate P in diets, but the phytate P may not be completely hydrolyzed. Even AP in the diets together with P hydrolyzed by phytase cannot meet the requirement for P of broilers, resulting in the low performance. Therefore, certain amount of AP in the diets should be guaranteed as part of P demand of broilers along with the supplemention of phytase.

The utilization of phytate P in broiler diets can be improved with the supplementation of KDN phytase and P excretion can be reduced. The current experiment showed significant decrease of P excretion by 26.7% and 24.4% (P<0.05) in group B and C while 20.6% (P>0.05) in group D. When TP is consistent with AP

Table 7. Comparison of economic benefit from each group.

	Feed Consumption (kg)		Feed Price (RMB/kg)		Feed Cost	Chick Cost	Market Weight	Bird Sold Price	Gross Profit	Difference
	0-21 day	21-42 day	0-21 day	21-42 day	RMB/ bird	RMB/ Bird	kg/ bird	RMB/ bird	RMB/ bird	RMB/ bird
Group A	0.87	2.63	2.20	2.15	7.56	3.00	1.836	12.85	2.29	0
Group B	0.84	2.52	2.18	2.06	7.02	3.00	1.709	11.96	1.94	-0.35
Group C	0.88	2.72	2.19	2.10	7.63	3.00	1.913	13.39	2.76	0.47
Group D	0.88	2.66	2.17	2.08	7.44	3.00	1.857	13.00	2.56	0.27

level, P excretion gradually decreased with phytase level improving in the diets.

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