

EFFECT OF PHYTASE OR LYSINE SUPPLEMENTATION ON PERFORMANCE OF BROILERS FED LYSINE DEFICIENT DIET FROM 21 TO 42 DAYS OF AGE

By

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Abstract: *An experiment was conducted to study the effect of dietary lysine and phytase supplementation levels on: body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broilers fed a lysine-deficient diet. A corn-soybean meal based diet, containing 0.90 % lysine (90% of the requirement NRC, 1994) was supplemented with L-lysine HCl to provide 0.95, 1.00 and 1.05 % lysine or with 125, 250, 375 and 500 U phytase/kg diet resulting eight experimental diets. Each diet was fed to 5 replicates of 20 chicks each from 21 to 42 days of age. The results showed that supplementation of phytase or lysine to the basal diet significantly ($P < 0.01$) improved BWG, FI and FCR. Increasing dietary lysine level from 0.90 to 1.00% or adding 375 U phytase /Kg to the basal diet increased BWG during the different intervals and the entire period by averages being 12 and 13%, respectively. Also, addition of either lysine or phytase increased FI. Feed conversion ratio was improved by the addition either phytase or increasing dietary lysine level. These results indicated that addition of phytase to low lysine diet significantly improved body weight gain and feed conversion ratio. Addition of phytase enzyme to corn-soybean meal broiler diets deficient in lysine was suggested. Further studies are recommended on adding phytase enzyme to broiler diets deficient in other amino acids.*

INTRODUCTION

Phytic acid can form electrostatic associations with the terminal amino acid group on proteins or with the free amino group on lysine and arginine residues within protein molecule. Moreover, phytate-mineral-protein complexes can form with multivalent cations acting as a bridge between phosphate groups on the phytate molecule and the terminal carboxyl group of proteins or free carboxyl group on aspartate and glutamate residues within protein molecule (Cheryan, 1980). Furthermore, phytate is able to bind endogenous proteases such as trypsin and chymotrypsin in the gastrointestinal tract and these complexes may inhibit activity of those enzymes with a subsequent decrease

in the digestibility of protein and amino acids (Caldwell, 1992).

Phytase addition to poultry diets, therefore, may improve not only the utilization of dietary phytate-P but also protein, although research dealing with the latter is inconsistent and controversial (Kornegay, 1996; Kornegay *et al.*, 1998; Kies and Selle, 1998, Boling *et al.*, 1999; Zhang *et al.*, 1999; Ravindran *et al.*, 1999; 2001 and Selle *et al.*, 2000, 2005 and 2007). Supplemental phytase improved availability of N and amino acids and AME in poultry (Ravindran and Bryden, 1998). Addition of microbial phytase to broiler diet could improve the digestibility of amino acids (AA) and crude protein (CP)

by 1- 3%. There were, however, large variations between trails, probably caused by differences in the compositions of the diets and in methods for determining digestibility values (Kies *et al.*, 2001). Supplementation of poultry diets with exogenous phytase did improve growth performance of broiler chicks due to the utilization of phosphorus from phytate and also enhance the digestibility of amino acids (Kies *et al.*, 2001, Rutherford *et al.*, 2002 and 2004, Selle *et al.*, 2000, 2005 and 2007). However, the effect of phytase on amino acid digestibility coefficient is variable and depend on a number of factors. These factors include; variation in dietary ingredients and type and age of poultry species (Selle *et al.*, 2000; Rutherford *et al.*, 2002). Selle *et al* (2005) reported that supplementation of lysine-

deficient diets with exogenous phytase or lysine (L-lysine HCl) improved weight gain and feed efficiency of broilers. However, the effects of phytase on growth performance were more pronounced in lysine-deficient diets. Selle *et al* (2007) determined the effects of broilers diets containing 10.0 and 11.8 g/kg lysine, without and with 500 U phytase /kg, on growth performance and nutrient utilization. Both lysine and phytase supplementation improved ($p<0.05$) weight gain and feed efficiency. However, phytase responses were more pronounced in lysine-deficient diets.

The objective of the present study was to investigate the effect of phytase supplementation on performance of broiler chicks fed lysine- deficient diet.

MATERIALS AND METHODS

A growing broiler trail was designed to study the effect of adding phytase enzyme to lysine- deficient diet on performance from 21 to 42 days of age.

A corn-soybean meal basal diet was formulated to contain 90% of the recommended level of lysine (0.9% lysine) for broiler growers (NRC, 1994). Formulation and nutrient composition of the starter and basal grower diets are shown in Table 1. This diet met or exceeded the recommended requirements of all amino acids, except lysine, for broiler growers. The basal diet was supplemented with four levels of lysine or phytase enzyme. Lysine was added to the basal diet at levels; 0.00, 0.05, 0.10 or 0.15 % (using L-lysine monohydrochloride) to perform four diets containing 90, 95, 100 or 105%, respectively, of recommended lysine levels. Phytase enzyme was added to the basal diet by levels of 125, 250, 375, and 500 U phytase/Kg diets. The Phytase enzyme (Ronozyme 2500 granulate) contained 2500 units (U/g) activity. It is produced by

Novo Nordisk A/S, DK-2880 Bagsvaerd, Denmark. Therefore, eight experimental diets were formulated containing varying levels of lysine or phytase.

Nine hundreds one-day old Arbor Acres broiler chicks were obtained from a commercial hatchery, brooded on floor and fed a commercial broiler starter diet (23% crude protein, 1.10% lysine and 3200 kcal ME/Kg) to 21 days of age. After fasting overnight birds were individually weighed and 800 chicks of uniform weights were used in this experiment. Every dietary treatment was fed to 5 replicates of 20 chicks each. Replicates were randomly allocated in batteries of three-tier system divided into 40 compartments (5 replicates x 8 dietary treatments). Gas heaters were used to keep the required temperature for the brooding period while light was provided 23 hr daily throughout the experimental period. Feed and water were offered for *ad libitum* consumption. Birds were individually weighed and feed intake was recorded per replicate at weekly

intervals. Gain in body weight, and feed conversion ratio (g feed/g gain) were calculated. Birds were vaccinated against AI, ND, IBD and IB disease. After such medical treatments, a dose of vitamins (AD₃E) was offered in the drinking water for the successive 3 days.

Data were statistically analyzed for analysis of variance (One-way) using the General Liner Model (SAS Institute, 1990). Differences among means were compared

($P < 0.05$) using Duncan's new multiple rang test (Duncan, 1955).

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RESULTS AND DISCUSSION

Tables 2, 3, 4 and 5 show the growth performance as affected by levels of lysine or phytase enzyme supplementation during the different weekly intervals and the entire period (21–42 days of age). The results of dietary treatments showed that birds fed the basal diet (0.90% lysine) recorded the lowest values of BWG during the different weekly intervals and the entire period. Addition of graded levels of lysine or phytase to the basal diet significantly ($P < 0.01$) increased BWG during the experimental periods.

Increasing levels of dietary lysine markedly increased BWG at all ages. Also, addition of phytase up to 375 U/Kg to the basal diet increased BWG. Increasing dietary lysine levels from 0.90 to 1.00% increased BWG by 11, 15, 9 and 12% during the periods of 21 to 28, 28 to 35, 35 to 42 and 21 to 42 days of age, respectively. Addition of 375 U/Kg phytase to the basal diet increased BWG by 11, 20, 9 and 13% during the periods of 21 to 28, 28 to 35, 35 to 42 and 21 to 42 days of age, respectively. The highest BWG was obtained during growing periods for birds fed 1.05% lysine. However, the highest BWG was recorded for birds fed on 0.90% lysine supplemented with 375 U phytase /Kg during 28 to 35 days of age.

These results indicated that addition of phytase enzyme to corn-soybean meal

broiler diets deficient in lysine (90% of the requirements, NRC 1994) significantly ($P < 0.05$) improved BWG. Addition of 375 U phytase/ Kg to the basal diet (0.90% lysine) showed an increase in BWG as that resulted from increasing the dietary lysine content by 0.1%.

Regarding the effect of the dietary treatments on feed intake, addition of graded levels of lysine or phytase to a corn-soybean meal basal diet significantly increased FI during the period 28 to 35 ($P < 0.001$), 35 to 42 ($P < 0.05$) and 21 to 42 ($P < 0.05$) days of age. Birds fed the basal diet of low lysine level (0.90%) without phytase consumed less feed compared to the other groups measured at the different intervals. Increasing levels of dietary lysine or addition of phytase up to 375 U/Kg to the basal diet markedly increased FI at all intervals.

The highest FI was recorded for birds fed 1.00% dietary lysine during the period 35 to 42 days of age and with birds fed the basal diet (0.90% lysine) supplemental with 375 U phytase /Kg diet during the period 28 to 35 and 21 to 42 days of age. The calculated values of feed/gain (FCR) indicated that increasing dietary lysine levels or addition of phytase enzyme to the basal diet (0.90% lysine) significantly ($P < 0.001$) improved FCR for the different intervals and the entire period.

No differences were detected among the dietary lysine levels of 0.95, 1.00 and 1.05% on FCR values during the period 21 to 28, 35 to 42 and 21 to 42 days of age. Also, no differences were detected among the dietary phytase levels of 250, 375 and 500 U/Kg on FCR for the different intervals and the entire period.

Addition of phytase enzyme (250 U/Kg) to the basal diet improved FCR by 6, 8, 4 and 5%, while, increasing lysine level from 0.90 to 1.00% improved feed/gain by 6, 10, 6 and 8% compared to the basal diet during the period 21 to 28, 28 to 35, 35 to 42 and 21 to 42 days of age, correspondingly.

It is important to mention that the result of broiler's of the present study is lower than the normal performance recorded nowadays. This may be explained by the fact that current basal diet was formulated based on the NRC, 1994 requirements which are lower than the recommended requirements of the modern commercial broilers

These results indicated that addition of phytase enzyme to corn-soybean meal broiler diets deficient on lysine (90% of the NRC 1994, requirements) significantly ($P<0.001$) improved FCR as the increasing level of lysine availability.

These results are in agreement with those of many earlier studies. Baker (1996) reported that phytase supplementation to a 12% crude protein diet with any single deficiency of four limiting AA (Met, Thr, Lys and Val), or with all them deficient, improved feed conversion ratio of chicks. Biehl and Baker (1997) found that phytase supplementation caused significant increases in gain: feed values of young chicks (13-d-old) fed AA-deficient diet. Kornegay *et al.* (1998) reported that when dietary protein/ AA levels were reduced from 95 to 85% of the NRC (1994) recommendation (1.5-2.0% Units of CP), addition of 300-450 unit phytase /Kg diet prevented the decrease in performance of

broiler. Also, Ravindran and Bryden (1998) used a low lysine (85% NRC, 1994) wheat-sorghum-soybean meal diet with multiple levels of crystalline lysine or microbial phytase and found that 500 Units phytase /Kg diet was equivalent to 0.7g lysine /Kg diets based on BWG and FCR.

The relevance of phytate protein complexes in lowering protein utilization in monogastric animals and the potential of phytase enzyme to release the phytate-bound protein have attracted considerable attention (Kies and Selle, 1998 and Ravindran *et al.*, 2001). Simons *et al.* (1990) suggested that the improvements in growth observed in chickens fed diet supplemented with phytase may result from one or more of the following (a) an increase in absorbed phosphorus (b) the release of other minerals from the phytate mineral complex, (c) the utilization of inositol, (d) an increase in digestibility, and/or (e) increased availability of amino acid.

Kornegay (1996) found that ileal digestibility of all AA except methionine and proline were linearly increased as phytase (250, 500 and 750 Units /Kg) was added to young broilers diets. Jongbloed *et al.* (1997) explained that a very strong complex is formed between soluble protein and phytate at pH values between 2 and 3 in different feedstuffs. Pretreatment of phytate with phytase prevented the formation of this complex. After formation the hydrolysis of protein from this complex by pepsin was considerably accelerated by the addition of phytase. Rutherford *et al.* (1997) showed that free lysine forms a complex with phytate. They incubated rice pollards (feedstuff rich in phytate) with lysine hydrochloride. Approximately 20% of lysine were bound, but half of this was liberated after the addition of phytase. Protein digestion may also be inhibited indirectly because proteolytic enzymes in the digestive tract form complexes with phytate. Johnston and Southern (1999) fed broiler chicks a single level (600 U/Kg) of phytase or multiple levels of crystalline lysine added to a low

lysine (80% of requirement) maize-soybean meal diet. They found an average equivalency, based on BWG and FCR, of 0.23g lysine /Kg diet for 600 U phytase /Kg diet. Ravindran *et al.* (2001) fed broiler chicks on diets containing graded levels of lysine (1.00, 1.06, 1.12 and 1.18%) or phytase (125, 250, 375, 500, 750 and 1000 U/Kg), with deficient in lysine (91% of the requirement NRC, 1994). The results indicated that addition of lysine to lysine-deficient diet linearly increased ($P<0.001$) body weight gain and gain /feed. Feed intake increased up to 1.12% dietary lysine. The response of body weight gain to added phytase reached a plateau at 500 U/Kg diet. Phytase had no effect on gain per feed to 250 U/Kg diet and then increased ($P<0.05$) with further addition. Rutherford *et al.* (2002) examined amino acid digestibility of five broiler diets, supplemented with microbial phytase (750 U/Kg). The results indicated that amino acid digestibility was significantly greater in the presence of microbial phytase for all amino acids examined in wheat, for

several amino acids in each of maize and rapeseed meal and for one amino acid in rice bran and soybean meal. Abd-Elsamee (2002) found that using either high level of sulphur amino acid (120%) or microbial phytase supplementation (750 U/Kg) significantly ($P<0.05$) improved the average values of broiler performance except for feed intake. Selle *et al.* (2005 and 2007) found that supplementation of lysine-deficient diets with phytase or lysine (L-lysine HCl) improved weight gain and feed efficiency of broilers. However, the effects of phytase on growth performance were more pronounced in lysine-deficient diets.

It could be concluded that, addition of phytase enzyme to broiler diets deficient on lysine improved performance to be comparable with high lysine diet. This may be due to hydrolysis of phytate protein complexes presents in feedstuffs or increasing availability of amino acids. Further studies are recommended on adding phytase enzyme to broiler diets deficient on other amino acids.

Table 1. Formulation and nutrient composition of starter (1-20 days) and basal grower (21-42 days) diets

Item	Starter diet	Grower diet
Ingredients %		
Yellow corn	50.43	60.59
Soybean meal (44%)	32.00	25.29
Corn gluten meal (60%)	8.00	6.68
Soy oil	5.63	4.05
Limestone	1.40	1.44
Dicalcium phosphate	1.66	1.18
Vitamins and mineral mix.*	0.40	0.40
Salt	0.35	0.35
L-lysine HCl	0.03	0.00
DL-methionine	0.10	0.02
Total	100	100
Calculated composition %**		
Crude protein	23.00	20.00
ME (kcal/kg)	3199	3198
Lysine	1.10	0.90
Methionine	0.54	0.39
Methionine and cystine	0.90	0.75
Calcium	1.00	0.90
Total phosphorus	0.70	0.58
Non-phytate P	0.45	0.35

(*) Vitamin - mineral mixture supplied per Kg of diet: Vit A, 12000 I.U; Vit D₃, 2200 I.U; Vit E, 10 mg; Vit K₃, 2 mg; Vit B₁, 1mg; Vit B₂, 4mg; Vit B₆, 1.5mg; Vit B₁₂, 10µg; Niacin, 20 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50 µg; Choline chloride, 500mg; Copper, 10 mg; Iodine, 1mg; Iron, 30 mg; Manganese, 55 mg; Zinc, 50 mg and Selenium, 0.1 mg.

(**) Calculated based on feed composition Tables of NRC (1994)

Table 2: Growth performance of broilers fed a lysine-deficient diet supplemented with varying levels of lysine and phytase from 21 to 28 days of age

Item		Body weight gain (g)	Feed Intake (g)	Feed conversion ratio (feed/gain)
Dietary treatments				
Lysine %	Phytase U/Kg			
0.90	0	352 ^b	675	1.92 ^a
0.95	0	389 ^a	705	1.81 ^b
1.00	0	392 ^a	706	1.80 ^b
1.05	0	393 ^a	714	1.82 ^b
0.90	125	360 ^b	681	1.89 ^a
0.90	250	383 ^a	690	1.80 ^b
0.90	375	390 ^a	711	1.82 ^b
0.90	500	385 ^a	696	1.81 ^b
SE of means		± 3.75	±5.09	± 0.01
Significances		**	NS	***

^{a-d} Means within each column with no common superscript are significantly different (P<0.05). NS: not significant ** P<0.01 *** P<0.001

Table 3: Growth performance of broilers fed a lysine-deficient diet supplemented with varying levels of lysine and phytase from 28 to 35 days of age

Item		Body weight gain (g)	Feed Intake (g)	Feed conversion ratio (feed/gain)
Dietary treatments				
Lysine %	Phytase U/Kg			
0.90	0	324 ^c	690 ^c	2.13 ^a
0.95	0	353 ^d	703 ^{bc}	1.99 ^{bcd}
1.00	0	371 ^c	708 ^{bc}	1.91 ^d
1.05	0	373 ^{bc}	714 ^{bc}	1.91 ^d
0.90	125	354 ^d	722 ^b	2.04 ^b
0.90	250	388 ^{ab}	764 ^a	1.97 ^{bcd}
0.90	375	388 ^{ab}	765 ^a	1.97 ^{bcd}
0.90	500	392 ^a	782 ^a	1.99 ^{bc}
SE of means		± 4.09	± 7.29	± 0.01
Significances		***	***	***

^{a-c} Means within each column with no common superscript are significantly different (P < 0.05).
 *** P < 0.001

Table 4: Growth performance of broilers fed a lysine-deficient diet supplemented with varying levels of lysine and phytase from 35 to 42 days of age

Item		Body weight gain (g)	Feed Intake (g)	Feed conversion ratio (feed/gain)
Dietary treatments				
Lysine %	Phytase U/Kg			
0.90	0	360 ^d	798 ^b	2.22 ^{ab}
0.95	0	375 ^{bcd}	800 ^b	2.13 ^{bc}
1.00	0	392 ^b	854 ^a	2.18 ^{abc}
1.05	0	409 ^a	836 ^{ab}	2.05 ^c
0.90	125	370 ^{cd}	847 ^{ab}	2.29 ^a
0.90	250	378 ^{bc}	808 ^{ab}	2.13 ^{bc}
0.90	375	392 ^b	846 ^{ab}	2.16 ^{bc}
0.90	500	392 ^b	816 ^{ab}	2.08 ^c
SE of means		± 4.11	± 8.01	± 0.01
Significances		***	*	*

^{a-c} Means within each column with no common superscript are significantly different (P < 0.05).
 *P < 0.05 *** P < 0.001

Table 5: Growth performance of broilers fed a lysine-deficient diet supplemented with varying levels of lysine and phytase from 21 to 42 days of age

Item		Body weight gain (g)	Feed Intake (g)	Feed conversion ratio (feed/gain)
Dietary treatments				
Lysine %	Phytase U/Kg			
0.90	0	1036 ^d	2163 ^c	2.09 ^a
0.95	0	1118 ^b	2208 ^{bc}	1.97 ^b
1.00	0	1155 ^a	2268 ^{ab}	1.96 ^b
1.05	0	1175 ^a	2265 ^{ab}	1.93 ^b
0.90	125	1084 ^c	2250 ^{ab}	2.07 ^a
0.90	250	1149 ^a	2262 ^{ab}	1.97 ^b
0.90	375	1170 ^a	2322 ^a	1.98 ^b
0.90	500	1169 ^a	2294 ^a	1.96 ^b
SE of means		± 9.94	± 12.21	± 0.01
Significances		***	*	***

^{a-c} Means within each column with no common superscript are significantly different (P < 0.05).
*P<0.05 ***P<0.001

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الملخص العربي

تأثير إضافة إنزيم الفيتيز على أداء كتاكيت اللحم المغذى على علائق منخفضة في الليسين من عمر ٢١ إلى ٤٢ يوم

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٢- المركز الإقليمي للأغذية والأعلاف- مركز البحوث الزراعية- الجيزة- مصر

تهدف هذه التجربة إلى دراسة تأثير إضافة الليسين أو إنزيم الفيتيز على أداء (الزيادة في وزن الجسم، الغذاء المستهلك ومعامل التحويل الغذائي) كتاكيت اللحم النامي المغذى على علائق منخفضة في الليسين. احتوت العليقة الأساسية والتي تكونت من الذرة وكسب فول الصويا على ٠.٩٠% ليسين (٩٠% من مقررات NRC, 1994). تم إضافة الليسين التجاري لتحتوى على ٠.٩٥، ١.٠٠، ١.٠٥ و ١.١٠% ليسين أو إضافة ١٢٥، ٢٥٠، ٣٧٥ و ٥٠٠ وحدة فيتيز/كجم علف إلى العليقة الأساسية لتكوين ثمان علائق تجريبية. كل عليقة قدمت لعدد ٥ مكررات بكل مكررة ٢٠ كتكوت خلال الفترة من ٢١ إلى ٤٢ يوم. سجلت القياسات المختلفة للمعاملات أسبوعياً.

أوضحت النتائج أن إضافة الليسين أو إنزيم الفيتيز إلى العليقة الأساسية أدى إلى زيادة معنوية (٠.٠١) في وزن الجسم والغذاء المستهلك وتحسن في معامل التحويل الغذائي. زيادة مستوى الليسين من ٠.٩٠ إلى ١.٠% أو إضافة ٣٧٥ وحدة من إنزيم الفيتيز/كجم إلى العليقة الأساسية أدى إلى زيادة في وزن الجسم خلال الفترات المختلفة والفترة الكلية بمعدل ١٢ و ١٣% على التوالي. أيضاً إضافة إنزيم الفيتيز أو زيادة الليسين أدى إلى زيادة في الغذاء المستهلك. معامل التحويل الغذائي تحسن بإضافة إنزيم الفيتيز أو زيادة مستوى الليسين.

أشارت هذه النتائج إلى أن إضافة إنزيم الفيتيز إلى العلائق المنخفضة في الليسين تؤدي إلى تحسن كبير في وزن الجسم ومعامل التحويل الغذائي. يقترح إضافة الفيتيز للعلائق المكونة من الذرة وكسب فول الصويا والمنخفضة في الليسين. بجانب معرفة تأثير الفيتيز على تحرير الفوسفور من الفيتات كذلك يمكنه تحرير الليسين. نوصى بمزيد من الدراسات حول إضافة إنزيم الفيتيز إلى علائق كتاكيت اللحم المنخفضة في أحماض أمينية أخرى.