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ADDITION OF PHYTASE AND OTHER ENZYME PREPARATION TO LOW PHOSPHORUS - LOW CALCIUM BROILER DIETS.

By

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Abestract: *Broiler growth experiment was conducted to evaluate the influence of adding phytase (Ronozyme 2500® granulate) individually or in combination with an enzyme preparation (Avizyme 1500®) containing amylase, protease and xylanase. The experimental diets contained lower levels of Ca and P than the recommended levels. The basal starter diet contained 0.85% Ca and 0.35% non-phytate phosphorus (NPP) while the basal grower diet contained 0.75%Ca and 0.26% NPP. Phytase and / or avizyme were added to the basal diets at levels 500 U phytase and 0.75 g. avizyme /kg diet. Performance, bone parameters and some carcass characteristics were determined at 35 days of age. The results showed that adding phytase or avizyme individually or in combinations to corn soybean meal broiler diets of low Ca and P content significantly (P<0.05) increased body weight gain and feed intake of 35 days old. Such addition did not affect feed gain ratio. Phytase significantly (P<0.05) improved bone parameters (tibia length, weights and ash percentage of tibia and toe). Adding phytase alone or with avizyme significantly (P<0.05) increased Ca % of tibia ash. Tibia P (%) and Zn (mg/kg) were not affected by either phytase or avizyme addition.*

The dressing and abdominal fat % were significantly (P<0.001) increased with addition of avizyme while addition of phytase did not significantly affect dressing, abdominal fat, liver or heart weights as percentage of body weight.

INTRODUCTION

Feed ingredients of plant origin contain some of components that cannot be digested by monogasteric animals because of the lack or insufficiency of endogenous enzyme secretions. These components also

decrease the utilization of other dietary nutrients, leading to depressed performance. Such antinutritive components include pentosans in wheat, beta-glucans in barley, and phytic acid, which is found in all plant feed ingredients. Inclusion of exogenous glycanase enzymes (xylanase and glucanase) to improve the performance of broilers fed diets based on wheat or barley has become a routine practice (Ravindran *et al.,* 1999). The nutritive value of corn-soybean diets has been improved by the inclusion of α-galactosidase enzymes, which hydrolyze the antinutritive factors present in soybean. Using phytase enzyme in broiler diets facilitates the release of phytate bound phosphorus. Supplementation of broiler diets with a combination of these enzymes may enhance nutrient utilization further, obtaining additional benefits on phosphorus availability and performance (Puchal and Mascarell, 1999). Amylase supplementation to corn soybean meal diets improves performance of turkey poults (Ritz *et al.,* 1995). Addition of a mixture of amylase, protease and xylanase did enhance performance of broiler fed diets based on corn and soybean meal (Zanella *et al.,* 1999; Douglas *et al.,* 2000 and Café *et al.,* 2002).

The objective of the present study was to examine the influence of phytase and other enzyme preparation in commercial forms, individually or in combination, on performance, bone parameters and some carcass characteristics of broilers fed low P – low Ca corn-soybean meal diets for 35 days.

MATERIAL AND METHODS

A basal starter diet (Table 1) was formulated to contain 23% crude protein, 3200 Kcal ME/Kg, 0.85% Ca and 0.35% NPP, and basal grower diet contained 20% crude protein, 3200 Kcal ME/Kg, 0.75% Ca and 0.26% NPP. The dietary Ca and P levels were lower than the current NRC (1994) recommendation. Phytase® (Ronozyme 2500 granulate) and/or avizyme® 1500 (a commercial enzyme preparation contains 300 U/g amylase, 400 U/g protease and 400 U/g xylanase) were added to such basal diets. Phytase and / or avizyme were added to the basal diets at levels 500 U phytase and 0.75 g avizyme /kg diet (Table 2) to perform the dietary treatments.

A number of 120 one day old Ross broiler chicks were used in this experiment. Every dietary treatment was fed to 3 replicates of 10 chicks each. The average initial live body weights of all replicates were nearly similar. Replicates were randomly allocated in batteries of three-tier system divided into 12 compartments (3 replicates X 4 dietary treatments). Gas heaters were used during the first two weeks of age to keep the required temperature for the brooding period while light was provided 23 hr daily during the experimental period. Feed and water were allowed for *ad libitum*

consumption. Birds were fed the starter diets from 1 to 21 days of age and the grower diets from 21 to 35 days of age. After fasting overnight, birds were individually weighed and feed consumption was recorded per replicate at 35 days of age. Gain in body weight and feed conversion ratio were calculated.

At 35 days of age, six representative chicks with body weight close to the group average were selected from every treatments group and slaughtered for bone measurements (as described by Mohamed *et al*. (2001) and some carcass characteristics. Determination of phosphorus and calcium in tibia ash was carried out based on the Official Methods of Analysis (AOAC, 1990). Zinc content of tibia ash was determined using flame atomic absorption spectrophotometer after ashing with 15 ml HNO_3 and 10 ml $HClO_4$ as described by Scancar *et al. (2000)* based on the method of Berg *et al.*(2000).

Data were statistically analyzed for analysis of variance as 2 x 2 factorial arrangements using the General Linear Model of SAS (1990). Significant differences among treatment means were separated by Duncan's new multiple rang test (Duncan, 1955) with a 5% level of probability.

RESULTS AND DISCUSSIONS

Performance of 35 Days Old Broiler Chicks.

Effect of phytase and avizyme supplementation, on performance of broiler chicks is presented in Table 3. Addition of phytase significantly ($P<0.05$) improved weight gain by 5.17 % (from 1411 to 1484 g), while avizyme showed only 2.41 % increase (from 1411 to 1445 g). The combination of phytase and avizyme caused further improvements in weight gain (7.73 %), being from 1411 to 1520 g. The main effects showed significant increase in weight gain due to phytase ($P<0.001$) and avizyme ($P<0.05$) addition. Weight gain increased by 5.18%with phytase addition while avizyme addition increased weight gain by 2.49% regarding the main effect of such supplementations. Effect of treatments on feed intake indicated that addition of phytase and/or avizyme increased feed intake. When these enzymes were supplemented individually, such increase was not significant. When a combination of the enzymes was used, a significant ($P<0.05$) increase (6.86%) in feed intake was observed. The main effect clearly showed significant increase in feed intake when phytase ($P<0.01$) or avizyme ($P<0.05$) were used separately by 3.57% and 3.22%, respectively.

The recorded values of feed conversion ratio ranged from 1.77 to 1.81. However, phytase or avizyme addition resulted in slight improvements

in feed utilization. Adding phytase enzyme, avizyme or combination of both enzymes did not significantly affect feed conversion ratio. No significant interaction (phytase x avizyme) was detected on the measured criteria.

These results indicate that added phytase or phytase and avizyme (amylase, protease and xylanase) together to a corn-soybean meal diet improves growth performance of broiler chicks as assessed by increasing body weight and feed intake. Phytase effect was more pronounced than avizyme in increasing weight gain and feed intake.

These findings are in agreement with previous studies of Zyla *et al.*, (1999); Zyla *et al.*, (2000); and Wu *et al.,* (2004). Puchal and Mascarell (1999) reported that α-galactosidase enzymes improved the nutritive value of corn-soybean diets. Phytase enzyme facilitates the release of phytate phosphorus and reduces the excreted phosphorus. A combination of phytase and α-galactosidase could enhance nutrient utilization further, giving additional benefits on phosphorus availability and also in performance. Wu *et al.* (2004) found that the addition of phytase to wheat-based diet improved weight gain and feed conversion ratio by 17.5 and 2.9%, respectively. Corresponding improvements due to the addition of xylanase were 16.5 and 4.9%, respectively. The combination of phytase and xylanase caused no further improvements in broiler performance.

Bone parameters of 35-Days Old Broiler Chicks.

The effect of phytase and avizyme supplementation on bone parameters of chicks fed the different dietary treatments are presented in Table 4. Adding phytase or avizyme increased length of tibia with no significant differences. Addition of phytase and avizyme together significantly ($P<0.05$) increased length of tibia compared to the basal diet of no enzyme addition. The main effect of phytase and avizyme supplementation showed that phytase addition significantly ($P<0.05$) increased tibia length, while avizyme addition did not.

Birds fed the basal diet without enzyme addition gave the lowest weights of fat free dry tibia and dry toe. Supplementation of phytase or avizyme enzyme to the basal diet slightly increased weight of tibia. Combination of phytase and avizyme significantly ($P<0.05$) increased tibia and toe dry weights compared to the basal diet. The main effects of phytase and avizyme supplementation indicated that weight of tibia and toe significantly ($P<0.05$) increased by adding phytase. Adding avizyme significantly ($P<0.01$) increased toe weight only.

Addition of phytase to the basal diet significantly (P<0.05) increased tibia and toe ash.. Addition of avizyme did not significantly affect tibia or toe ash percentages. The maximum ash percentage was recorded for birds fed the basal diet supplemented with phytase and avizyme together, being 41.68 and 10.66 % for tibia and toe, respectively.

Tibia Mineral Concentrations

The effects of phytase and avizyme supplementation on concentrations of Ca%, P% and Zn mg/Kg in tibia ash are shown in Table 5. Adding phytase significantly (P<0.05) increased concentration of Ca in tibia ash, while it did not significantly affect concentrations of both P and Zn.

Avizyme supplementation did not affect P, Ca or Zn concentration of tibia ash. Adding phytase and avizyme together to the basal diet significantly (P<0.05) increased Ca % of tibia ash. No significant differences in Ca% of tibia ash were detected between birds fed the basal diet with phytase and birds fed the basal diet with phytase and avizyme together.

The main effects of phytase addition showed significant (P<0.05) increase in Ca concentration in tibia ash. No significant effect was observed in P and Zn concentrations by phytase addition. Avizyme supplementation did not significantly affect P, Ca and Zn concentrations of tibia ash.

These data clearly indicated that the addition of phytase to broiler diets from 1 to 35 days of age significantly (P<0.05) improved bone parameters. The percentage of tibia ash was improved by the addition of phytase and this improvement was further increased when avizyme was added with phytase. These results are in agreement with the previous studies of Zyla *et al. (*1996), Sohail and Roland (1999), and Zyla *et al. (*2000). The improvement in ash percentage in the tibia indicated an increase in the availability of minerals liberated by phytase from phytate-mineral complex. Zyla *et al.* (1999) reported that concentrations of ash in the toes of birds increased by phytase supplementation, the effect being more pronounced in birds consuming feed that also contained xylanase.

Some Carcass Characteristics of 35-Days Old Broiler Chicks.

The effects of phytase and avizyme supplementation on carcass characteristics of chicks fed the different dietary treatments are shown in Table 6. Carcass characteristics included dressing, abdominal fat, liver, heart and gizzard (% of BW) at 35 days of age. Addition of phytase enzyme did not significantly affect dressing, abdominal fat, liver, gizzard and heart

weights (% of body weight). Addition of avizyme individually or with phytase significantly ($P<0.05$) increased dressing and abdominal fat % of body weight. The highest dressing, gizzard and abdominal fat percentage values were recorded for birds fed the basal diet supplemented with avizyme. The main effect of phytase supplementation showed significant ($P<0.05$) decrease in gizzard weight % only. Supplementation of avizyme significantly ($P<0.001$) increased abdominal fat % and dressing percentages ($P<0.01$).

It could be concluded that the dressing and abdominal fat % significantly ($P<0.001$) increased with avizyme while addition of phytase did not significantly affect dressing, abdominal fat, liver and heart weight percentages. Increasing dressing percentage and abdominal fat by the addition of enzymes has been reported in many previous studies (Jamroz *et al.,* 1996; Pisarski and Wojcik 1995 and Alam *et al.,* 2003). They attributed the increased in carcass yield by the addition of enzymes in diets to the higher fat deposition in carcass and also for increased meat yield.

Alam *et al.* (2003) found that addition of three different enzymes Roxazyme-G® (Beta glucanase, cellulase and xylanase), Alquerzim® (pepsin, pancreatin, lipase and cellulase) and Feedzyme® (Alpha amylase, proteinase, Beta-glucanase and pentosanase)] to broiler diets significantly increased ($P<0.01$) dressing and liver weight. Garcia *et al.* (2003) found that weights of gizzard and liver as a percentages of BW decreased ($P>0.05$) with α-amylase supplementation to broiler diets based on corn-soybean meal.

It could be concluded from the results that addition phytase and avizyme or combination of both to low P-low Ca broiler diets increased weight gain and feed intake significantly. No significant effect on feed conversion ratio was detected. Phytase effect was more pronounced than avizyme in increasing weight gain and feed intake. The addition of phytase to such broiler diets significantly ($P<0.05$) improved bone parameters. The percentage of tibia ash was improved by the addition of phytase and this improvement was further increased when avizyme was added with phytase. The dressing and abdominal fat % significantly ($P<0.001$) increased with avizyme while addition of phytase did not significantly affect dressing, abdominal fat, liver and heart weight.

Table 1: Formulation and nutrient composition of the starter and grower diets.

Item	Starter diet From 1 to 21 d	Grower diet From 21 to 35 d
Ingredients %		
Yellow corn	51.62	61.35
Soybean meal (44%)	32.00	26.00
Corn gluten meal (60%)	7.80	6.00
Vegetable oil	5.26	3.88
Limestone	1.31	1.31
Dicalcium phosphate	1.13	0.73
Vitamin and Mineral mix[(1)]	0.40	0.25
Salt (NaCl)	0.35	0.35
L-Lysine HCl	0.03	0.10
DL-Methionine	0.10	0.03
Calculated Composition [(2)] %		
Crude protein	22.99	20.03
ME (Kcal/Kg)	3200	3200
Lysine	1.10	1.00
Methionine	0.54	0.39
Methionine + Cystine	0.90	0.75
Calcium	0.85	0.75
Total phosphorus	0.60	0.50
Nonphytate P	0.35	0.26

[(1)] Vitamin - mineral mixture supplied per Kg of diet: Vit A, 12000 I.U; Vit D_3, 2200 I.U; Vit E, 10 mg; Vit K_3, 2 mg; Vit B_1, 1mg; Vit B_2, 4mg; Vit B_6, 1.5mg; Vit B_{12}, 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.
[(2)] Calculated values based on feed composition Tables of NRC (1994)

Table 2: Levels of the supplemental enzymes

Diets No.	Phytase U/kg	Avizyme g/kg
1	0	0
2	500	0
3	0	0.75
4	500	0.75

Table 3: Effect of phytase and avizyme supplementation on body weight gain, feed intake and feed conversion ratio of 35 days old broiler chicks

Item		Body weight gain (g)	Feed intake (g)	Feed/gain
Dietary treatments				
Phytase U/kg	**Avizyme g/kg**			
0	0	1411 ^{c}	2538 ^{b}	1.80
500	0	1484 ^{ab}	2625 ^{ab}	1.77
0	0.75	1445 ^{bc}	2616 ^{b}	1.81
500	0.75	1520 ^{a}	2712 ^{a}	1.79
SE of means		13.91	22.07	0.01
Main effects				
Phytase U/kg				
0		1428 ^{b}	2577 ^{b}	1.81
500		1502 ^{a}	2669 ^{a}	1.78
Avizyme g/kg				
0		1447 ^{b}	2581 ^{b}	1.79
0.75		1483 ^{a}	2664 ^{a}	1.80
Significances				
Source of variation				
Phytase effect		***	**	NS
Avizyme effect		*	*	NS
Phytase **x** Avizyme		NS	NS	NS

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

* P< 0.05 ** P<0.01 *** P<0.001 NS: not significant (P>0.05)

Table 4: Effect of phytase and avizyme supplementation on bone measurements of 35-day old broiler chicks.

Item		Tibia Length (cm)	Weight (g)		Ash %	
			Tibia [(1)]	Toe [(2)]	Tibia	Toe
Dietary treatments						
Phytase U/Kg	**Avizyme g/kg**					
0	0	8.50 [b]	4.31 [b]	1.01 [c]	39.32 [b]	9.66 [b]
500	0	8.70 [ab]	4.81 [ab]	1.13 [bc]	41.28 [a]	10.45 [a]
0	0.75	8.60 [ab]	4.41 [ab]	1.26 [ab]	39.75 [b]	9.53 [b]
500	0.75	8.73 [a]	5.22 [a]	1.39 [a]	41.68 [a]	10.66 [a]
SE of means		0.04	0.13	0.05	0.32	0.16
Main effects						
Phytase U/Kg						
0		8.55 [b]	4.51 [b]	1.13 [b]	39.53 [b]	9.59 [b]
500		8.72 [a]	5.02 [a]	1.26 [a]	41.48 [a]	10.56 [a]
Avizyme g/kg						
0		8.60	4.56	1.07 [b]	40.30	10.06
0.75		8.67	4.97	1.33 [a]	40.72	10.10
Significances						
Source of variation						
Phytase effect		*	*	*	***	***
Avizyme effect		NS	NS	**	NS	NS
Phytase **x** Avizyme		NS	NS	NS	NS	NS

[a--c] Means within each column for each effect with no common superscript are significantly different (P< 0.05).

[(1)] Fat free dry weight [(2)] Dry weight

* P<0.05 ** P<0.01 *** P<0.001 NS: not significant (P>0.05)

Table 5: Effect of phytase and avizyme supplementation on tibia minerals of 35-day old broiler chicks.

Item		Tibia ash minerals		
		P%	Ca%	Zn mg/Kg
Dietary treatments				
Phytase U/Kg	**Avizyme g/kg**			
0	0	19.90	39.73 [b]	367
500	0	19.94	39.97 [a]	383
0	0.75	19.84	39.75 [b]	366
500	0.75	19.96	40.02 [a]	371
SE of means		0.6	0.05	3.63
Main effects				
Phytase U/kg				
0		19.87	39.74 [b]	366
500		19.95	39.99 [a]	377
Avizyme g/kg				
0		19.92	39.85	375
0.75		19.90	39.88	369
Significances				
Source of variation				
Phytase effect		NS	*	NS
Avizyme effect		NS	NS	NS
Phytase **x** Avizyme		NS	NS	NS

[a-b] Means within each column for each effect with no common superscript are significantly different (P< 0.05).

* P<0.05 NS: not significant (P>0.05)

Table 6: Effect of phytase and avizyme supplementation on dressing, abdominal fat and giblets weights as percent of live body weight of 35-day old broiler chicks.

Item		Dressing %	Abdominal fat %	Giblets % (of BW)		
				Liver	Heart	Gizz-ard
Dietary treatments						
Phytase U/kg	**Avizyme g/kg**					
0	0	70 [b]	1.62 [b]	2.09	0.50	1.64 [bc]
500	0	70 [b]	1.67 [b]	2.04	0.52	1.66 [b]
0	0.75	71 [a]	2.12[a]	2.13	0.51	1.71 [a]
500	0.75	71 [a]	2.06[a]	2.09	0.51	1.63 [c]
SE of means		0.22	0.07	0.01	0.01	0.01
Main effects						
Phytase U/kg						
0		70	1.87	2.11	0.51	1.68 [a]
500		70	1.87	2.07	0.51	1.64 [b]
Avizyme g/kg						
0		70 [b]	1.65 [b]	2.07	0.51	1.65
0.75		71 [a]	2.09 [a]	2.11	0.51	1.67
Significances						
Source of variation						
Phytase effect		NS	NS	NS	NS	*
Avizyme effect		**	***	NS	NS	NS
Phytase **x** Avizyme		NS	NS	NS	NS	**

[a--c] Means within each column for each effect with no common superscript are significantly different (P< 0.05).

* P<0.05 ** P<0.01 *** P<0.001 NS: not significant (P>0.05)

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الملخص العربي

اضافة أنزيم الفيتيز ومستحضر أنزيمي آخر الى علائق دجاج اللحم المنخفضة فى محتواها من الفسفور والكالسيوم

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قسم الإنتاج الحيوانى – كلية الزراعة – جامعة القاهرة.

تهدف هذه التجربة الى دراسة تأثير إضافة إنزيم الفيتيز بمفرده أو فى وجود إنزيمات أخرى (الأفيزيم والذي يحتوى على زيلانيز, بروتيز, أميليز) إلى علائق كتاكيت اللحم (ROSS) المحتوية على مستوى منخفض من الكالسيوم والفوسفور على الأداء الإنتاجى وبعض صفات العظام والذبيحة. تم تكوين عليقة أساسية احتوت على ٠,٨٥ و ٠,٧٥% كالسيوم ؛ ٠,٣٥ و٠,٢٦% فوسفور متاح خلال الفترة من ١-٢١ يوم ومن ٢١-٣٥ يوم من عمر الكتاكيت على الترتيب. أضيف إنزيم الفيتيز إلى كل العلائق بمعدل صفر، ٥٠٠ وحدة /كجم عليقة وأضيف الأفيزيم بمعدل صفر، ٠,٧٥جرام/كجم. قدمت هذه المعاملات لعدد ١٢٠ كتكوت (٤ معاملات X ٣ مكررات X ١٠ كتاكيت).

أوضحت ألنتائج المتحصل عليها أن إضافة إنزيم الفيتيز أو الافيزيم أو كلاهما إلى عليقة مكونة من الذرة وكسب الصويا والمنخفضة فى محتواها من الكالسيوم والفوسفور إلى تحسن الأداء الإنتاجى لكتاكيت اللحم. و قد وجد أن تأثير إضافة إنزيم الفيتيز أكثر وضوحا.

كما أدت إضافة إنزيم الفيتيز إلى زيادة معنوية فى محتوى رماد عظمة الساق من الكالسيوم. إضافة الأفيزيم لم تؤثر على محتوى عظمة الساق من الكالسيوم أو الفوسفور أو الزنك.و عند إضافة إنزيم الفيتيز مع الأفيزيم أدى إلى زيادة معنوية فى محتوى عظمة الساق من الكالسيوم فى حين لم يتأثر محتواها من الفوسفور أو الزنك. إضافة إنزيم الفيتيز لم تؤثر على أي من نسبة التصافي ووزن كلا من الدهن، القلب، الكبد (% من وزن الجسم). بينما إضافة ألأفيزيم أدت إلى زيادة معنوية فى نسبة التصافي ووزن الدهن (% من وزن الجسم). لوحظ أن إضافة المستحضرين معاً أدت ايضا إلى زيادة معنوية فى نسبة التصافي ووزن الدهن (% من وزن الجسم).

وقد أوضحت النتائج أن إضافة إنزيم الفيتيز أو الفيتيز مع الافيزيم إلى العلائق المكونة من الذرة وكسب الصويا والمنخفضة فى محتواها من الفوسفور والكالسيوم تؤدى إلى تحسن معنوي فى الأداء الإنتاجى وفى صفات العظام وهذا يدل على زيادة الاستفادة من المركبات الغذائية التي كانت مرتبطة بالفيتات.