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## **PRODUCTION PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILERS FED DIETS WITH OR WITHOUT PHYTASE SUPPLEMENTATION**

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### **ABSTRACT**

To evaluate the effect of phytase supplementation, 300 day-old broiler chicks were distributed to five treatments following a completely randomized design: T1 - Positive Control (PC); T2 - Negative Control 1 (diet with available phosphorus [P], calcium [Ca] and metabolizable energy [ME] lower than PC by 0.15%, 0.18% and 75 kcal/kg, respectively); T3 - T2 + 100 g phytase per ton of feed; T4 - Negative Control 2 (diet with available P, Ca and ME lower than PC by 0.18%, 0.23% and 95 kcal/kg, respectively); and T5 - T4 + 200 g phytase/ton of feed. Phytase supplementation improved body weight, weight gain and feed efficiency of broilers fed the negative control diets. Significant differences among treatments were observed in % abdominal fat, % leg and % thigh. Higher income over feed and chick cost was obtained in broilers fed diets with phytase compared to those fed the negative and positive control diets. The results suggest that growth performance of broilers fed diets with lower specification in available P, Ca and ME is not adversely affected if phytase is added in the diet.

Keywords: broilers, carcass, growth performance, phosphorus, phytase

### **INTRODUCTION**

Phytate phosphorus (P) present in feed ingredients of plant origin is poorly utilized by monogastric animals. In order to satisfy the phosphorus requirement of animals, feeds are supplemented with inorganic feed phosphate. Unfortunately, mineral phosphate is a limited resource, both in quantity and quality, and its price has quadrupled since 2006. Supplementation with mineral phosphate and the presence of phytate P in animal diets increase P runoff from animal farms resulting in pollution of bodies of water. In addition, phytate has also been found to bind nutrients such as calcium, magnesium and amino acids, making them unavailable to the animal (Dilger *et al.*, 2004).

Phytase, an enzyme that dephosphorylates phytate, has gained popularity for decades (Wodzinski and Ullah, 1996). Commercial forms of phytase are derived

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from either bacteria or fungi. Phytase added to broiler diets has been shown to improve the availability of P (Rutherford *et al.*, 2004; Cowieson and Adeola, 2005), macro-minerals such as calcium, zinc, magnesium and potassium (Ravindran *et al.*, 2008; Saima *et al.*, 2009) and some amino acids (Rutherford *et al.*, 2004).

Recently, a new 6-phytase, produced by expression of synthetic gene in *Aspergillus oryzae* (Aureli *et al.*, 2011) was introduced in the market. This novel phytase has higher P release (0.15 to 0.18% available P) compared to current commercial phytases (Fru *et al.*, 2012). The efficacy of this novel phytase in broilers fed low-P corn-soybean meal diets has been reported by Aureli *et al.* (2011).

The objective of the study was to determine the effect of supplementing broiler diets with the new phytase on the growth performance and carcass characteristics of broilers. In addition, the economics of using phytase in broiler production was evaluated.

## MATERIALS AND METHODS

The study was conducted from March to April, 2012 using 300 day-old broiler chicks distributed at random to 5 treatments, with 6 replications per treatment and 10 chicks per replicate, following a completely randomized design. The treatments were as follows: Treatment 1: Positive Control (PC); Treatment 2: Negative Control 1 (diet with available P, Ca and ME lower than PC by 0.15%, 0.18% and 75 kcal/kg, respectively); Treatment 3: same as treatment 2 + 100 g phytase per ton of feed; Treatment 4: Negative Control 2 (diet with available P, Ca and ME lower than PC by 0.18%, 0.23% and 95 kcal/kg, respectively); and Treatment 5: same as treatment 4 + 200 g phytase per ton of feed. The ingredient composition, calculated nutrient analysis and cost of the different diets are presented in Tables 1 to 3. The cost of the different diets was calculated based on the purchase price of the different ingredients as of March 22, 2012.

The birds were fed *ad libitum* with booster (day 1 to day 14), starter (day 15 to day 28) and finisher (day 29 to day 35) diets. Water was made available at all times. Standard management and vaccination programs in the farm were followed.

### Data collection

Weighing was done on days 1, 14, 28 and 35. The gain in weight was determined by subtracting the initial body weight from the weights obtained on d14, d28 and d35. The average feed consumption was calculated by subtracting the weights of feed left from the total amount feeds offered per period. Feed efficiency was calculated by dividing the total feed consumed by the total weight gain of the bird per period. Percent mortality was calculated by dividing the total number of dead birds from day 1 to day 35 by the total number of birds at the start of the study. At the end of the feeding trial, 60 birds were slaughtered and subjected to carcass analysis. Dressing percentage was calculated by dividing the dressed weight by the live weight multiplied by 100. The weights of the abdominal fat, breast, leg, thigh, head, neck, feet, wings, back and giblets were obtained and expressed as percentages of the dressed weight.

Table 1. Ingredient composition, calculated nutrient analysis and cost of booster diets.

Ingredient	T1 Positive Control	T2 Negative Control 1	T3 Negative Control 1 + 100g/ton phytase	T4 Negative Control 2	T5 Negative Control 2 + 200g/ton phytase
Yellow corn	53.86	57.50	57.50	57.74	57.74
US Soya Hi Pro	36.40	35.80	35.80	35.80	35.80
Rice bran D1	3.00	3.00	3.00	3.00	3.00
Powdered fat	3.40	1.33	1.33	1.32	1.32
Monodicaphos	1.62	0.79	0.79	0.60	0.60
Limestone	0.94	0.83	0.83	0.77	0.77
L-lysine	0.04	0.04	0.04	0.03	0.03
DL-methionine	0.19	0.18	0.18	0.17	0.17
Choline chloride	0.08	0.08	0.08	0.08	0.08
Toxin binder	0.10	0.10	0.10	0.10	0.10
Mineral premix	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
Phytase	0	0	0.01	0	0.02
Vitamin premix	0.03	0.03	0.03	0.03	0.03
Antioxidant	0.01	0.01	0.01	0.01	0.01
Total	100	100	100	100	100
Calculated Analysis:					
Crude protein, %	22.0	22.0	22.0	22.0	22.0
Crude fat, %	5.92	4.0	4.0	4.0	4.0
Crude fiber, %	3.10	3.18	3.18	3.18	3.18
Metabolizable energy, kcal/kg	3050	2975	2975	2960	2960
Calcium, %	0.85	0.67	0.67	0.62	0.62
Avail. P, %	0.40	0.25	0.25	0.22	0.22
Dig. lysine, %	1.12	1.11	1.11	1.11	1.11
Dig. met+cys, %	0.82	0.81	0.81	0.81	0.81
Dig. threonine, %	0.74	0.74	0.74	0.74	0.74
Dig. tryptophan, %	0.25	0.25	0.25	0.25	0.25
Cost/kg, PhP	22.91	21.94	22.09	21.90	2.20

Table 2. Ingredient composition, calculated nutrient analysis and cost of starter diets.

Ingredient	T1 Positive Control	T2 Negative Control 1	T3 Negative Control 1 + 100g/ton phytase	T4 Negative Control 2	T5 Negative Control 2 + 200g/ton phytase
Yellow corn	59.85	56.03	56.03	55.32	55.32
US Soya Hi Pro	30.40	28.70	28.70	28.10	28.10
Rice bran D1	5.35	10.00	10.00	10.00	10.00
Copra meal	0	2.54	2.54	4.21	4.21
Powdered fat	1.02	0.28	0.28	0.14	0.14
Monodicaphos	1.52	0.66	0.66	0.47	0.47
Limestone	0.97	0.88	0.88	0.83	0.83
L-lysine	0.08	0.10	0.10	0.12	0.12
DL-methionine	0.18	0.18	0.18	0.18	0.18
L-threonine	0.02	0.03	0.03	0.03	0.03
Choline chloride	0.08	0.08	0.08	0.08	0.08
Toxin binder	0.10	0.10	0.10	0.10	0.10
Mineral premix	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Phytase	0	0	0.01	0	0.02
Vitamin premix	0.03	0.03	0.03	0.03	0.03
Antioxidant	0.01	0.01	0.01	0.01	0.01
Total	100	100	100	100	100
Calculated Analysis:					
Crude protein, %	20.00	20.00	20.00	20.00	20.00
Crude fat, %	4.00	4.00	4.00	4.00	4.00
Crude fiber, %	3.13	3.42	3.42	3.42	3.42
ME ,kcal/kg	2950	2875	2875	2855	2855
Calcium, %	0.82	0.64	0.64	0.59	0.59
Avail. P, %	0.38	0.23	0.23	0.20	0.20
Dig. lysine, %	1.02	1.01	1.01	1.01	1.01
Dig. met+cys, %	0.77	0.76	0.76	0.76	0.76
Dig. threonine, %	0.69	0.68	0.68	0.68	0.68
Dig. tryptophan,%	0.22	0.22	0.22	0.22	0.22
Cost/kg, PhP	21.68	20.64	20.79	20.42	20.72

Table 3. Ingredient composition, calculated nutrient analysis and cost of finisher diets.

Ingredient	T1 Positive Control	T2 Negative Control 1	T3 Negative Control 1 + 100g/ton phytase	T4 Negative Control 2	T5 Negative Control 2 + 200g/ton phytase
Yellow corn	65.70	61.60	61.60	60.95	60.95
US Soya Hi Pro	25.50	24.00	24.00	23.30	23.30
Rice bran D1	4.60	10.00	10.00	10.00	10.00
Copra meal	0	1.90	1.90	3.60	3.60
Powdered fat	1.00	0.24	0.24	0.10	0.10
Monodicaphos	1.40	0.53	0.53	0.34	0.34
Limestone	1.03	0.94	0.94	0.89	0.89
L-lysine	0.06	0.07	0.07	0.08	0.08
DL-methionine	0.15	0.15	0.15	0.15	0.15
L-threonine	0.02	0.02	0.02	0.03	0.03
Choline chloride	0.08	0.08	0.08	0.08	0.08
Toxin binder	0.10	0.10	0.10	0.10	0.10
Mineral premix	0.08	0.08	0.08	0.08	0.08
Salt	0.30	0.30	0.30	0.30	0.30
Phytase	0	0	0.01	0	0.02
Vitamin premix	0.02	0.02	0.02	0.02	0.02
Antioxidant	0.01	0.01	0.01	0.01	0.01
Total	100	100	100	100	100
Calculated Analysis:					
Crude protein, %	18.00	18.00	18.00	18.00	18.00
Crude fat, %	4.00	4.00	4.00	4.00	4.00
Crude fiber, %	3.06	3.32	3.32	3.44	3.44
ME, kcal/kg	3000	2925	2925	2905	2905
Calcium, %	0.80	0.62	0.62	0.57	0.57
Avail. P, %	0.35	0.20	0.20	0.17	0.17
Dig. lysine, %	0.88	0.87	0.87	0.87	0.87
Dig. met+cys, %	0.69	0.68	0.68	0.68	0.68
Dig. threonine, %	0.62	0.61	0.61	0.616	0.61
Dig. tryptophan, %	0.19	0.19	0.19	0.19	0.19
Cost/kg, PhP	19.30	18.36	18.51	18.16	18.46

### **Phytase**

The phytase product used in the experiment is a 6-phytase produced by the expression of synthetic genes that have been incorporated in *Aspergillus oryzae*. The genes are synthesized based on the protein sequence of the phytase enzyme in *Citrobacter braakii*. DSM Nutritional Products Philippines Inc. provided the phytase sample used in this study.

### **Environmental condition**

During the experiment, the average daily temperature was  $27.93 \pm 1.36^{\circ}\text{C}$ , with a range of  $24.7\text{-}30.10^{\circ}\text{C}$ . Average relative humidity was  $79.31 \pm 5.29\%$  with a range of 70-93% (National Agromet Station, UPLB  $14^{\circ}11'\text{N}$ ,  $121^{\circ}15'\text{E}$ ). The heat index calculated during the experimental period was  $31.89 \pm 2.84^{\circ}\text{C}$  with a range of  $26.00\text{-}37.00^{\circ}\text{C}$ . Based on the calculated heat index, 65.7% of the trial period was considered under extreme caution category ( $32\text{-}39^{\circ}\text{C}$ ).

### **Analyses**

Data on growth performance and carcass characteristics were subjected to analysis of variance of the General Linear Model procedures of SAS software (SAS Institute, 1989). Treatment means were compared using Duncan's Multiple Range Test. Income over feed and chick cost was computed based on the prevailing prices of chicks, feeds and live weight price of broilers during the conduct of study.

## **RESULTS AND DISCUSSION**

### **Body weight and body weight gain**

The body weights of broilers at day 14 and day 28 were not significantly different among treatments (Table 4). However, phytase supplementation of the negative control diets had a tendency to improve body weight at day 14 ( $P=0.07$ ) and day 28 ( $P=0.06$ ). Body weight of broilers at day 35 was significantly improved ( $P<0.05$ ) by phytase supplementation of diets with reduced content of available P, Ca and ME to the level of the positive control diet. The same results were observed for body weight gain. The results indicate that phytase supplementation was able to uplift the available P, Ca and ME level of the negative control diets resulting in a similar growth performance as that of the positive control diet. Using the same novel phytase, Aureli *et al.* (2011), Shaw *et al.* (2011) and Rutherford *et al.* (2012) also reported significant improvement in body weight and gain of broilers fed low-P diet supplemented with phytase.

### **Feed intake and feed efficiency**

No significant differences ( $P>0.05$ ) among treatments were observed in the feed intake of broilers. This is contrary to the report of Rutherford *et al.* (2012) wherein differences were noted in the feed intake of birds given low-P diet, adequate P diet as well as those fed phytase-supplemented low-P diets. The differences in the results could be due to the high heat index that may have affected the feeding behavior of the birds. It should be noted that the heat index calculated during the experimental period was  $31.89^{\circ}\text{C} \pm 2.84$  with a range of  $26.00\text{-}37.00^{\circ}\text{C}$ .

Table 4. Growth performance and mortality of broilers fed diets with or without phytase supplementation.

Variables*	Treatments**					CV, %
	T1	T2	T3	T4	T5	
Initial weight, g	45.0	45.0	45.8	45.8	45.0	2.9
Body weight at day 14, g	365.8	360.8	384.2	344.8	374.2	6.3
Body weight at day 28, g	1,091.1	1,015.0	1,103.5	1,016.3	1,097.2	6.4
Body weight at day 35, g	1,451.4 <sup>a</sup>	1,351.2 <sup>b</sup>	1,526.6 <sup>a</sup>	1,338.2 <sup>b</sup>	1,445.1 <sup>a</sup>	5.1
Weight gain, g						
Day 1 to 14	320.8	315.8	338.3	299.0	329.2	7.3
Day 1 to 28	1,046.1	970.0	1,057.7	970.5	1,052.2	6.7
Day 1 to 35	1,406.4 <sup>a</sup>	1,306.2 <sup>b</sup>	1,480.8 <sup>a</sup>	1,292.3 <sup>b</sup>	1,400.1 <sup>a</sup>	5.3
Feed intake, g						
Day 1 to 14	478.3	476.7	493.3	461.7	480.0	9.0
Day 1 to 28	1,770.0	1,680.0	1,771.7	1,675.0	1,720.0	5.7
Day 1 to 35	2,678.3	2,568.3	2,690.0	2,508.3	2,565.0	5.6
Feed conversion ratio						
Day 1 to 14	1.48	1.50	1.46	1.55	1.45	9.8
Day 1 to 28	1.69	1.73	1.68	1.72	1.63	4.8
Day 1 to 35	1.91	1.96	1.82	1.94	1.83	5.3
Mortality, %						
Day 1 to 35	1.67	1.67	3.33	5.00	3.33	179.8

\*Means in the same row followed by different letters are different ( $P < 0.05$ , DMRT).

\*\*Treatments: T1 = Positive control (PC); T2 = Negative control 1 (available P, Ca and ME lower than PC by 0.15%, 0.18% and 75 kcal/kg, respectively); T3 = Negative control 1 + 100 g phytase/ton; T4 = Negative control 2 (available P, Ca and ME lower than PC by 0.18%, 0.23% and 95 kcal/kg, respectively); T5 = Negative control 2 + 200 g phytase/ton.

The different treatments had no significant ( $P > 0.05$ ) effect on feed efficiency of broilers. Rutherford *et al.* (2012) obtained the same results wherein there were no differences seen in the feed intake to weight gain ratio of birds fed unsupplemented and phytase-supplemented low-P diets. However, phytase supplementation of negative control diets had a tendency to improve ( $P = 0.07$ ) the overall feed efficiency

of broilers. The result indicates that in diets with reduced available P, Ca and ME, the addition of phytase increased the availability of these nutrients resulting in improvement in feed efficiency. Shaw *et al.* (2011) reported a significant improvement in feed conversion of broilers when fed low-P diet supplemented with phytase.

#### **Mortality rate**

No differences ( $P>0.05$ ) were observed in the mortality rate among treatments. While some birds died during the trial, the cause was not related to the different treatments used in the study.

#### **Dressing percentage and carcass composition**

Dressing percentage was not significantly affected by addition of either 100 g/ton or 200g/ton phytase to low phosphorus diets compared to those fed low phosphorus diets without phytase and diets with adequate phosphorus level (Table 5). Addition of phytase increased ( $P<0.05$ ) the abdominal fat weight of those given low phosphorus diets with added phytase (100 g/ton) compared to those without phytase, but was similar to that of the positive control. This may be due to the freed-up phytate-bound nutrients such as proteins, carbohydrates and lipids (Dilger *et al.*, 2004) that could have led to excess energy and eventually stored in the abdominal area as fat. Among the prime cuts, no significant differences ( $P>0.05$ ) were observed between the breast weight of those broilers given treatment diets but differences ( $P<0.05$ ) were noted in the leg and thigh weights; however, no trend was observed. The weight of back, wings, head, feet, neck, giblets were similar ( $P>0.05$ ) among treatments.

#### **Income over feed and chick cost**

The income over feed and chick cost (IOFCC) was highest in Treatment 3 followed by Treatment 5 (Table 6). As expected, the lowest IOFCC was observed in Treatments 2 and 4 due to poor growth performance of broilers as a result of low levels of available P, Ca and ME in these negative control diets. The higher IOFCC of broilers fed diets supplemented with phytase is due to the lower feed cost and better feed efficiency compared to the positive control diet. The result indicates that addition of the new phytase in broiler diets with reduced specification for available P, Ca and ME can lower feed cost without adverse effect on growth and feed efficiency resulting in higher economic return.

Based on the results of this study, growth performance of broilers fed diets with lower specification in available P, Ca and ME is not adversely affected if phytase is added in the diet.

### **ACKNOWLEDGEMENT**

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Table 5. Dressing percentage and carcass composition of broilers fed the diets with or without phytase supplementation.

Variables**	Treatments**					CV, %
	T1	T2	T3	T4	T5	
Dressing percentage, %	74.35	75.89	76.00	71.62	74.20	5.2
Abdominal fat, %	0.88 <sup>ab</sup>	0.52 <sup>c</sup>	1.01 <sup>a</sup>	0.64 <sup>bc</sup>	0.77 <sup>abc</sup>	50.6
Breast, %	32.41	32.01	31.90	35.04	32.26	9.7
Thigh, %	14.46 <sup>ab</sup>	14.12 <sup>b</sup>	13.64 <sup>b</sup>	15.41 <sup>a</sup>	14.21 <sup>b</sup>	8.8
Neck, %	6.31	6.32	6.01	6.40	6.72	12.0
Giblets, %	5.27	4.91	4.77	5.12	5.34	12.1
Leg, %	13.49 <sup>ab</sup>	13.09 <sup>bc</sup>	12.68 <sup>c</sup>	13.89 <sup>a</sup>	14.10 <sup>a</sup>	5.7
Back, %	19.30	18.62	18.86	18.66	18.75	8.9
Wings, %	12.05	11.78	11.49	12.08	12.01	7.9
Head, %	3.52	3.67	3.54	3.87	3.73	13.5
Feet, %	5.71	5.62	5.36	5.79	5.62	12.3

\*Means in the same row followed by different letters are different ( $P < 0.05$ , DMRT).

\*\*Treatments: T1 = Positive control (PC); T2 = Negative control 1 (available P, Ca and ME lower than PC by 0.15%, 0.18% and 75 kcal/kg, respectively); T3 = Negative control 1 + 100 g phytase/ton; T4 = Negative control 2 (available P, Ca and ME lower than PC by 0.18%, 0.23% and 95 kcal/kg, respectively); T5 = Negative control 2 + 200 g phytase/ton.

## REFERENCES

- Aureli R, Umar-Faruk M, Cechova I, Pedersen PB, Elvig-Joergensen SG, Fru F and Broz J. 2011. The efficacy of a novel microbial 6-phytase expressed in *Aspergillus oryzae* on the performance and phosphorus utilization in broiler chickens. *Int J Poult Sci* 10 (2): 160-168.
- Cowieson AJ and Adeola O. 2005. Carbohydrases, proteases and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chickens. *Poult Sci* 84: 1860-1867.
- Dilger RN, Onyango EM, Sands JS and Adeola O. 2004. Evaluation of microbial phytase in broiler diets. *Poult Sci* 83: 962-970.
- Fru F, Wilson JW and Glitsoe V. 2012. Choosing the right phytase for your application. *Proc Poultry Feed Quality Conf.* Bangkok, Thailand. July 9-10, 2012.

Table 6. Income over feed and chick cost.

Variables	Treatments***				
	T1	T2	T3	T4	T5
Total feed cost/bird, PhP*	56.72	51.42	54.60	50.04	52.64
Chick cost, PhP	20.00	20.00	20.00	20.00	20.00
Feed and chick cost, PhP	76.72	71.42	74.60	70.04	72.64
Final weight, g	1451.39	1351.16	1526.62	1338.16	1445.14
Sale**, PhP	123.37	114.85	129.76	113.74	122.84
Income over feed and chick cost, PhP	46.65	43.43	55.16	43.71	50.20

\*Computed based on the purchased price of ingredients as of March 22, 2012.

\*\*Price per kg live weight Php 85.00.

\*\*\*Treatments: T1 = Positive control (PC); T2 = Negative control 1 (available P, Ca and ME lower than PC by 0.15%, 0.18% and 75 kcal/kg, respectively); T3 = Negative control 1 + 100 g phytase/ton; T4 = Negative control 2 (available P, Ca and ME lower than PC by 0.18%, 0.23% and 95 kcal/kg, respectively); T5= Negative control 2 + 200 g phytase/ton.

Ravindran V, Cowieson AJ and Selle PH. 2008. Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization, and excreta quality of broiler chickens. *Poult Sci* 87: 677-688.

Rutherford SM, Chung TK, Morel PCH and Moughan PJ. 2004. Effect of microbial phytase on the ileal digestibility of phytate phosphorus, total phosphorus and amino acids in a low phosphorus diet for broilers. *Poult Sci* 83: 61-68.

Rutherford SM, Chung TK, Thomas DV, Zou ML and Moughan PJ. 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. *Poult Sci* 91: 1118-1127.

Saima MZ, Khan U, Jabbar MA, Ijaz M and Qadeer MA. 2009. Efficacy of microbial phytase at different levels on growth performance and mineral availability in broiler chickens. *J Anim Plant Sci* 19: 58-62.

SAS Institute. 1989. *SAS User's Guide: Statistics*. (4<sup>th</sup> ed). Cary, North Carolina: SAS Institute Inc.

Shaw AL, Hess JB, Blake JP and Ward NE. 2011. Assessment of an experimental phytase enzyme product on live performance, bone mineralization and phosphorus excretion in broiler chickens. *J Appl Poult Res* 20 (4): 561-566.

Wodzinski RJ and Ullah AHJ. 1996. Phytase. *Adv Appl Microbiol* 42: 263-303.