Journal of Society & Technology 4:183-192 (2014)

Nutrient Utilization of Different Strains of Broilers Fed with Varying Levels of Phytase-Supplemented Diets

Teofilo S.Cerera* & Aida N. Cerera
Naval State University-Biliran, Biliran, Philippines

Abstract

A feeding trial was conducted to study the effect of microbial phytase on the production performance and energy, protein, calcium and phosphorus utilization in the three broiler strains (Starbro, Arbor Acre-70 and Lohmann). Phytase was incorporated in the broiler diet at the recommended rate of 0.020% in T_2 diet, 0.015% in T_1 diet and 0.025% in T3 diet. Data were analyzed using the analysis of variance (ANOVA) in a randomized complete block design (RCBD). The least significant difference (LSD) test was used to compare treatment means. Consistently, Arbor Acre-70 fed with T3 diet consumed more feed (2651.00 g), significantly obtained the highest body weight (1313.33 g), body weight gain (1271.67 g) and average daily gain (36.33 g). The feed conversion of the birds in the different treatments was not significantly different. However, Arbor Acre-70 on T_3 diet showed the lowest feed conversion ratio (2.10) which indicates that this strain is a better feed converter. Among the strains, Arbor Acre-70 on T_3 diet had the highest metabolizable energy availability (89.08%) and the highest percentage of available calcium for utilization (90.00%). The utilizable protein of Starbro on T_2 diet (70.37%) and Arbor Acre-70 fed with T_3 diet (70.34%) was comparable. The percentages of phosphorus utilized by Arbor Acre-70, Starbro and Lohmann on T_2 diet were 68.03, 59.18 and 58.50%, respectively. Raising Arbor Acre-70 broilers using a diet supplemented with 0.025% phytase is very profitable. Supplementation of broiler diets with phytase improved production performance and nutrient utilization of the birds.

Keywords: : phytase, phytate, broiler strains, treatment diets, non-ruminant animals

Introduction

If broilers are expected to remain healthy and productive, they must consume adequate amounts of all the necessary nutrients. They convert feeds into food products quickly and efficiently; their high rate of productivity results in relatively high nutrient needs such as energy, protein, calcium and phosphorus, among many others.

Since feeds mainly contain ingredients from plants, particularly cereal grains and their by-products which are not good sources of phosphorus for non-ruminant animals, nutritional problems may arise because these plant feed materials contain phytic acids or their salts known as phytates. Phytates are associated with a number of anti-nutritional effects, largely because they can chelate divalent cations such as Ca, Mg, Fe, Zn, Cu and Mn and can reduce protein availability (Ravindran et al., 2006; Liu et al., 1998; and Bedford and Schulze, 1998).

Furthermore, interactions between protein/phytate and starch/phytate obstruct the digestion of protein and carbohydrates because phytate tends to bind with enzymes produced by the animals and thus, limit their full activity. The higher the phytate content, the lower is the performance of the animal.

The proportion of phytate phosphorus varies from 60% to 90% in these plant materials (BASF 2000). The ability of poultry and pigs to use phytate phosphorus is poor (Ravindran et al., 2006; Wu et al., 2003; NRC, 1994) due to either the animals do not have the enzyme to break the phosphorus bond on the ring, or their enzyme cannot release phosphorus from phytate. This means that up to 90% of the phosphorus in feed ingredients cannot be used by the animals and is excreted as manure; thus, pollution with phosphorus and nitrogen from manure is becoming man's major problem.

*Correspondence: tcerera@gmail.com

Hence, the more important concern of the human population as a whole is the negative effect of phosphorus on the environment. This phenomenon is due to intensification of animal production which is considered a potential source of air pollution and a threat to soil and drinking water quality. Phosphorus is a limiting nutrient in algal growth, and the phosphorus in poultry manure can stimulate algal blooms that deplete dissolved oxygen in surface water. For this reason, phytase, an enzyme that catalyses the stepwise removal of phosphate from phytate, making phosphorus and other nutrients available to animals, is of considerable interest for biotechnological and environmental concerns.

While the use of phytase in broiler feed has been done especially in other countries, where phosphorus pollution is a limitation to animal production, information on the effect of phytase enzyme in broiler diets in our country, particularly in the province of Biliran, is not available.

This work, which determines the influence of phytase on the performance of broilers and establishes the required level considered beneficial to nutrient utilization is a big help to the intensive poultry and swine operations in the province of Biliran by way of generating reasonable income since the use of phytase in diets enhances animal performance; and more importantly improves environmental quality (Gill,2008).

Materials and Methods

Stock Procurement and Preparation of House and Facilities. One hundred eighty day-old broiler chicks consisting of three strains were ordered from a single supplier to ensure age uniformity. A house with 36 cages and with a capacity of five birds per cage was prepared, including all the facilities needed therein such as drinkers, feeders, and litter materials. These cages served as the brooders and birds were randomly distributed to each brooder. Since there were only five birds per cage, light was shared among experimental cages for maximum distribution and utilization of warmth.

Preparation of the Experimental Diets. Two kinds of feeds were used – the chick booster mash and the broiler starter mash. A reduced protein amino acid-balanced diet was formulated with respective levels of phytase supplementation in every treatment. At the inclusion rate of 0.020% in T2 per manufacturer's recommendation, phytase enzyme was reduced by 0.005% in T1 (0.015%) and increased by the same level in T3 (0.025%).

All feeds used in this study were formulated and mixed at the University Feedmill, Department of Animal Science, Visayas State University, Visca, Baybay, Leyte.

Tables 1 and 2 present the feeds formulated and used in this study.

Experimental Design

A total of 180 broiler chicks of three different strains were used in this study, with five birds per cage as an experimental unit. Four treatment diets were randomly distributed to 36 cages following the 3 x 4 factorial experiment in a randomized complete block design (RCBD) replicated three times (Figure 1).

The three broiler strains and the treatment diets with varying levels of phytase are shown below:

Strains	Treatment Diets
S_1 – Starbro	T_0 (Control) – no phytase
S_2 – Arbor-Acre	T_1 – 0.015% phytase
S_3 – Lohmann	T_2 – 0.020% phytase
	T_3 – 0.025% phytase

Collection of Data

Collection of data started immediately during brooding until the study ended. The study consisted of two parts. The first part was carried out until 35 days of age in order to

REP. I

S_2T_3	S_1T_1
S_3T_2	S_1T_0
S_3T_0	S_3T_3
S_1T_3	S_3T_1
S_2T_1	S_1T_2
S_2T_2	S_2T_0

REP. II

S_1T_3	S_3T_3
S_2T_3	S_3T_2
S_2T_2	S_2T_1
S_3T_0	S_1T_2
S_2T_0	S_1T_1
S_1T_0	S_3T_1

REP. III

S_3T_3	S_2T_1
S_3T_0	S_2T_2
S_1T_1	S_1T_2
S_1T_3	S_3T_1
S_3T_2	S_2T_0
S_2T_3	S_1T_0

Figure 1: The cage layout of a 3x4 factorial experiment involving three broiler strains (S_1, S_2, and S_3) and four levels of phytase ($\mathbf{T}_0$ $\mathbf{T}_1$ $\mathbf{T}_2$ and $\mathbf{T}_3$) treatment arranged in a randomized complete block design (RCBD) with three replications.

determine the effects of phytase supplementation on the production performance of the birds such as feed intake, growth rate, and feed conversion efficiency. The profitability of this study was determined in terms of return on investment (ROI) which was calculated per treatment. The determination of metabolizable energy, protein, calcium and phosphorus of broilers using the Sibbald TME procedure constituted the second part of the study. Right after the last feeding day, one bird per treatment combination in all replications (or a total of 36 birds), was randomly selected and confined in individual cages to collect feces. A fecal sample from one bird representing each strain and enzyme level was adequate for fecal analysis.

To collect excreta, the experimental and control birds were separately confined in cages with plastic acetate underneath. Feces were collected the following day. From this sample, the excreta was dried in a forced-draft oven, weighed and brought to the Nutrition Laboratory, Department of Animal Science, Visayas State University (VSU), Baybay, Leyte to determine its metabolizable energy. Analysis of metabolizable protein, calcium and phosphorus and the nutrients in feeds was done at PhilRootcrops, also in VSU.

Analysis of Data

Data were analyzed using the analysis of variance (ANOVA) in a randomized complete block design. The least significant difference test (LSD) was used to compare treatment means. All statistical analyses were done through a computer using Statistical Package for Social Sciences (SPSS).

Results and Discussion

Food intake

As shown in Table 3, the feed intake of the three broiler strains showed highly significant differences with Arbor Acre-70 consuming more feeds (2572.71 g) than Starbro (2318.58 g) and Lohmann (2274.32 g). Feed intake was likewise affected by enzyme level. Feed intake of the birds on T_3 diet was significantly the highest (2525.79 g) although not statistically different from those in the control diet (2395.47 g). This suggests that T_3 diet without inorganic phosphorus (MCDP) produced the same effect as the control (T_0) diet with MCDP.

Table 1: *Composition of experimental chick booster diet (21% CP)*

	Treatments			
Ingredients	**T_0**	**T_1**	**T_2**	**T_3**
Yellow corn	44.8	44.8	44.8	44.8
Rice bran (D_1)	10	10.7	10.7	10.7
Copra meal	10	10	10	10
Fish meal (Peruvian)	3	3	3	3
Soybean meal	27.04	27.08	27.08	27.08
Monocalcium diphosphate	1.32	0	0	0
Salt	0.5	0.05	0.05	0.05
Vit. Min. premix	0.5	0.5	0.5	0.5
Limestone	0.84	1.4	1.4	1.4
Molasses	2	2	2	1.995
Phytase	0	0.015	0.02	0.025
Total	**100**	**100**	**100**	**100**
Nutrients				
CP, %	21	21	21	21
ME (kcal/kg)	2816	2816	2816	2816
Ca, %	0.9	0.9	0.9	0.9
Available P, %	0.55	0.55	0.55	0.55
Lysine, %	1.9	1.9	1.9	1.9
Methionine, %	0.34	0.34	0.34	0.34
Feed cost/kg (P)	**16.52**	**16.58**	**16.59**	**16.6**

The combined effect of strain and enzyme level revealed that Arbor Acre-70 on T_3 diet consumed the greatest amount of feed (2651.00 g). Starbro fed with T_2 diet had the lowest feed intake (2065.27 g) (Table 4). This finding agrees with that of De Carmo Maria et al (1998) which claims that daily feed intake significantly increases by the addition of phytase to the diet. Increased feed intake through phytase supplementation was also reported by Broz et al (1994), Schoner et al (1993) and Sebastian et al (1996).

It was observed that when the amount of phytase was increased, feed intake was also increased. This may be the optimum level (T_3) where the enzyme can use the available form of phosphorus more efficiently and release the bound materials making it available for intestinal absorption so that more feeds are needed to meet the demand for the body's active metabolic processes.

Body weight of the Birds

Throughout the duration of the study, Arbor Acre-70 consistently had significantly heavier body (1212.08 g) than Starbro (1077.92 g) and Lohmann (1050.83 g). Similarly, body weight of the birds was affected by enzyme level. The growth of the birds on T_3 diet (1203.33 g) was significantly higher than the birds in T_1 (1050.83 g) and T_2 diets (1082.78 g) but not those on the control diet (1117.50 g). (Table 3).

The combined effect of strain and enzyme level revealed that final body weight of Arbor Acre-70 on T_3 diet was significantly the heaviest among treatments with an average weight of 1313.33 g and the lowest was Starbro fed with T_2 diet (910.00 g). (Table 4). This confirms the results of Petersen and Chung (2000) that when phytase is used in broilers, their growth performance can be

Table 2: *Composition of experimental chick booster diet (21% CP)*

Ingredients	Treatments			
	T_0	**T_1**	**T_2**	**T_3**
Yellow corn	44.3	44.3	44.3	44.3
Rice bran (D1)	11.12	11.88	11.88	11.88
Soybean meal	25.18	25.18	25.18	25.18
Copra meal	10	10	10	10
Fish meal (Peruvian)	3	3	3	3
Monocalcium diphosphate	1.3	0	0	0
Salt	0.5	0.5	0.5	0.5
Vit. Min. premix	0.5	0.5	0.5	0.5
Limestone	2.1	2.62	2.62	2.62
Molasses	2	2	2	2
Phytase	0	0.015	0.02	0.025
Total	**100**	**100**	**100**	**100**
Nutrients				
CP, %	19	19	19	19
ME (kcal/kg)	2829	2829	2829	2829
Ca, %	0.9	0.9	0.9	0.9
Available P, %	0.55	0.55	0.55	0.55
Lysine, %	1.9	1.9	1.9	1.9
Methionine, %	0.34	0.34	0.34	0.34
Feed cost/kg (P)	**16.47**	**16.23**	**16.3**	**16.33**

maintained even when inorganic phosphorus is dramatically reduced. Likewise, Broz et al (1994), Schoner et al (1993) and Sebastian et al (1996) corroborate this view that phytase supplementation increased body weight of broilers.

Body weight gain of the Birds

Arbor Acre-70 had significantly higher weight gain (1170.00 g) than Starbro (1033.75 g) and Lohmann (1009.58 g). Likewise, the enzyme level had an influence on the body weight gain of the birds. Birds on T_3 diet exhibited the highest weight gain (1160.00 g) which was significantly higher than the other treatments, except that the T_0 (1075.28 g) and T_3 (1160.00 g) values were statistically the same (Table 3).

The combined effect of strain and enzyme level showed that the weight gain of Arbor Acre-70 on T_3 diet (1271.67 g) was significantly higher than that on T_1 diet (1065.00 g) (Table 4) These findings concur with those of Chung (2000) that weight gain of broilers fed with microbial phytase-supplemented diet is comparable to that of those given the control diet containing normal levels of calcium and available phosphorus. However, Oh et al. (1998) reported no significant differences in body weight gain among chicks fed with kenzyme, phytase, yeast and KPY.

Average Daily Gain (ADG) of the Birds

The three broiler strains showed highly significant differences in ADG with Arbor Acre-70 obtaining the highest ADG of 33.43 g while Starbro (29.46 g) and Lohmann (28.84 g) got statistically comparable values. On the

Table 3: *Production performance of the birds grown for five weeks as affected by strain and enzyme level**

Variable	Parameter				
	Feed Intake (g)	**Body Weight (g)**	**Body Weight Gain (g)**	**ADG (g)**	**FCR**
Broiler Strain					
S_1 (Starbro)	2318.58b	1077.92b	1033.75b	29.46b	2.26
S_2 (Arbor Acre-70)	2572.71a	1212.08a	1170.00a	33.43a	2.21
S_3 (Lohmann)	2274.32b	1050.83b	1009.58b	28.84b	2.26
Enzyme Level					
T_0 (Control, 0%)	2395.47ab	1117.50ab	1075.28ab	30.72ab	2.24
T_1 (0.015%)	2296.89b	1050.83b	1006.39b	28.75b	2.29
T_2 (0.020%)	2335.46b	1082.78b	1039.44b	29.70b	2.26
T_3 (0.025%)	2525.79a	1203.33a	1160.00a	33.14a	2.19
CV (%)	**6.44**	**8.14**	**8.32**	**8.55**	**4.81**

*Within a factor, means in the same column with or without the same letter superscript are not significantly different at 5% level of probability.

other hand, the effect of enzyme level on ADG was noted. The birds on T_3 diet (33.14 g) had significantly higher ADG than those on T_1 (28.75 g) and T_2 diets (29.70 g). (Table 3).

Similarly, the combined effects of strain and enzyme level on ADG were found to be significant. Arbor Acre-70 on T_3 diet obtained higher ADG (36.33 g) than the rest of the birds with this strain fed with other treatment diets (Table 4). This observation contradicts the claim of Gervacio (2001) that inclusion of phytase at the manufacturer's recommended level (0.020%) did not significantly increase average daily gain.

Feed Conversion Ratio (FCR) of the Birds

Analysis of variance indicated that feed conversion ratio of the birds was not influenced by strain and enzyme level. However, the FCR of Arbor Acre-70 (2.21) was lower than that of Starbro and Lohmann both of which obtained an FCR value of 2.26. On the other hand, birds fed with T3 diet had the lowest FCR value (2.19) compared with the rest of the treatment diets (Table 3).

The FCR values of the three strains in all treatment diets are statistically similar. However, Arbor Acre-70 on T3 diet is numerically the most efficient feed converter since it required the least amount of feed (2.10) to produce a kilogram of live body weight (Table 4).

Metabolizable Energy (ME)

Table 5 shows the metabolizable energy of the diets fed to broilers. Phytase supplementation in broiler diets resulted in improved energy metabolism with increase in ME observed among three strains. Starbro and Arbor Acre-70 fed with T_3 diet had an increase in metabolizable energy of 2.8 and 6.03%, respectively while Lohmann on T_1 diet had 6.30%. However, Arbor Acre-70 on $T_(3)$ diet gave the highest ME availability of 89.08%. The lowest was noted in Lohmann on the control diet (79.33%.

This result is confirmed by Chung (2000) who stressed that microbial phytase-supplemented diet produces higher metabolizable energy than the control diet

Table 4: *Interaction effect of strain and enzyme level on the production performance of the birds for five weeks**

Broiler Strain/ *Phytase Level*	**Parameter**				
	Feed Intake (g)	**BodyWeight (g)**	**Body Weight Gain (g)**	**ADG (g)**	**FCR**
S_1 (Starbro)					
T_0	2466.17^a	1190.83^a	1145.83^a	32.74^a	2.15
T_1	2171.86^b	1025.00^b	973.33^b	27.81^b	2.25
T_2	2065.27^b	910.00^b	865.00^b	24.71^b	2.39
T_3	2571.00^a	1185.83^a	1140.83^a	32.59^a	2.26
S_1 (Arbor Acre-70)					
T_0	2630.07	1223.33^{ab}	1181.67^{ab}	33.75^{ab}	2.23
T_1	2458.67	1106.67^b	1065.00^b	30.43^b	2.31
T_2	2551.12	1205.00^{ab}	1161.67^{ab}	33.19^{ab}	2.2
T_3	2651	1313.33^a	1271.67^a	36.33^a	2.1
S_3 (Lohmann)					
T_0	2090.17^b	938.33^b	898.33^b	25.67^b	2.34
T_1	2261.75^{ab}	1020.83^{ab}	980.83^{ab}	28.02^{ab}	2.31
T_2	2390.00^a	1133.33^a	1091.67^a	31.19^a	2.19
T_3	2355.38^a	1110.83^a	1067.50^a	30.50^a	2.22
CV (%)	**6.44**	**8.14**	**8.32**	**8.55**	**4.81**

*Within the same strain, means in the same column without or with the same letter superscript are not significantly different at 5% level of probability by LSD.

with an improvement of 2.30%. The improved ME availability in the diet maybe attributed to the presence of phytase which releases phytate-bound starch, minerals and proteins and makes them available for utilization by the birds.

Metabolize Crude Protein (MCP)

The addition of microbial phytase improved the digestibility of proteins in the experimental diets. Starbro fed with T_2 diet had metabolizable crude protein of 70.37% with an increase of 8.92%. The metabolizable crude protein of Arbor Acre-70 and Lohmann, which were both fed with T_3 diet were 70.34 and 67.37% with an increase of 11.14 and 4.87%, respectively. Arbor Acre-70 had the highest increase in metabolizable crude protein (Table 5). This suggests that improvement in protein availability maybe due to the breakdown of phytate-protein complexes by microbial phytase. This observation supports the claim of Chung (2000) that the digestibility of protein and amino acids in plant feed ingredients is improved by the presence of microbial phytase.

Calcium Utilization

Table 5 shows that irrespective of strain, birds fed with the control diet had lesser percentage of utilizable calcium than those receiving the phytase-supplemented diets.The amounts were 47.92% (Arbor Acre-70), 71.53% (Lohmann)and 72.22% (Starbro).On the other hand, birds fed with microbial phytase-supplemented diets had improved percentage of available calcium for body

Table 5: *Interaction effect of strain and enzyme level on the production performance of the birds for five weeks**

Broiler Strain/ *Phytase Level*	**Parameter**			
	Metabolizable Energy (ME), %	**Metabolizable Crude Protein (MCP), %**	**Utilizable Calcium, %**	**Utilizable Phosphorus, %**
S_1 (Starbro)				
T_0	82.67	61.45	72.22	57.75
T_1	81.49	53.91	72.00	50.35
T_2	83.55	70.37	86.74	59.18
T_3	85.47	57.20	57.33	58.50
S_1 (Arbor Acre-70)				
T_0	83.05	59.20	47.92	63.64
T_1	84.69	62.83	50.28	48.23
T_2	84.86	60.92	88.95	68.03
T_3	89.08	70.34	90.00	63.47
S_3 (Lohmann)				
T_0	79.33	62.50	71.53	50.26
T_1	85.63	61.96	60.57	48.23
T_2	84.60	65.37	75.14	58.50
T_3	84.16	67.37	53.07	54.15

Table 6: *Average profit and return on investment (ROI) of the birds fed diets supplemented with varying levels of phytase enzyme*

Treatment	**Ave. Weight (kg)**	**Gross Sales (PhP)***	**Expenses (PhP)**	**Profit (PhP)**	**ROI (%)**
S_1 (Starbro)					
T_0	1.191	80.99	65.4	15.59	23.84
T_1	1.025	69.7	60	9.7	16.17
T_2	0.91	61.88	58.44	3.44	5.89
T_3	1.185	80.58	66.76	13.82	20.7
S_2 (Arbor Acre-70)					
T_0	1.223	83.16	67.1	16.06	23.93
T_1	1.107	75.28	63.68	11.6	18.22
T_2	1.205	81.94	65.36	16.58	25.37
T_3	1.313	89.28	67.07	22.21	33.11
S_3 (Lohmann)					
T_0	0.938	63.43	59.2	4.58	7.74
T_1	1.021	69.43	61.49	7.94	12.91
T_2	1.133	77.04	63.74	13.3	20.87
T_3	1.111	75.55	63.24	12.31	19.46

*The bird was priced at PhP 68.00/kg liveweight utilization with Arbor Acre-70 on T_3 diet having the highest value (90.00%) followed by Starbro on T_2 diet (86.74%). The lowest was noted in Lohmann fed with T_2 diet (75.14%).

This observation clearly indicates that the inclusion of phytase in the feed ration of broiler at either 0.020% or 0.025% level minimizes calcium wastage via fecal excretion. Supporting this view, Lyson (1999) said that the excretion of calcium, phosphorus, zinc and nitrogen can be reduced significantly when diets are properly formulated using phytase.

Phosphorus Utilization

Birds on phytase-supplemented diets showed better or improved utilization than those fed with the control diet. In Arbor Acre-70 and Starbro, improved phosphorus digestibility was observed in T_2 diet with utilizable phosphorus of 68.03% and 59.18%, respectively as against Lohmann on the same diet with only 58.50% (Table 5).

This observation suggests that feed ration containing 0.020% phytase (T_2) is already adequate to get optimum phosphorus utilization. This finding confirms the report of NRC (1994) that phytase-supplemented treatments showed greater reduction in phosphorus excretion than the control diet without phytase supplementation, and improved the availability of phosphorus in feedstuffs of plant origin (Weremko et.al,1997).

Economic Returns

The most important yardstick in measuring project performance is profit which is indicated by the return on investment (ROI). ROI is a measure of profitability relative to the capital invested in the project.

Among the strains of broiler and treatment diets evaluated, Arbor Acre-70 on T_3 diet (S_2T_3) obtained the highest average profit of P22.21, while the least profitable was Starbro on T_2 diet (P3.44). Likewise, among the strains fed with control diet (T_0), Arbor Acre-70 obtained the highest profit of P16.06 while the least profitable was Lohmann (P4.58.

When strains and treatment diets were considered in the determination of profit,Arbor Acre-70 on T_3 diet obtained the highest ROI of 33.11% which simply means that every peso invested in the study gives a net profit of 33 centavos. Hence, it implies that the project is feasible (Table 6).

Conclusion

Supplementation of broiler diets with phytase improved growth performance and nutrient utilization. On the strength of these findings, the following conclusions are drawn:

1.The growth performance and response to phytase of broilers vary with strain.

2.Incorporation of 0.025% microbial phytase enhances feed intake, increases body weight and percentage of energy and protein metabolized in broilers.

3.Supplementation of 0.020% microbial phytase increases percentage of calcium and phosphorus availability.

4.Birds with faster growth rate have lower feed conversion ratio (higher feed efficiency).

5.High economic benefits are obtained with the supplementation of 0.025% phytase in broiler feed.

References

BASF (2000). Phosphate a problem posed by nature. *Feed International*, 21(9):28.

Bedford, M. R.& Schulze, H. (1998). Exogenous enzymes for pigs and poultry. *Nutr. Res. Rev.*, 11:91–114.

Broz, J., Oldale, A., Volts, P., Rychen, G., and Schulze, J. & Nunes, C. (1994). Effect of supplemental phytase on performance and phosphorus utilization in broiler chickens fed at low phosphorus diet without addition of inorganic phosphate. *Br. Poult. Sci*, 35:273–280.

Chung, T. (2000). Exploring other benefits of supplementing broiler diets with microbial phytase. *Feed Focus*, 100. Roche Vitamins Asia Pacific Pte Ltd.92.

De Carmo Maria, M., Ludke, M., and Lopez, J. & De Brum, P. (1998). Effect of phytase with or without the addition of l-lysine on the digestibility of nitrogen, phosphorus and calcium in the diets for pigs. In *Proc. 8th World Conference on Animal Production*, volume 1, page 692, Seoul National University,Seoul, Korea.

Gervacio, N. (2001). The effect of phytase supplementation on the growth performance and phosphorus and protein utilization in broilers. Unpublished MS Thesis.LSU, Visca, Baybay, Leyte,22-23.

Gill, C. (2008). Reducing phytase excretion. *Feed Inter*, 2(9):18.

Liu, B. L., Rafiq, A., and Tzeng, Y.M. & Rob, A. (1998). The induction and characterization of phytase and beyond. *Enzyme Microbial Technology*, 22:415–424.

Lyson, J. (1999). Biotechnology in the feed industry. In *Proc. of Altech 15th Annual Symposium*, pages 461–480.

NRC (1994). Nutrient requirement of poultry.national academy press, washington dc. *National Research Council*, 9^{th} re.ed.:28.

Oh, H., Piao, X., Han, I., and Kim, J. L. C. (1998). Effect of kenzyme, phytase, and yeast supplementation on the growth performance and pollution reduction of broiler chicks. In *Proc. 8th World Conference on Animal Production, June 28-July 4, 1998*, Seoul National University, Seoul, Korea.

Petersen, S.T. & Chung, T. (1998). The new phytase, Ronozyme. *P. Feed Focus, Roche Vitamins Asia Pacific Pte Ltd.*, Q400:852.

Ravindran, V. and Bryden, W. L. & Kornegay, E. (2006). Phytates: Occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev*, 6:125–143.

Schoner, B., Hoppe, P., and Schwarz, G. & Wieshe, H. (1993). Effect of microbial phytase and inorganic phosphate in broiler chickens: performance and mineral retention at various calcium levels. *J. An. Physiol. An. Nutr.*, 69:235–244.

Sebastian, S., Touchburn, S., and Cleaver, E.R. & Lague, P. (1996). The effect of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed corn-based diets. *Poult. Sci.*, 75:72–736.

Weremko, D., Fandrejewski, H., Zebroska, T., Han, N. K., and Kim, J.H. & Cho, W. (1997). Bioavailability of phosphorus in feces of plant origin for pigs. *AHAS*, 10(6):551–566.

Wu, Y. B., P. J. and Hendricks, W.H., . R. V. (2003). Comparison of in vitro nutrient release by three enzyme preparations in wheat-and maize-based diets. *Proc. Aust. Poult. Sci. Symposium*, 15:114–118.