



## ORIGINAL RESEARCH ARTICLE

### Egg quality and laying performance of pullets fed different protein sources and Ronozyme-P supplementation

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#### ABSTRACT

A study was designed to assess the laying performance of pullets in response to different protein sources and phytase supplementation. One hundred and twenty Nera-Brown pullets of 26 weeks (8 weeks in lay) were randomly allotted to five experimental diets replicated four times with six birds per replicate in a completely randomized design. Five diets were formulated such that diet 1 contained protein sources of both plant and animal origin, Diets 2 and 3 had groundnut cake as protein source, but diet 3 had phytase supplementation, diets 4 and 5 had soyabean meal as protein source, but diet 5 had phytase supplementation. The birds were fed experimental diets for 36 weeks during which laying performance, egg internal and external quality traits were assessed. The result obtained showed that hen day production of birds on diet 3 recorded highest ( $P < 0.05$ ) value ( $66.35 \pm 0.44\%$ ) and highest egg weight was obtained for birds on diets 1 and 3. The internal egg quality characteristics of laying birds were not affected by the experimental diets except Haugh units. This study reveals that phytase inclusion in laying pullets diets enhance egg quality. However, birds fed groundnut cake diets with phytase laid heavier eggs and better egg production.

**Keywords:** Egg laying, Phosphorus Utilisation, Protein Sources, Ronozyme P.

#### INTRODUCTION

Phytate exists widely in feed ingredients and is poorly degradable in the gastro intestinal tract of poultry because of the lack of endogenous phytase. Its presence in poultry feed ingredients restricts the effective use of organic phosphorus and other nutrients including calcium, energy and amino acids in the alimentary tract (Chenyan, 1980; Ravindran *et al.*, 1995). This is due to the chelation of Ca (Vohra *et al.*, 1965; Oberleas, 1973; Cheryan, 1980) and amino acids (DeRham and Jast, 1979) by phytate. This antinutritional effect was demonstrated in broilers, in which the digestibility of energy and amino acids declined with an increase in dietary phytate (Ravindran *et al.*, 2006). Although it has been well documented that phytase hydrolyzes phytate and increases the digestion of phosphorus. Consequently reducing the excretion of phosphorus and lowering environmental pollution. Recently, several studies have demonstrated that the application of phytase to conventional diets improves the

digestion of energy and amino acids in broilers (Rutherford *et al.*, 2004; Onyango *et al.*, 2005; Cowieson *et al.*, 2006b). Based on a large amount of data, the matrix value of phytase has been developed to optimize profits in the broiler production system (Shelton *et al.*, 2004, Patridge, 2006; Selle *et al.*, 2006).

The efficacy of phytase on performance and phosphorus digestibility in layers fed a corn and soyabean based diet has been well established by Vanderklis *et al.* (1997), Lim *et al.*, (2003), Panda *et al.* (2005), and Wu *et al.* (2006). Jalal *et al.* (1999) reported that supplementation of phytase in corn and soyabean meal diets for layers resulted in significant improvements in the digestibility of Methionine, cystine, Alanine, and Glutamine. This research aimed at assessing the effect of phytase on the utilization of nutrients on laying and egg quality characteristics of laying hen.

Table 1: Composition of experimental diets (g/100g) for Laying birds

Ingredients	Dietary treatments				
	1	2	3	4	5
		Phytase inclusion			
		–	+	–	+
Maize	49.00	49.00	49.00	49.00	49.00
Groundnutcake	8.00	20.00	20.00	0.00	0.00
Soyabean	12.00	0.00	0.00	20.00	20.00
Wheat offal	18.00	20.00	20.00	20.00	20.00
Fishmeal (72%)	2.00	0.00	0.00	0.00	0.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Oystershell	7.00	7.00	7.00	7.00	7.00
Nacl	0.25	0.25	0.25	0.25	0.25
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	0.25	0.25	0.25	0.25	0.25
Premix	0.50	0.50	0.50	0.50	0.50
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>
<b>Calculated composition</b>					
Crude protein (%)	17.02	16.53	16.53	16.33	16.33
Crude fibre (%)	4.23	4.08	4.08	4.62	4.62
Metabolisable energy (Kcal/kg)	2557.38	2504.56	2504.56	2523.86	2523.86

–, No phytase inclusion; +, phytase inclusion; 250 FTU phytase inclusion, diet 1- Reference diet,

## MATERIALS AND METHODS

One hundred and twenty Nera-Brown pullets at 18 weeks of age were used for the research and reared in a two tier cages with cell dimension of 38cm x 40cm at three birds per cell in an open-sided house in the Teaching and Research Farm, Ekiti State University, Ado-Ekiti.

Five experimental diets were formulated to contain 16.5% crude protein and 2500kcal/kg metabolisable energy. The diets were adequately furnished with minerals and vitamins. At 26 weeks (8 weeks in lay) they were randomly allotted to five experimental diets replicated four times with six birds per replicate in a completely randomised design. The birds had an average initial weight of 1.47kg.

The five diets were formulated such that plant products predominantly made up the diets as presented in Table 1. Diet 1 was the reference diet with no phytase supplementation but protein sources of both plant and animal origins. Diets 2

and 3 were duplicate diets with enzyme supplementation in diet 3. Diets 4 and 5 were also duplicate diets with enzyme supplementation in diet 5. Diets 2 and 3 had groundnut cake as the main protein ingredients while diets 4 and 5 had soyabean meal as the main protein ingredient. All diets were supplemented with methionine and lysine. All the diets were iso-nitrogenous and iso-caloric.

They were fed their respective experimental diets and water ad-libitum. The birds were weighed at the beginning and at the end of the experimental period to obtain the body weight gain during the experiment. The feed intake of the birds was determined on a daily basis by the difference between weight (g) of the feed offered and weight (g) of feed leftover. The 5 diets were analysed for phytic acid and phytin-phosphorus.

The extraction and precipitation of phytin in the experimental diets were done by the method of Wheeler and Ferrel (1971) while iron in the precipitate was determined by the method of

Makower (1970). Phytin was determined by using a Fe/P (4:6) ratio to calculate phytin-phosphorus and multiplying the phytin-phosphorus by 3.55 as suggested by young and Greaves (1940).

The weight of eggs, weekly egg production and percentage hen-day production were determined.

Table 2: Proximate composition of experimental diets fed to laying birds

Parameters	Dietary treatments				
	1	2	3	4	5
	Phytase Inclusion				
	-	+	-	+	
Crude protein (%)	16.98±0.06	16.37±0.14	16.39±0.02	16.46±0.06	16.48±0.04
Crude fibre (%)	5.30±0.08	5.10±0.28	5.12±0.46	5.83±0.21	5.80±0.03
Ether extract (%)	3.04±0.19	2.98±0.10	2.98±0.20	2.99±0.08	3.00±0.10
Nitrogen free extract (%)	52.75±0.39	51.60±1.76	52.55±0.10	52.12±0.42	52.53±1.26
Ash (%)	11.40±0.03	11.49±0.02	11.90±0.26	11.56±0.11	11.61±0.30
Moisture content (%)	10.42±0.06	10.50±0.11	10.90±0.17	10.74±0.44	10.76±0.33

-, No phytase inclusion; +, phytase inclusion ;determination in duplicate, diet 1- Reference diet,

#### Determination of internal egg quality

The weight of the thick albumen was measured with a tripod micro meter as described by Haugh (1937). The yolk colour was scored with the aid of Roche Yolk Colour Fan. The yolk weight was measured with a tripod micrometer. The albumen height and yolk height were obtained using vernier caliper.

The yolk egg weight and Albumen egg weight were expressed as percentage of egg weight.

The Haugh's unit was calculated per replicate from the values obtained from albumen height and egg weight by employing the formular proposed by Oluyemi and Roberts (1979).

#### Determination of external egg quality

The egg length and egg diameter were determine using vernier caliper. The egg shape index was calculated as a ratio of the egg diameter to egg length after Allen and Young (1980). The shell egg weight (SEW) was expressed as the percentage of egg weight.

The two egg membranes were pulled off the shells immediately and the shells peeled were air-dried after which the egg shell thickness was determined with a micrometer screw gauge. Egg Shell Surface Area (ESSA) was calculated

according to the formular used by Lewis and Perry (1987).

#### Statistical Analysis

Data obtained were subjected to descriptive statistics and analysis of variance using the Minitab computer software package (2005 version). Duncan's multiple Range Test of SPSS 14.0 was used for means separation at P=0.05.

#### RESULTS

The performance characteristics of laying birds fed the experimental diets are presented in Table 3. The final liveweight of birds on diet 3 had a significantly highest ( $p<0.05$ ) value (1673.11±6.20), while birds on diets 1, 2 and 4 were not significantly different with mean value of 1596.80±15.0g, 1602.23±13.3g and 1612.88±25.8g respectively. The feed intake of laying birds across the treatments was not significantly influenced by phytase supplementation. A significantly highest ( $p<0.05$ ) hen day production was recorded for birds on diet 3 (66.35%). The weight change, feed intake, feed efficiency were not significantly different among the treatments.

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Table 3: Performance characteristics of laying birds fed experimental diets

Parameters	Dietary treatments				
	1 Reference Diet	2 -	3 +	4 -	5 +
Initial					
Live weight (g)	1508.96±16.0	1509.64±19.7	1577.36 ±6.3	1521.36±12.3	1533.04±8.2
Final live weight (g)	1596.80±15.0 <sup>c</sup>	1602.23±13.3 <sup>c</sup>	1673.11±6.2 <sup>a</sup>	1612.88±25.8 <sup>c</sup>	1629.40±11.2 <sup>b</sup>
Weight changes (g)	87.84±6.94	92.59±6.65	95.75 ± 2.52	91.24 ± 5.89	96.36±3.00
Feed intake (g/b/d)	110.40±0.40	110.49±0.38	110.64±0.27	110.96±0.29	110.60±0.33
Feed efficiency (feed/kg egg)	1.78±0.01	1.82±0.01	1.78±0.01	1.83±0.01	1.80±0.00
Hen day production (%)	65.75±0.65 <sup>ab</sup>	65.59±0.62 <sup>ab</sup>	66.35±0.44 <sup>a</sup>	65.51±0.55 <sup>ab</sup>	65.07±0.28 <sup>b</sup>

Means with different superscripts in the same row differ significantly ( $P < 0.05$ ), diet 1- Reference diet, -, No phytase inclusion; +, phytase inclusion

Table 4: External egg quality characteristics of laying birds fed experimental Diets

Parameters	Diets				
	1 Reference Diet	2 -	3 +	4 -	5 +
Egg weight(g)	62.12±0.18 <sup>a</sup>	60.89±0.12 <sup>c</sup>	61.95±0.33 <sup>ab</sup>	60.39±0.18 <sup>d</sup>	61.68±0.26 <sup>b</sup>
Egg Length (mm)	61.68±0.97	60.16±0.17	61.15±0.28	60.25±0.74	61.10±0.67
Egg diameter (mm)	52.25±0.27	51.10±0.30	51.93±0.29	51.02±0.45	52.53±0.07
Egg Shell wt (g)	4.64±0.13	4.44±0.11	4.64±0.15	4.52±0.16	4.63±0.13
Egg Shell thickness (mm)	0.41±0.03	0.40±0.02	0.40±0.01	0.40±0.01	0.40±0.01
Shell egg weight (%)	7.49±0.14	7.36±0.06	7.51±0.12	7.34±0.18	7.62±0.03
Egg Shell surface area	72.99±0.20	72.70±0.10	73.23±0.26	72.28±0.73	73.47±0.38
Egg shape index	0.85±0.01	0.85±0.00	0.85±0.01	0.85±0.01	0.86±0.01

abcd: Means with different superscripts on the same row differ significantly ( $P < 0.05$ ), diet 1- Reference diet, -, No phytase inclusion; +, phytase inclusion

The result of external egg quality characteristics of laying birds fed experimental diets are shown in Table 4. The highest egg weight value was recorded for birds on diets 1 and 3. The egg

weight value of 60.89±0.12g recorded in diet 2 was also significantly ( $p < 0.05$ ) different from the birds on diet 4 (60.39±0.18g). The egg length, egg diameter, egg shell weight, egg shell

thickness, shell egg weight, egg shell surface and egg shape index were not significantly affected by the experimental diets.

The internal egg quality characteristics of laying birds fed experimental diets are shown in Table 5. Haugh units value recorded for birds on diet 1

was significantly ( $P<0.05$ ) highest. Birds on diets 2 and 4 had similar haugh unit but significantly ( $p<0.05$ ) higher or lower than haugh units of other diets. The yolk egg weight, yolk weight, yolk height, albumen egg weight, albumen weight, albumen height and egg yolk colour were not significantly affected by experimental diets.

Table 5: Internal egg quality characteristics of laying birds fed experimental diets

Parameters	Dietary treatments				
	1 Reference Diet	2 -	3 +	4 -	5 +
Haugh units	65.03±0.65 <sup>a</sup>	62.22±0.30 <sup>c</sup>	63.29±0.57 <sup>b</sup>	62.29±1.00 <sup>c</sup>	63.45±0.76 <sup>b</sup>
Yolk egg weight (%)	22.85±0.08	22.17±0.28	22.56±0.26	22.31±0.24	22.69±0.09
Yolk weight (g)	13.84±0.15	13.54±0.18	13.69±0.36	13.53±0.61	14.04±0.66
Yolk height (mm)	15.08±0.71	14.48±0.32	15.02±0.71	14.32±0.66	14.96±0.61
Albumen					
Egg weight (%)	51.32±0.03	49.69±3.00	50.92±0.34	51.20±0.35	51.45±0.30
Albumen weight (g)	32.05±0.75	31.03±0.57	31.08±0.70	31.07±0.49	31.99±0.71
Albumen height (mm)	5.05±0.63	5.05±0.73	4.64±0.71	4.41±0.17	4.69±0.07
Egg yolk colour	3.88±0.15	3.72±0.04	3.72±0.13	3.74±0.06	3.90±0.13

Means with different superscripts on the same row differ significantly ( $P<0.05$ ), diet 1- Reference diet, -, No phytase inclusion; +, phytase inclusion

## DISCUSSION

The final liveweight of birds on diets 3 and 5 (diets with phytase inclusion) were superior to other birds fed other dietary treatments. The increase in final liveweight of birds on diets 3 and 5 (diets with phytase inclusion) may be due to benefit derived from phytase supplementation in poultry diets and such benefits are improvement in nutrient digestibility and reduction on the variation of nutrient quality of feed ingredients when feeding diets with phytase (Bedford 2000). The similarity in egg shell qualities is an indication that nutrient composition of the diets, especially minerals (calcium and phosphorus) were adequate and fairly constant and therefore caused no variation in these parameters. Smith (1990) reported that there is a strong correlation between shell strength and shell thickness while Oluyemi and Roberts (1979) also stated that an average shell thickness of 0.34mm is considered normal for any flock of layers. Stadelman (1994) reported that the lowest egg shell thickness

necessary is 0.33mm in order for an egg to have over 50% chance to go through the normal market chain without being broken. In regards to phytase supplementation, birds fed diets with phytase laid heavier eggs in comparison to birds fed without phytase supplement. It was seen that the treatment with phytase improved egg shell weight. The results also indicate that phytate did not inhibit calcium and phosphorus absorption in the diets. Therefore, the average shell thickness value of 0.40mm in this study is an indication that the diets satisfied that requirement. Carnarius *et al.* (1996) studied the egg shell thickness of broken eggs, eggs with damaged egg shell and egg with good egg shell. They found that egg with good shell quality was 0.38mm. Having compared the various analyses in egg shell thickness it was observed that the diet with or without phytase had no significant effect on egg shell thickness. Improvement in shell strength can be achieved by ensuring that layers ration contain the right amounts of calcium (Nesheim *et*

*al.*, 1979). This is important in commercial table eggs production where egg breakage in cages can cause a considerable loss or waste of revenue (Washington Bureau, 1991). The values of shell quality parameters obtained in this study are similar to those reported by Oluyemi and Roberts (1979), Patrick and Schaible (1980), CTA (1992), Ologhobo *et al.* (1997) and Ayanwale *et al.* (2003). The egg length, egg diameter and egg shell surface areas are indices that are associated with the general size of the egg. The egg length and egg diameter of hen fed diet supplemented with phytase are higher than those without phytase these result are in agreement with Jalal and Scheideler (2001), Um and Palk (1999) and Panda *et al.* (2005) reported that beneficial effects of phytase supplementation on egg shell thickness in corn-soya diet improved egg mass. The similarities of the reference diet to other diets (with or without phytase) did not adversely affect the egg size since dietary deficiencies in total protein or their amino acids are associated with reduction in egg size (Oluyemi and Roberts, 1979). It means that all the diets were able to furnish adequately the required total protein or other amino acids for normal egg size. The similarity of values of egg shape index for all the diets means that no abnormal egg shape developed when the layers were fed the experimental diets.

The Haugh unit which is an index of protein utilisation was similar for all the diets except reference diet which had the highest haugh unit. This further confirms that dietary protein provided by the diets were equally and effectively utilised by the laying birds. The range of haugh values obtained in this study are similar to those earlier reported for layers in the tropics (Odunsi, 1991 and Akpodiete, 1992). Available literatures show that Haugh unit and albumen height (mm) have positive correlation. The height of the albumen and the weight of an egg determines the Haugh unit of the egg and the higher the height of the albumen, the greater the value of Haugh unit and the better the quality of the egg. The similarities of yolk weight, yolk height and yolk weight as a percent of the egg weight further revealed the nutritional adequacy, compatibility and quality of the experimental diets and therefore a comparable nutritive value of groundnut cake and soyabean meal for laying

birds, this could be an indication that the physiological activities involved in yolk formation were not adversely affected by with or without phytase. The similarity of egg yolk colour for all diets is a positive indication that phytase can be added into layers diets without adversely affecting consumer preference for yolk colour although there was little increase in yolk colour in birds fed diet 5 (soyabean with phytase inclusion) this however did not follow a trend to clearly attribute the difference to the inclusion of phytase or not. Perhaps it may be due to individual differences in the ability to utilize the colour pigment in yolk formation. The yolk colour is of commercial importance in the egg industry and is affected by the composition of the diet (Belyavin and Marangos, 1989).

## CONCLUSION

The findings from this study revealed that groundnut cake and soybeans can serve as main protein source in laying pullet diets with or without phytase without adversely affecting laying performance or egg quality traits. However, phytase supplementation in a groundnut cake based diet of laying pullets enhanced hen day production and egg weight.

## CONFLICT OF INTEREST

Authors declare that no conflict of interest within, during or after performing the experiment that could influence this work

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