

EFFECT OF PROTEASE SUPPLEMENTATION ON THE PERFORMANCE OF LAYING CHICKENS FED LOW PROTEIN DIETS IN EARLY PRODUCTION CYCLE

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ABSTRACT

A 10-week study was conducted to investigate the effect of diets supplemented with protease on the performance of 32 weeks old Isa Brown hens housed in cages and fed low Crude Protein (CP) diets in a completely randomized design. A total of 60 Isa Brown laying birds were randomly allotted to 5 dietary treatments each having six replicates of 2 birds per replicate. The diets were formulated to contain 16% crude protein (Treatment 1) which was the control diet; T2, 15.2% CP diet (5% CP reduction); T3, 15.2% CP diet + 0.05% protease; T4, 14.4% CP diet (10% CP reduction) and T5, 14.4% CP diet + 0.05% protease. Feed and water were supplied *ad libitum*. Results showed that feed intake, Haugh unit and hen day production were not significant ($p > 0.05$). Addition of enzyme to 15.2 and 14.4% CP diets improved egg weight, shell weight, shell thickness and albumen weight. Yolk weight was improved by 14.4% CP diet. In conclusion, 10% CP reduction plus protease gave optimum performance of the laying hens and could be adopted.

Keywords: Isa Brown, Performance, Protease, Supplementation, Performance characteristics.

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INTRODUCTION

Dietary protein is an essential key nutrient of animal feed absolutely necessary for growth, body maintenance, reproduction and products such as eggs, milk and wool. Protein is however an expensive item in feeds for poultry. Feed formulation should therefore be manipulated in such a way that optimum performance is achieved with minimal expenditure. Protein is the most expensive nutrient in laying hen diets. Production of eggs is of great economic importance in the poultry industry. The success of the enterprise however depends on the total number of eggs and the quality of eggs produced (Ojedapo *et al.*, 2009).

Evaluation of the internal and external qualities of chicken egg is important in commercial egg production (Parmer *et al.*, 2006). The inclusion of appropriate crude protein level in the poultry diet improves feed utilization and reduces environmental pollution due to decrease output of nitrogen in manure (Novak *et al.*, 2007). The gut environment can incapacitate endogenous enzymes in poultry in the full release of nutrients in feeds. This leads to the release of nitrogen to the environment.

Various approaches have been studied to optimize crude protein utilization in laying hen diets. One of such approaches is to reduce the crude protein content in the

diets of laying hen. Blair *et al.* (1999) found that layers performance could be maintained with low protein diets (13.5% CP) but if supplemented with essential amino acids. Khajali *et al.*, (2008) also reported that layers performance remained satisfactory on reduced crude protein diets only for short periods, but long term feeding of reduced Crude Protein (CP) diets may not be advisable because it reduced performance in the late stage of production. Another approach is to use exogenous enzymes to enhance the feeding value of the diets. Enzymes such as xylanases (Mirzaie *et al.*, 2012), proteases (Angel *et al.*, 2011) have been used extensively in layer's diet. However some poultry farmers tend to supplement diets with exogenous enzymes without taking into consideration the targeted substrates (Abu *et al.*, 2011). Proteases are protein-digesting enzymes that are used in pig and poultry nutrition to complement endogenous enzymes to break down proteins in various plant and animal sources (Tempira, 2013). Angel *et al.*, (2012) observed significant improvements in performance of laying hens when their diets were supplemented with a protease than was found with young broilers (Angel *et al.*, 2011). Addition of exogenous enzymes to diets has been found to elicit beneficial changes on the microbial intestines of consuming animals (Ferket, 2004). The introduction of single exogenous enzyme in monogastric nutrition are gradually replacing curtail enzymes and new mechanisms have also been proposed for their actions (Adeola and Cowieson, 2011). This study was therefore carried out to determine the effect of protease supplementation on the performance of Isa Brown laying chickens fed low protein diets.

MATERIALS AND METHODS

The feeding trial was carried out at the Poultry Unit of Teaching and Research Farm, University of Ibadan, Nigeria, Nigeria.

Experimental birds and diets: Sixty commercial layer strain of Isa brown at 28 weeks of age were purchased from a reliable farm. The birds were fed standard layer diet until the start of the experiment. The birds were randomly allotted to five dietary treatments. Each treatment was replicated six times with 2 birds per replicate in a cage. Diet 1 (control) was formulated to supply 16% CP, Diet 2 contained 15.2% CP, Diet 3, 15.2% CP + 0.05% Protease Dp 100; Diet 4, 14.4% CP; and Diet 5, 14.4% CP + 0.05% Protease Dp 100. The percentage crude protein reduction in treatments 2 and 3 was 5% respectively and treatment 4 and 5 had 10% reduction. But treatments 1, 2 and 4 had no protease supplementation while treatments 3 and 5 had 0.05% protease supplementation. The gross composition of the experimental diets is as presented in Table 1.

Data collection and Analysis: Feed intake was recorded daily, hen- day production and Haugh unit were calculated. Internal egg quality parameters such as albumen weight (g), albumen height and width (mm), yolk height (mm), yolk colour (Roche Colour Fan), yolk weight (g) were taken weekly. External egg quality parameters such as egg weight (g), egg length and width (mm), shell weight (g) and thickness (mm) were also recorded weekly for ten weeks the experiment lasted

All data were subjected to one-way Analysis of variance (ANOVA) using SAS (version 9.2) package (SAS, 2002) and means were separated using Duncan multiple range test of the same software.

Table 1: Gross Composition Of The Experimental Diets (%)

Ingredients (%)	T1	T2	T3	T4	T5
Maize	41.82	41.82	41.82	41.82	41.82
Soyabean meal	16.18	15.23	15.23	14.43	14.43
Corn bran	17.68	16.81	16.76	15.79	15.54
Wheat offal	10.37	10.37	10.37	10.37	10.37
Oyster shell	7.50	7.50	7.50	7.82	7.82
Di-calcium phosphate	3.50	3.50	3.50	3.82	3.82
Fish meal	2.00	2.00	2.00	2.00	2.00
Premix	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.15	0.15	0.15	0.15
Table salt	0.30	0.30	0.30	0.30	0.30
Palm oil	0.00	1.82	1.82	3.00	3.00
Protease	0.00	0.00	0.05	0.00	0.05
Total (%)	100	100	100	100	100
Calculated value					
Crude protein (%)	16.00	15.20	15.20	14.40	14.40
Crude fibre (%)	4.80	4.71	4.71	4.53	4.53
Calcium (%)	3.64	3.63	3.63	3.63	3.63
Phosphorus (%)	0.90	0.90	0.90	0.90	0.90
L- lysine	0.80	0.80	0.80	0.73	0.73
DL-Methionine	0.30	0.30	0.30	0.30	0.30
ME (kcal/Kg)	2500	2500	2500	2500	2500

T1: Basal diet with 16% CP (without enzyme inclusion)., T2: 15.2% CP diet, T3: 15.2% CP diet + 0.05% Protease Dp 100, T4: 14.4% CP diet, T5: 14.4% CP diet+ 0.05% Protease Dp 100

RESULTS AND DISCUSSION

The results of performance characteristics of laying hens fed protease supplemented diets are presented in Table 2. There were no significant differences ($p > 0.05$) among treatments for daily feed intake, hen-day production and Haugh unit. Jalal *et al.*, (2007) and Lee *et al.*, (2014) reported that reducing crude protein in laying hens fed with or without enzyme

supplementation had no effect on feed intake. This reason may be that laying hens consume feed to first meet their daily energy requirement (Latshaw *et al.*, 1990). And the diets fed were isocaloric. Roberts *et al.* (2007) also reported no effect of crude protein reduction on feed intake in laying hens because regardless of the level of crude protein in the diet, amino acid content was the same. Lee *et al.* (2014) also reported no

significant difference ($p > 0.05$) in egg production of layers fed diets with reduced CP and protease inclusion. However, Ru (2009) reported positive effects of protease supplementation on egg production from laying hens. These variations could be attributed to differences in layers' strains, differences in activities and concentration of protease preparations including the use of multi-enzyme complex compared with

purified and microbial protease (Tempra *et al.*, 2013). The results of the Haugh unit reported in this study contradicted the report of Torki *et al.* (2014) who found significant difference ($P < 0.05$) in Haugh unit of multi-enzyme supplemented diets for laying hens. The reasons for these contradictions could be due to strain and age of hens as explained by Silversides and Scott (2001)

Table 2: Performance characteristics of laying birds fed diets supplemented with protease

Performance	T1	T2	T3	T4	T5	SEM
HDP (%)	51.79	53.45	52.86	50.36	53.10	0.56
Feed intake (g)	124.77	124.74	124.75	124.76	116.45	1.70
Haugh unit	74.01	70.83	75.32	71.67	76.35	1.05

T1= Basal diet with 16% CP (without enzyme inclusion), T2 = 15.2% CP diet, T3 = 15.2% CP diet + 0.05% Protease Dp 100, T4 = 14.4% CP diet, T5 = 14.4% CP diet+ 0.05% Protease Dp 100. HDP = Hen day production.

The results of the external characteristics of eggs produced by laying hens fed protease supplemented diets are presented in Table 3. Egg weight was positively influenced by protease supplementation. A 10% reduction in protein supplemented with protease enzyme produced the highest egg weight. This finding however contradicted the results of Tempra *et al.*, (2013) who concluded that addition of multi-enzyme complex has no positive effects on egg weights. However, Egg shell weight and shell thickness were however significant ($p < 0.05$) across treatment with protease supplemented diets showing significantly higher values than the control diet and non-protease supplemented diets. This result supported the findings of Torki *et al.* (2014) who reported significant increase in egg shell weight between enzyme supplemented diets and control. Protease would have played a significant role in calcium and phosphorus utilization as it has been implicated in the utilization of calcium and phosphorus by laying hens

(Tempra *et al.*, 2013). The egg length and width showed that enzyme supplementation had a significant ($p < 0.05$) effect. This contradicted the report of El Full *et al.*, (2000) and Yoruk *et al.*, (2006) who reported that diets containing multi-enzymes had no effect ($p > 0.05$) on egg performance. They concluded that this index could differ due to the age and strain of laying hens and the source and levels of enzyme used. Egg length and width also showed similar trend as the egg weight.

The results of the internal characteristics of egg produced by laying hens fed protease supplemented diets are presented in Table 4. The albumen weight, height and width were positively influenced by enzyme inclusion in the diet and differed significantly ($p < 0.05$) from other diets without enzyme supplementation. This indicated that protease inclusion up to 5% with 10% reduction in crude protein improved the quality of the egg. This result contradicted the findings of Santos-Ricalde *et al.*, (2013) and Reem (2013) who found

no significant difference ($p > 0.05$) in albumen weight, height and width of Avizyme[®] supplemented layers diet. Internal egg quality depends partly on the presence and stability of the dense layer of albumen, which is given by the protein ovomucin. This quality is however influenced by factors such as age and strain of hen, nutrition and environmental conditions (Leandro *et al.*, 2005). Egg yolk traits showed significant differences ($p < 0.05$) across treatment except yolk height and yolk index where non-significant ($p > 0.05$) increase were observed. The result of the yolk index reported in this study is corroborated with the findings of Geraldo *et*

al., (2012) and Torki *et al.*, (2014) who found no significant difference ($p > 0.05$) in yolk index of hens fed carbohydrases and phytase supplemented diets. However, it is contradicted by Yoruk *et al.*, (2006) who fed a multi enzyme supplemented diet to laying hens and reported significant difference ($p < 0.05$). However, a 5% and 10% reduction in CP with protease inclusion had significant effects on yolk traits. There were significant differences ($p < 0.05$) in egg yolk colour. Diet with 14.4% CP produced the highest colour index followed by diet with 14.4% CP plus enzyme and that of control which were similar. Diets with 15.2% CP and 15.2% CP plus were similar and the lowest.

Table 3: External characteristics of eggs laid by hens fed diets supplemented with protease

Parameters	T1	T2	T3	T4	T5	SEM
Egg weight (g)	63.25 ^{bc}	59.54 ^d	65.71 ^{ab}	62.65 ^c	67.35 ^a	1.34
Egg length (mm)	5.40 ^c	5.34 ^c	5.57 ^{ab}	5.47 ^{bc}	5.60 ^a	0.05
Egg width (mm)	4.16 ^b	4.06 ^c	4.23 ^a	4.12 ^{bc}	4.25 ^a	0.04
Shell weight (g)	6.55 ^{bc}	6.32 ^c	6.68 ^b	6.45 ^{bc}	7.08 ^a	0.13
Shell thickness (mm)	0.32 ^{bc}	0.31 ^c	0.33 ^b	0.33 ^b	0.35 ^a	0.006

T1= Basal diet with 16% CP (without enzyme inclusion)., T2 = 15.2% CP diet, T3 = 15.2% CP diet + 0.05% Protease Dp 100, T4 = 14.4% CP diet, T5 = 14.4% CP diet+ 0.05% Protease Dp 100.

Table 4: Internal characteristics of eggs laid by hens fed diets supplemented with protease

Parameters	T1	T2	T3	T4	T5	SEM
Albumen weight (g)	41.05 ^b	38.28 ^c	44.05 ^a	39.44 ^{bc}	43.77 ^a	1.15
Albumen height (mm)	0.64 ^a	0.55 ^b	0.62 ^a	0.57 ^b	0.65 ^a	0.02
Albumen width (mm)	6.06 ^{ab}	5.71 ^{bc}	6.39 ^a	5.63 ^c	6.30 ^a	0.15
Yolk weight (g)	15.48 ^{bc}	14.98 ^c	15.84 ^b	15.66 ^b	16.38 ^a	0.23
Yolk height (mm)	1.59	1.47	1.51	1.46	1.56	0.03
Yolk width (mm)	3.38 ^{bc}	3.32 ^c	3.47 ^a	3.35 ^{bc}	3.42 ^{ab}	0.03
Yolk colour	5.43 ^b	4.68 ^c	4.80 ^c	5.80 ^a	5.30 ^b	0.21
Yolk Index	0.47	0.44	0.44	0.44	0.46	0.007

T1= Basal diet with 16% CP (without enzyme inclusion)., T2 = 15.2% CP diet, T3 = 15.2% CP diet + 0.05% Protease Dp 100, T4 = 14.4% CP diet, T5 = 14.4% CP diet+ 0.05% Protease Dp 100.

CONCLUSIONS

- Supplementation of protease in layers diet even with 5 – 10% crude protein reduction had positive effects on the performance, internal and external qualities of egg.
- Inclusion of protease in laying hens diet elicited best internal egg parameters like albumen height, albumen weight, egg length, egg width, shell thickness among other diets.
- It is however recommended that protease could be included in laying chicken's diet containing as low as 14.4% CP. A positive effect of this study is the possible reduction in the nitrogen released in the environment since birds performed well on a reduced protein diet.

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REFERENCES

- Abu, O.A., Iyayi, E.A. and Tewe, O.O. 2011. Agro-industrial by-products and farm wastes for poultry production in Nigeria: Challenges and opportunities. *Ibadan Journal of Agriculture*, Vol. 7: 56-65.
- Adeola, O., Cowieson, A.J. 2011. Board-Invited review: Opportunities and challenges in using exogenous enzymes to improve non ruminant animal production. *Journal of Animal Science*, 89: 3189-3218.
- Angel, C.R., Saylor, W.W., Vieira, S.L., Ward, N.E. 2011. Effects of a mono-component protease on performance and protein utilisation in 7-22 day-old broiler chickens. *Poultry Science*, 90, 2281-2286.
- Angel, C.R., Sell, J.R. and Zimmerman, D.R. 2012. Autolysis of α -galactosides of defatted soy flakes: Influence of nutritive value for chickens. *Journal of Agricultural and Food Chemistry*, 36, 542–546.
- Blair, G., Catchpoole, D. and Horne, P. 1999. Forage tree legumes; their management and contribution to the nitrogen economy of wet and humid tropical environments. *Advances in Agronomy*, 44: 27-54.
- El-Full, E.A., Asker N.E., Ali M.M., Abdel-Wahed, H.M. and Omar, E.M. 2000. The use of rice bran in broiler and layer diets with reference to enzyme supplementation. *Egyptian Poultry Science Journal*, 20: 517-543.
- Ferket, P.R., 2004. Alternatives to antibiotics in poultry production: Responses, practical experience and recommendations. In: *Alltech's 20th, Annual Symposium 2004, Lexington. Proceedings*, Lexington, Alltech, pp: 54 - 56.
- Geraldo, A., Filho, J.A.V., Machado, L.C., Brito, J.A.G., Bertechini, A.G. 2012. Associação De Carbohidrase E fitase em dietas valorizadas para poedeiras semipesadas. *Acta Scientiarum. Animal Sciences*, v. 34, n. 3, p. 34 (3): 253-258.
- Jalal, M.A., Scheideler, S.E., Pierson, E.M. 2007. Strain response of laying hens to varying dietary energy levels with and without Avizyme supplementation. *Journal of Applied Poultry Research*, 16 (3): 289 – 295.
- Khajali, F., Khoshouie, E.A., Dehkordi, S.K. and Hematian, M.H. 2008. Production performance and egg quality of Hy-line W36 laying hens fed reduced-protein diets at a constant total sulfur amino acid: Lysine ratio. *Journal of Applied Poultry Research*, 17: 390-397.
- Lee, K.W., Lillehoj, H.S., Jang, S.I., Lee, S.H., Bautista, D.A. and Siragusa, G.R. 2014. Effect of Bacillus subtilis-based directfed microbials on immune status in broiler chickens raised on fresh or used litter. *Asian Australas. Journal of Animal Science* 26:1592 - 1597.
- Leeson, S. and Summer, J.D. 1997. *Ingredient Evaluation and Diet Formulation: Commercial Poultry Nutrition*. Publishers: University Books, Canada; 2nd Revised edition (April 1997).

- Mirzaie, S., Zaghari, M., Aminzadeh, S., Shivazad, M. and Mateos, G.G. 2012. Effects of wheat inclusion and xylanase supplementation of the diet on productive performance, nutrient retention and endogenous intestinal enzyme activity of laying hens. *Poultry Science*, 91: 413-425.
- Novak, C., Yakout, H.M. and Remus, J. 2007. Response of varying dietary energy and protein with or without enzyme supplementation on growth and performance of Leghorns in growing period. *Journal of Applied Poultry Research*, 16; 481-493.
- Ojedapo, L.O., Adedeji, T.A., Ameen, S.A., Olayemi, T.B., Amao, S.R., Ige, A.O., Rafin, T.A., Ojediran, T.K. and Akinniran, T.N. 2009. Effects of strain and age on egg quality characteristics of two different strains of layer chickens kept in cages in Derived Savannah Zone of Nigeria. Proceedings of 14th Annual Conference, Animal Science Association of Nigeria. Ogbomoso, pp: 42-43.
- Parmer, R.A.E., Guerne Bleich, E., and Hoffman, I. 2006. The relative contribution of indigenous chicken breeds to poultry meat and egg production and consumption in the developing countries of Africa and Asia. Proceedings of the 11th European Poultry Conference, Verona, Italy.
- Reem, R.W. 2013. The effects of dietary xylanase, phytase and phosphorus on the performance of laying hens. *Poultry Science*, 72:17.
- Roberts, S.A., Xin, H., Kerri, B.J., Russell, J.R. and Bregendahl, K. 2007. Effects of dietary fibre and reduced crude protein on nitrogen balance and egg production in laying hens. *Poultry Science*, 86:1716-1725.
- Ru. 2009. The Key to More Profitable Egg Production. Press Release. Danisco A/S, Communications, Langebrogade 1, Copenhagen, Denmark.
- Santos-Ricalde, Sarmiento-Franco, L. and Segura-Correa, J. 2013. Effect of three protein levels and an enzyme blend on egg quality of laying hens. *Pakistan Journal of Biological Sciences*, 16: 1056-1060.
- SAS. 2002. SAS-STAT User's Guide Statistics. 4th Edn. SAS Inst. Inc., Carry, NC.
- Silversides, F.G., Scott, T.A., Korver, D.R., Afsharmanesh, M. and Hruby, M. 2006. A study on the interaction of xylanase and phytase enzymes in wheat-based diets fed to commercial white and brown egg laying hens. *Poultry Science* 85: 297-305.
- Silversides, F.G. and Scott, T.A. 2001. Effect of storage and layer age on quality of eggs from two lines of hens. *Poultry Science* 80:1240-1245.
- Tempra, M.A., 2013. Proteases for broilers and layers: do they work? *Asian Poultry*, 2013 July : pp. 34-35
- Tempra, M.A., Luis, E.S., Merca, F.E., Batungbacal, M.R. and Hurtada – Nuevo Milenio, W.A. Philippines. 2013. The effect of dietary alkaline protease supplementation on the performance of broilers and layers. A paper presented at the 24th Australian Poultry Science Symposium (APSS). The annual symposium will be held at the Veterinary Science Conference Centre, University of Sydney, Feb. 17-20, 2013.
- Torki M., Zangiabadi, H. and Ghasem, I.H.A. 2014. Effects of enzyme supplementation on productive performance and egg quality of laying hens fed diets containing graded levels of whole date waste. *Poultry Science Journal* 2014, 2 (2): 139-151
- Yörük, M.A., Gül, M., Hayirli, A. and Karaoglu, M. 2006. Multi-enzyme supplementation to peak producing hens fed corn-soybean meal based diets. *International Journal of Poultry Science* 5 (4): 374-380