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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 (Tempra, 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 a the Poultry Unit of Teaching and Resear Farm, University of Ibadan, Nigeri Nigeria.

Experimental birds and diets: Six commercial layer strain of Isa brown at 3 weeks of age were purchased from a reliab farm. The birds were fed standard layer di until the start of the experiment. The bir were randomly allotted to five dietar treatments. Each treatment was replicat six times with 2 birds per replicate in a cag Diet 1 (control) was formulated to supp 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 cru protein reduction in treatments 2 and 3 wa 5% respectively and treatment 4 and 5 ha 10% reduction. But treatments 1, 2 and had no protease supplementation whi treatments 3 and 5 had 0.05% protea supplementation. The gross composition o the experimental diets is as presented i Table 1.

Data collection and Analysis: Feed intak was recorded daily, hen- day production an Haugh unit were calculated. Internal eg quality parameters such as albumen weig (g), albumen height and width (mm), yol height (mm), yolk colour (Roche Colo Fan), yolk weight (g) were taken weekl External egg quality parameters such as eg weight (g), egg length and width (mm), sh weight (g) and thickness (mm) were als recorded weekly for ten weeks th experiment lasted

All data were subjected to one-wa Analysis of variance (ANOVA) using SA (version 9.2) package (SAS, 2002) an means were separated using Dunca multiple range test of the same softwar

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 un reported in this study contradicted the repo of Torki *et al.* (2014) who found significa difference ($P < 0.05$) in Haugh unit multi-enzyme supplemented diets for lay hens. The reasons for these contradictio could be due to strain and age of hens a explained by Silversides and Scott (200 .

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

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% C diet + 0.05% Protease Dp 100, T4 = 14.4% CP diet, T5 = 14.4% CP diet+ 0.05% Protease D 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 an width showed that enzyme supplementati had a significant ($p < 0.05$) effect. Th contradicted the report of El Full *et a* (2000) and Yoruk *et al.*, (2006) wh reported that diets containing multi-enzyme had no effect ($p > 0.05$) on eg performance. They concluded that this ind could differ due to the age and strain o laying hens and the source and levels o enzyme used. Egg length and width als showed similar trend as the egg weight.

The results of the interna characteristics of egg produced by layin hens fed protease supplemented diets ar presented in Table 4. The albumen weigh height and width were positively influence by enzyme inclusion in the diet and differe significantly ($p < 0.05$) from other die without enzyme supplementation. Thi indicated that protease inclusion up to 5% with 10% reduction in crude prote improved the quality of the egg. This resu contradicted the findings of Santos-Ricald *et al.* (2013) and Reem (2013) who foun 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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