EFFECTS OF ENZYME SUPPLEMENTION OF LOW PROTEN DIETS ON NITROGEN ECONOMY, NUTRIENT DIGESTIBILITY AND FEACAL MINERAL CONTENT OF LAYER HENS

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ABSTRACT
The study evaluated the effect of enzyme supplementation of low protein diet on nitrogen economy, nutrient retention and faecal mineral content of laying hens. Twenty (20) weeks old layer hen were randomly allotted to 13.5% protein diets whose methionine and lysine levels has been adjusted to meet the NRC (1994) requirements for laying hens with two enzymes and without enzyme supplementation. Enzyme A is a Non-Starch Polysaccharide (NSP) degrading enzyme with some protease activities while Enzyme B is an NSP-degrading enzyme. The hens were divided into five (5) treatments groups containing enzyme A (0.1g/kg), enzyme A (0.2g/kg), enzyme B (0.1g/kg), enzyme B (0.2g/kg) and control diet without enzyme. Nitrogen economy, nutrient retention and faecal minerals i.e., calcium, potassium, magnesium, phosphorus, and sodium in (%) and manganese, iron, zinc and copper in (ppm), of experimental layers were evaluated. Nitrogen, fibre, crude fat and ash retention were significantly ($p<0.05$) higher while nitrogen output intensity and anthropogenic propensity were significantly ($p<0.05$) improved in the layers fed enzyme supplemented low protein diets compared to those in the control group. All minerals in the faeces of layer chicken fed enzyme supplemented diets were significantly affected ($p<0.05$) by treatment effect. The study concluded that enzymes supplementation of low protein diets holds promise for improving the environmental footprint in layers production.

Keywords: Enzyme, Laying hens, dietary protein, nitrogen economy and faecal characteristics.

INTRODUCTION
Sustainability is an increasingly important consideration in livestock production, with emphasis on profitable production that does not negatively impact on the environmental footprint of the animals. Various measures including feeding of low protein diets, ideal amino acids, enzymes supplementation and faecal management, among others, have been adopted in achieving sustainable poultry production.

Exogenous enzymes are added to poultry feed to increase the availability of starches, proteins and minerals, which are enclosed within the fibrous cell walls of the ingredients or bound in chemical forms that the animal is unable to digest and therefore inaccessible to their digestive enzymes. Degradation of non-starch polysaccharides (NSP) has been proposed as the underlying mechanism to improve bird performance by releasing nutrients trapped within the cell and lowering digesta viscosity to enhance nutrient digestion and subsequent absorption (Bedford and Schulze 1998). Common NSPs present in raw materials used for poultry diets include pectin, cellulose, mixed linked β-glucans and arabinoxylans (Parsippany, 2008). Improvements in animal performance due to use of exogenous enzyme additives have also been attributed to further release of minerals by phytase inclusion or incremental digestible energy, amino acids or fatty acids release with addition carbohydrates, proteases or Lipases, respectively (Abu 2014).

The objective of this study is therefore to evaluate the effect of enzyme supplementation of low protein diet on nitrogen economy, nutrient retention and faecal mineral content of laying hens.

MATERIAL & METHOD
A total of two hundred and ten (210) 20-week-old Isa Brown layers were randomly allotted to 13.5% protein diets whose methionine and lysine levels have been adjusted to meet the NRC (1994)

requirements for laying hens with and without enzyme supplementation. The study was carried out in battery cages fitted with automatic drinkers. Each treatment had three (3) replicates of fourteen layers/replicate. There were five (5) dietary treatments containing Enzyme A (0.1g/kg), Enzyme A (0.2g/kg), Enzyme B (0.1g/kg), Enzyme B (0.2g/kg) and the control diet without enzyme supplementation. The experimental diets were administered to the birds for a period of 12 weeks (Table 1).

Table 1: Composition of Basal low protein, enzyme supplemented layers' diet* (% DM)

Diets	Basal
Maize	57.90
SBM	10.20
Wheat Bran	20.50
Fish meal (72% CP)	1.00
Bone meal	3.00
Lysine	0.20
Methionine	0.20
Salt	0.25
Premix**	0.25
Oyster shell	7.00
Analyzed Nutrient Composition (%)	
Energy (ME/kg kcal)***	2520.3
Crude protein	13.54
Crude Fibre	8.05
Crude Fat	7.01
Ash	8.07

* Diet 1 (Ro_1) – Basal diet + Enzyme A 0.1g/kg feed; Diet 2 (Ro_2) – Basal diet + Enzyme A 0.2g/kg feed; Diet 3 (RG_1) – Basal diet + Enzyme B 0.1g/kg feed; Diet 4 (RG_2) – Basal diet + Enzyme B 0.2g/kg feed; Diet 5 (Control) – Basal diet + No enzyme supplementation

**Supplied the following per kg of diet as specified by the manufacturer: Vitamin A, 12500 IU; Vitamin D_3, 2500 IU; Vitamin E, 50.00 mg; Vitamin K_3, 2.50mg; Vitamin B_1, 3.00 mg; Vitamin B_2, 6.00 mg; Vitamin B_{12}, 0.25 mg; Panthothenic acid, 5.00 mg; Nicotinic acid, 20.00 mg; Folic acid, 1.00 mg; Choline chloride, 300 mg; Manganese, 100 mg; Iron, 50 mg; Zinc, 45 mg; Copper, 2.00 mg; Iodine, 1.55 mg; Cobalt, 0.25 mg; Selenium, 0.1 mg

***Calculated value

The Enzymes

Enzyme A and Enzyme B were supplied by Adisseo France S. A. S. and DSM Nutritional Products Ltd. Poland, respectively. Enzyme A is a non-starch polysaccharide degrading enzyme preparation (from non-genetically modified fungus *Penicillium funiculosum*) containing β-glucanase, xylanase, cellulase, pectinase and protease. Enzyme B is a specific blend of beta-glucanases, cellulases and xylanases produced by a strain of *Trichoderma reesei*. The enzymes are naturally stable and efficient for degrading NSP of feedstuff.

Data Collection

Nutrient retention, and faecal minerals were determined according to the methods described by Ojebiyi et al. (2018) and AOAC (1984), respectively. Ellerman and Wing (2003)'s formula for emissions intensity was adapted for nitrogen output relative to egg production of layers in this study while anthropogenic propensity (AP) was calculated according to Bolu *et al*. (2011),

RESULTS AND DISCUSSION

Nutrient Retention of Layers Fed Enzyme Supplemented Low CP Diets

Nitrogen economy in this study revealed that there was no significant difference (p>0.05) in N intake (g/b/d), feacal N (%) and total N output (g/b/d) of layers fed enzyme supplemented low CP diets (Table 2).

Table 2: Effects of Enzymes Supplemented Low CP Diets on Nutrient Retention of Layers

Parameters	Diets					SEM
	Enzyme A (0.1g/kg)	Enzyme A (0.2g/kg)	Enzyme B (0.1g/kg)	Enzyme B (0.2g/kg)	Control	
Feacal N	2.99	3.05	3.16	2.39	2.96	0.30
N-intake (g/b/d)	1.85	1.84	1.81	1.82	1.74	0.24
N-output (g/b/d)	0.58	0.77	0.77	0.67	0.80	0.15
N retention (%)	68.25[a]	58.15[c]	57.19[c]	63.19[b]	54.02[c]	2.31
N-output intensity (g/egg)	0.009[c]	0.013[b]	0.014[ab]	0.011[b]	0.016[a]	0.002
Anthropogenic propensity	2.26[c]	3.00[ab]	3.00[ab]	2.61[bc]	3.11[a]	0.21
Fibre retention (%)	78.26[a]	74.30[a]	76.51[a]	75.24[a]	66.4[b]	2.18
Crude fat retention (%)	74.58[b]	75.95[ab]	78.24[ab]	79.68[a]	69.79[c]	2.06
Ash retention (%)	79.80[b]	78.56[b]	80.92[b]	85.89[a]	70.6[c]	1.92

[a,b,c] Values on the same row with different superscripts are significantly different ($p<0.05$)

Nitrogen output intensity (NOI) estimates nitrogen emissions to the environment per unit production. Significantly ($p<0.05$) lower NOI and anthropogenic propensity (AP) in layers fed low protein diets suggests that enzyme supplementation led to improved performance without increasing overall environmental footprint of the hens. The implication of the nitrogen retention data suggests that Enzyme A is more effective in promoting N-efficiency at 0.1 g/kg inclusion level than at 0.2 g/kg, while Enzyme B was more efficient at 0.2 g/kg. Better N-retention in Enzyme A supplemented group than the others could be as a result of the protease improve nitrogen and amino acids utilization indirectly by increasing access of protein to digestive proteases (Rehman *et al.*, 2017).

Furthermore, nitrogen, fibre, crude fat and ash retention were significantly ($p<0.05$) higher in the enzymes supplemented groups than the control group. A possible mechanism of improved dietary nutrients and growth in chicken fed cereal-based diets was associated with fibre (NSP) degradation with resultant lowered digesta viscosity and enhanced nutrients digestibility including cell wall entrapped nutrients (Lee *et al.*, 2003). A combination of undigested fat, protein and starch contributes to this energy loss, and use of exogenous enzymes has been suggested as a good strategy to make this energy available to birds (Cowieson, 2010).

Feacal Characteristics of Layers Fed Enzyme Supplemented Low CP Diets

All the elemental minerals in the feaces of layers fed enzyme supplemented diets were significantly affected ($p<0.05$) by treatment effect (Table 3).

Table 7.5: Feacal minerals of layers fed low protein diets supplemented with enzyme

Parameters	Diets*					SEM
	Enzyme A (0.1)	Enzyme A (0.2)	Enzyme B (0.1)	Enzyme B (0.2)	Control	
Calcium (%)	0.99[a]	1.21[b]	1.41[b]	0.79[a]	0.97[a]	0.07
Magnesium (%)	0.40[bc]	0.35[ab]	0.29[a]	0.45[cd]	0.50[d]	0.02
Potassium (%)	0.31[a]	0.38[b]	0.44[b]	0.25[a]	0.31[a]	0.02
Phosphorus (%)	0.67[b]	0.63[b]	0.63[b]	0.51[a]	0.66[b]	0.31
Sodium (%)	0.45[c]	0.33[b]	0.22[ab]	0.18[a]	0.23[ab]	0.03
Manganese (ppm)	49.40[a]	60.80[b]	70.30[b]	39.50[a]	48.80[a]	3.50
Iron (ppm)	39.90[a]	48.00[b]	54.70[b]	33.00[a]	39.50[a]	2.47
Zinc (ppm)	25.22[a]	30.99[b]	35.78[b]	20.26[a]	24.95[a]	1.76
Copper (ppm)	11.56[a]	14.21[b]	16.32[b]	9.27[a]	11.43[a]	0.81
Nickel (ppm)	0.062[a]	0.076[b]	0.089[b]	0.049[a]	0.061[a]	0.004
Lead (ppm)	0.015[a]	0.018[a]	0.021[b]	0.012[a]	0.015[a]	0.002

[a,b,c] Values on the same row with different superscripts are significantly different ($p<0.05$). * Enzyme inclusion level is in g/kg.

Feacal Ca, K, Mn, Zn, Fe, Cu, and Ni were significantly lower ($p<0.05$) for layers fed diets with 0.1 g Enzyme A/kg feed, 0.2 g Enzyme B/kg feed and the control diets compared with the levels observed in layers fed 0.2 g Enzyme A/kg feed and 0.1 g Enzyme B/kg feed. This observation could be an indication that dietary exogenous enzymes inclusion levels in the diets exerted some influence on the amount of elemental nutrients excretion in layers. However, enzyme supplementation did not generally result in excessive elemental nutrient excretions when compared with the control diet as the value observed in this study were within the normal range reported for chickens by Edward and Daniels (2002).

CONCLUSION

The result of this study showed that nitrogen and other nutrient retention were enhanced. The implication of these findings is that layers benefited from enzyme supplementation of low CP by reducing nitrogenous excretions into the environment. However, care must be taken in ensuring that optimum levels of enzymes are utilized in diets in order to promote sustainable production in chickens.

REFRENCES

Abu, O. A. (2014). Application of exogenous enzymes to improve feed utilization by poultry farm animals – A Review. Book of Abstracts, Third ASAN-NIAS Joint Annual Meeting, p. 96.

Association of Official Analytical Chemists (AOAC) (1984). Official Methods of Analysis (14th Ed.). Association of official Analytical Chemists, Arlington,VA.

Bedford, M. R. and Schulze, H. (1998). Exogenous enzymes of pigs and poultry. *Nutrition Resources Review.*, 11: 91-114.

Bolu, S. A., Ojo, V., Malomo, G. A., Oladele, P. O. and Olonijolu, T. O. (2011). Dietary levels of protein and sustainable broiler production. *Journal of Applied Agricultural Research,* 3: 133-139.

Cowieson, A. J. (2010). Strategic selection of exogenous enzymes for corn/soy-based poultry diets. *Japan Poultry Science*, 47: 1-7.

Edwards, D. R. and Daniel, T. C. (1992). Environmental impacts of on-farm poultry waste disposal - A review. *Bioresource Technology*, 41: 9–33.

Ellerman, A. D. and Wing, I. S. (2003). Absolute vs. Intensity-Based Emission Caps. MIT Joint Program on the Science and Policy of Global Change, Report No. 100, July, 2003,15 pp.

Lee, J. T., Bailey, C. A. and Cartwright, A. L. (2003). β-Mannase ameliorates viscosity-associated depression of growth in broiler chickens fed guar germ and hull fractions. *Poultry Science*, 82:1925–1931.

Ojebiyi, O. O., Shittu, M. D., Omojoro, T. D. and Alabi, O. M. (2018). Laying performance and some egg quality characteristics of brown egg type layers on direct-fed microbial (DFM) or antibiotics. *Ghanian Journal of Animal Science,* 9(1): 19-24.

Parsippany, N. J. (2008). Non-starch polysaccharide enzymes for poultry. Proceedings of the 6th MID Atlantic Nutrition Conference, University of Maryland, 2008.

Rehman, Z. U., Kamran, J., Abd El-Hack, M. E., Alagawany, M., Bhatti, S. A., Ahmad, G., Saleem, A, Ullah, Z., Yameen, R. M. K. and Ding, C. (2017). Influence of low-protein and low-amino acid diets with different sources of protease on performance, carcasses and nitrogen retention of broiler chickens. *Animal Production Science. http://dx.doi.org/10.1071/AN16687.*