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Supplementation of Exogenous Enzyme to Laying Hens Diets Containing Heat Treated Sheep Manure: Effects on Performance and Egg Quality

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***Abstract* - A seventy-day feeding trial was conducted to evaluate the performance and egg quality of laying hen fed heat treated sheep manure (HSM) based diets supplemented with Roxazyme G enzyme. Seven experimental layer diets were formulated with oven-dried HSM such that diets 2, 4 and 6 contained 5%, 10% and 15% HSM without enzyme supplementation respectively. Diets 3, 5 and 7 contained 5%, 10% and 15% HSM supplemented with 100mg Roxazyme enzyme per kilogram weight of feed respectively. Diet 1 (control) contained 0% sheep manure. Two hundred and fifty-two (252) 35 weeks old (Black Nera Strain) birds were divided into seven groups of thirty-six birds each and randomly assigned to the seven dietary treatments in a completely randomized design (CRD). Each treatment was replicated three times with twelve birds/replicate. Results showed that there was no significant (P>0.05) difference in weight gain, feed conversion ratio and egg quality characteristics of laying hens fed HSM diets (with or without enzyme) but for percentage yolk and egg yolk colour where significant (P<0.05) differences existed. Hen day egg and total egg production significantly decreased with increase in HSM inclusion. However, enzyme supplementation significantly (P<0.05) enhanced hen-day and total egg production. Supplementation of HSM diets with exogenous enzyme significantly (P<0.05) reduced the feed intake of the birds at all levels of HSM inclusion. The results also indicated that laying hens could tolerate up to 10% unsupplemented HSM diets without any deleterious effects on weight gain, hen day, and total egg production, thus making HSM a potential feed stuff for laying hens. It's utilization at up to 15% dietary level in laying hens diets is achievable with enzyme supplementation.**

***Keywords* - Egg Characteristics, Enzyme Supplementation, Heat Treated Sheep Manure, Laying Hens, Performance.**

I. Introduction

In Nigeria, the consumption of poultry meat and egg has consistently increased over the years. This trend is expected to continue because there is a change in the eating patterns of the populace leading to a greater demand for poultry products. However, the poultry industry in Nigeria is confronted with a number of challenges, especially the pressures to produce high quality products to satisfy customer needs in a cost effective manner. In poultry productions, feed cost has always been one of the major issues, accounting for up to 70% of total production cost [1]. The high cost of feeding is attributed to the high cost and scarcity of conventional feed ingredients like maize, groundnut cake, soyabean meal and fish meal. This scenario has caused the prices of poultry products (egg and meat) to rise far beyond the purchasing ability of an average Nigerian.

A possible approach to reducing the relatively high feed cost in commercial laying hen production is the utilization of animal by-products such as heat-treated sheep as feed ingredients [2] –[4]. This is necessary since the production levels of conventional feed ingredients are not increasing proportionately to meet the increasing demand. Heat-treated sheep manure (HSM) contains 91.80% dry matter, 21.18% crude protein, 4.95% ether extract, 21.90% crude fibre, 32. 80% ash and 1817 ME (kcal/kg) [5]. However, the presence of high level of undegradable and complex carbohydrates (fibre) limits its widespread use in layers feed. These components are considered as antinutritional factors since they affect the digestion and absorption of nutrients in the intestine [6]. Chickens are not capable of hydrolyzing NPS's that mask protein and carbohydrate [7] because of lack of the needed enzymes. One good way of improving the utilization of NSPs is the use of exogenous enzymes on layers diets to improve the birds' performance.

Enzyme supplementation as a feed additive has become common since last four decades [8]. This is due to primarily their positive effects on animal performance as well as their lacking harmful effects on consumers [9]. Addition of exogenous feed grade contrasting results has been reported [10] –[14].

Roxazyme G is an enzyme complex derived from *Trichoderma viride* with glucanase and zylanase activity that has been developed to complement the digestive enzyme of poultry [15] so that polysaccharides in feedstuffs can be broken down into simpler molecules which can be digested and utilized. To date, there is dearth of information about the use of enzyme supplemented sheep manure in layers diets. This study therefore aims at bridging this gap in knowledge by evaluating the effects of exogenous enzymes in overcoming the adverse effects of the low energy and high fibre contents of sheep manure on the performance and egg quality characteristics of laying hens

II. Materials and Methods

This experiment was conducted at the Teaching and Research Farm (Poultry Unit) of the Department of Animal Science, Ebonyi State University, Abakaliki, Nigeria with the approval of the Animal Ethics Committee of the University.

The manure was oven- dried at 80^{oc} for 3 hours and ground in a hammer mill into a meal suitable for incorporation into poultry feed.

A. Experimental Diets

Seven experimental layer diets were formulated such that diets 2, 4 and 6 contained 5%, 10% and 15% sheep manure without enzyme supplementation respectively. Diets 3, 5 and 7 contained 5%, 10% and 15% sheep manure supplemented with 100mg Roxazyme an enzyme per kilogram weight of feed respectively. Diet 1 (control) contained 0% sheep manure (Table 1). The ingredients were measured out and mixed with a spade on a concrete floor. Turning was vigorously done to ensure good mixing of ingredients and homogeneity.

B. Experimental Birds, Design and Management

A total of two hundred and fifty two (252) 35 weeks old (Black Nera Strain) birds were wing banded, weighed individually and distributed randomly to the seven treatment groups of thirty six (36) birds each and each group randomly assigned to an experimental diet in a completely randomized design (CRD). Each treatment was further sub-divided into three (3) replicates of twelve (12) birds per replicate.

The birds were all raised in standard battery cages with feed and water given *ad libitum*. Standard commercial management practices of layers were observed throughout the experimental period. Prior to the commencement of the experiment, the birds were weighed to obtain their initial weights. Records of egg production, egg weight and feed intake were kept on daily basis. The feeding trial lasted 10 weeks.

C. Determination of Egg Quality

A total of eight (8) eggs per replicate of 12 birds were sampled at random weekly. Each egg was assessed separately for internal and external egg quality traits. For external parameters the eggs were weighed within 24 hrs of lay, thereafter the length and breadth of each egg were measured to the nearest 0.01 cm using vernier callipers. For internal egg quality parameters, the eggs were broken onto a flat glass plate and the height of the albumen determined using a spherometer. The yolk was separated from the albumen with the aid of an egg separator and yolk weight determined. Yolk colour was determined using a Hoffman la – Roche colour fan [16]. The thickness of the shell was measured by the use of a micrometer screw gauge. The shell was then air-dried to constant weight before the weight of the shell was determined. Haugh unit values were calculated using the formula of Haugh (17)

$$HU = 100\text{Log}10\,(H - 1.7W^{0.35} + 7.6)$$

Where Hu = Haugh unit (%), H = observed albumen height. The egg shape index was measured as the ratio of the length and width of the egg.

D. Data Analysis

Data obtained were analysed by ANOVA using SAS program [18]. Fisher's least significant difference (LSD) at 5% level (P<0.05) was used to test the differences between means of treatments [19].

III. Results and Discussion

The effects of exogenous enzyme supplementation of heat-treated sheep manure diets on the performance and egg quality characteristics of laying hens are presented in Tables 2 and 3 respectively.

There was no significant difference in final weight, weight gain and feed conversion ratio of the birds. Supplementation of exogenous enzymes did not significantly affect weight gain of the laying hens.

The effect of fibre on average weight gain depends on fiber level, fibre source and the age of the animal [20]. The lack of effect of feeding a high fibre diet on average weight gain and feed conversion ratio recorded in this experiment could have been due to an increase in digesta weight resulting from the high water holding capacity of the GIT and/or increase in gastro intestinal tract (GIT) weight resulting from the age of the birds [21], [22]. It could also be due to the specific degradation potential and chemical composition of the fibrous diets [23], [24]. Thus, when the hens were fed a high fibre diet for a long time the size of the GIT and digesta weight increased.

The result of this study show that heat-treated sheep manure can be fed at levels as high as 15% without having any detrimental effect on weight gain. This agrees with the reports of [25] - [27] and also strengthens the finding of [28], [29], who reported that enzyme supplementation of layers' diets containing fibrous ingredients did not increase or decrease body weight gain significantly. The lack of response to enzyme supplementation by laying hens may be due to the age of the birds. [30] had also noted that the effects of enzymes on weight gain decline with age. Old birds, because of the enhanced fermentation capacity of the microflora in their intestines, have a greater capacity to deal with the effects of high viscosity [31], [32].

Supplementation of exogenous enzymes did not significantly affect FCR of the laying hens. The lack of significant response to enzyme supplementation on feed conversion observed in study is in agreement with the reports of [14], [33], [34] who reported that enzyme supplementation of laying hens diets had no significant effect on their feed conversion ratio.

Effect of dietary treatments on protein efficiency ratio showed that protein efficiency ratio of the hens were depressed at 10% and 15% unsupplemented HSM dietary levels. This might be due to the interference of protein metabolism and utilization as a result of high fibre contents of the diets. High fibre intake probably led to partial digestibility of the protein and the component amino acids, rendering the undigested portion useless and unavailable for egg production. Hence, the depressed hen-day and total egg production noticed in birds fed these diets.

There were significant ($P < 0.05$) variations in the feed intake of the birds. Hens fed 15% (T_6) unsupplemented diet consumed significantly the highest amount of feed followed by those fed 10% (T_4) unsupplemented diet. Birds on 5% (T_3) enzyme supplemented diets consumed significantly ($p < 0.05$) the least amount of feed. The feed consumption of birds fed 5% (supplemented and unsupplemented) did not differ significantly (P > 0.05) from the control diet.

The effect of dietary fibre on feed intake depends on the level and source of fibre [35] especially with respect to energy concentration [36]. In the present study, the fibre and the energy levels were different between the treatments. The increase in feed intake as the level of sheep manure in the diet increased without enzyme supplementation could have arisen from high fibre content with low energy density of the diet as the level of HSM increased. Increase in fibre content of a feed leads to reduction in energy content compelling birds to eat as much as they can to satisfy their energy requirements.This observation lends credence to the reports of [4], [20], [37], [38] that high fibre diets tend to increase feed intake in birds. However, birds can only eat to their crop maximum capacity. Enzyme supplementation of the diets however reduced feed intake significantly, which may have emanated from improved nutritive value (energy content) of the diets through the hydrolysis of polysaccharides that encapsulated starch or protein [39], [40] and the subsequent reduction in the amount of undigested substrate available for ileal fermentation [41], [42].

Significant treatment effects (P< 0.05) were noticed in the values recorded for both hen day egg and total egg production. Hen-day egg production was between 59.83 and 74.43%, while the total egg produced per bird was between 41.88 to 52.10 eggs. Both hen-day and total egg production decreased significantly at 15% unsupplemented HSM inclusion. Enzyme supplementation significantly improved hen-day and total egg production. The decrease in percentage hen day egg production at 10% and 15% HSM inclusion without enzyme supplementation could be attributed to the marginal poor feed utilization observed at 10% and 15% HSM diets. The result agrees with the reports of preliminary studies of [3], [43] that increase in HSM in layers diets reduced hen day production. The reason for this is obvious. The quality of protein (amino acid profile) and energy of conventional feed stuffs such as soyabeans and maize is far better than HSM. According to [43], [44], it is the quality of the ingredients in terms of nutrient profile and the bioavailability of their nutrient, that is more important than the absolute value of such ingredients in the diets.

In contrast to non-supplemental diets, enzyme supplementation enhanced hen-day egg production and total egg production of the hens. The enhanced hen-day and total egg production of birds fed enzyme supplemented diets reveals that the mineral and protein contents of the diets are readily available for utilization by the birds. According to [45], enzyme supplementation can only have a negative effect on egg production if the protein and mineral contents of the diet are not readily available to the birds. This could also be attributed to the sparing effects of enzymes on losses of endogenous amino acids and energy [46] and the hydrolysis of the protein anti nutrients and non-starch polysaccharides present in the diet [47, 42]. The results are supported by the reports of [13], [14], [28] who observed significant improvement in egg production of hens fed diets supplemented with exogenous feed enzymes.

The egg quality characteristics are presented on Table 3. Results show that egg weight, egg length, egg breadth, egg shape index, egg shell thickness, yolk weight, yolk length, yolk breadth, yolk index, albumen weight, albumen length, albumen breadth, percent albumen weight and albumen index did not differ significantly among the treatments. Significant differences existed only in percent yolk weight and egg yolk colour.

Results from this study showed that neither unsupplemented nor enzyme supplemented HSM diets influenced the size of egg produced by the pullets. The weight of eggs obtained in this study ranged between 61.16g to 63.56. These values are higher than the standard egg weight of 58g reported by [48] - [50]. This observation is in consonance with the findings of [10], [14], [51], [52] that there were no significant effects on the weight of eggs laid by pullets fed diets supplemented with enzyme.

According to [53], yolk index is a measure of the standing up quality of the yolk and that yolk value for a normal fresh egg ranges between 0.40 and 0.42. This is within the range obtained in this study (0.41 to 0.44). The values obtained for yolk index were similar to the reports of [54] - [56]. However, these values were higher than the values reported by [57] but lower than those reported by [58]. This disparity can be explained with the observation of [53], that the holding conditions during storage and more importantly the genetic conditions in the hen rather than the quality of the diet have the most influential effects on the yolk index. Therefore, the observed difference and disparity of the egg yolk index from the previous studies may have arisen from the genetic make up of the poultry strain used in this study.

The results also indicated that supplementation of heat-treated sheep manure diet with or without enzymes failed to influence the shell thickness of eggs produced. The values obtained compare favourably with the values reported by [48], who reported an average of 0.35mm for the humid tropics. Feeding is one of the factors responsible for good quality shells. Since the shell thickness did not decrease as HSM inclusion increased, it indicates that the quality was not affected either positively or negatively by HSM inclusions. The results are in agreement with the findings of [28], [52] who found no significant effect on the eggshell thickness of pullets fed diets with enzyme supplements.

Haugh unit was not significantly (P < 0.05) affected by the treatments. Haugh unit is one of the important parameters for determining the quality of an egg. [56] reported that haugh unit of 72 and above are regarded as an indicator of freshness in egg. None of the values obtained for the different treatments in this study showed value below 72; indicating that all the eggs are of good quality.

The yolk colour improved significantly (P< 0.05) as the level of heat treated sheep manure (with or without enzyme supplementation) in the diets increased. This is due to higher intakes of xanthophyll present in HSM leading to its deposition in the yolk. Ruminant manure has been reported to contain a high level of xanthophyll, a precursor for carotene [60], which is an effective pigmenter for egg yolk colour. The Roche yolk colour fan (RYCF) scores increased from the lowest value of 1.23 recorded for the control diet, which had no HSM to 11.75 at 15% dietary level of HSM. The deeper coloured yolk is an indication of better quality egg yolk, because it means that its content of vitamin A and carotene are higher. [59] reported that RYCF score of 4 was the minimum acceptable egg yolk colour by individual consumers and pastry industries. This minimum was exceeded at only 5% dietary inclusion level of HSM. Therefore, if egg yolk colour is the main target, HSM can be included in laying hens diets at only 5% dietary level.

Enzyme inclusion in the diet had no impact on the yolk colour. The observations are in agreement with the findings of [60], [61] who found no significant effect on yolk colour of eggs fed wheat-peas based diets supplemented with enzymes. [62] also did not find significant effect on egg yolk colour of eggs fed maize-sunflower diets supplemented with enzymes.

In conclusion, HSM contributed to the deeply pigmentation of the egg yolk with yellow. In the meantime, incorporation of HSM at up to 10% dietary level without enzyme supplementation in this experiment did not influence layers performance and egg quality. It's utilization at up to 15% dietary level in laying hens diets is achievable with enzyme supplementation.

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Table 1: Ingredient and chemical composition of experimeMntal layers diets (%)

	Diets						
Ingredients	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Maize	46.00	43.00	43.00	40.00	40.00	37.00	37.00
HSM	00.00	5.00	5.00	10.00	10.00	15.00	15.00
Wheat Offal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
SBM	22.00	20.00	20.00	18.00	18.00	16.00	16.00
Fish Meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00
PKC	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated Chemical Composition							
Dry matter	79.26	79.41	79.41	79.56	79.56	79.71	79.71
Crude protein	16.92	17.11	17.11	17.31	17.31	17.51	17.51
Crude Fibre	5.23	6.14	6.14	7.04	7.04	7.95	7.95
Ether extract	3.81	3.87	3.87	4.00	4.oo	4.06	4.06
Total ash	2.92	4.44	4.44	5.98	5.98	7.56	7.56
Energy	2666.00	2604.86	2604.86	2543.72	2543.72	2482.57	2482.57
Phosphorus	1.32	1.30	1.30	1.29	1.29	1.26	1.26
Calcium	2.74	2.75	2.75	2.76	2.76	2.77	2.77

* The vitamin premix supplied the following per kilogramme diet: - Vit.A (I.U) 4000, 000; Vit.D (I.U) 1000, 000; Vit.E (I.U) 4,800; Vit. (g) 0.8; BI(g) 0.4; Vit.B2(g) 1.2; Nicotinic acid (g) 4.8; Folic acid (g) 0.12; Ascorbic acid (g) 20.0; choline chloride (g) 120.0; Mn(g) 40.0; Fe (g) 20.0; Zn(g) 18.9; Cu (g) 0.80; Iodine(g) 0.62.HSM = Heat-treated Sheep Manure, SBM = Soya Bone Meal, PKC = Palm Kernel Cake and E = Enzyme.

Table 2: Performance of laying hens fed enzyme supplemented heat treated sheep manure based diets

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	
	Heat-treated sheep manure and Roxazyme G inclusion							
Performance criteria	0%	5%	5%+E	10%	10%+E	15%	15%+E	SEM
Av. Initial body weight (g)	1965.77	1949.93	1960.14	1955.32	1958.86	1963.61	1951.60	
A.V Final body weight (g)	2046.34	2028.97	2042.21	2034.80	2040.74	2040.03	2032.25	3.10^{NS}
Av. Body weight gain (g)	80.61	79.04	82.07	79.48	81.88	76.42	80.65	4.19^{NS}
Av. Daily feed intake (g)	137.09^{c}	136.94^{c}	134.25^{d}	140.81^{b}	137.88^{c}	144.39^{a}	138.98^{c}	1.04
Feed conversion ratio	2.21	2.24	2. 19	2.26	2.18	2.27	2.21	0.13^{NS}
Av. Daily protein intake (g)	23.18^{d}	23.43^{cd}	23.31^{cd}	$24. 37^{b}$	23.87^{bc}	25.28^{a}	24.33^{b}	0.50
Protein efficiency ratio	3.48^{a}	$3. 43^{a}$	$3. 51^{a}$	$3. 30^{bc}$	$3. 38^{a}$	$3. 18^{c}$	$3. 32^{b}$	0. 20
Hen-day egg production (%)	68.43^{ab}	65.13^{bc}	71.65^{a}	64.87^{bc}	74.43^{a}	59.83^{c}	68.23^{ab}	1.33

a, b, c, d means with different superscripts on same row differ significantly ($P < 0.05$)

Table 3: Egg quality characteristics of laying hens fed enzyme supplemented heat treated sheep manure based diets

Measurements	0%	5.0%	5.0%+E	10.0%	10.0%+E	15.0%	15.0%+E	SEM
External Characteristics								
Egg weight (g)	61.92	61.16	61.23	62.49	63.29	63.56	62.91	0.74
Egg length (cm)	6.37	6.38	6.33	6.40	6.40	6.41	6.36	0.14
Egg breadth (cm)	4,52	4.51	4.49	4.52	4.53	4.49	4.52	0.14
Egg shape index	70.96	70.73	70.92	70.64	70.21	70.52	71.12	0.61
Egg shell thickness (mm)	0.35	0.37	0.36	0.39	0.37	0.38	0.39	0.08
Egg shell weight (g)	5.50	5.91	5.52	5.70	5.61	6-01	5.79	0.33
Egg shell weight (%)	8.88	9.66	9.02	9.12	8.87	9.46	9.03	0.40
Internal Characteristics								
Yolk weight (g)	16.45	16.12	16.21	16.34	15.69	15.39	16.47	0.45
Yolk length (cm)	1.50	1.47	1.49	1.48	1.52	1.48	1.52	0.13
Yolk breadth (cm)	3.53	3.45	3.52	3.50	3.52	3.39	3.53	0.15
Yolk index	0.43	0.43	0.42	0.41	0.43	0.44	0.43	0.07
Yolk weight (%)	26.58[a]	26.35 [ab]	26.47 [a]	26.15 [ab]	24.80 [bc]	24.23 [c]	26.18 [ab]	0.60
Albumen weight (g)	39.98	39.13	40.72	40.45	41.92	42.16	40.72	0.13
Albumen weight (%)	64.55	63.99	63.53	64.72	66.23	66.31	64.73	0.75
Albumen height (cm)	0.78	0.82	0.80	0.80	0.82	0.79	0.81	0.09
Albumen breadth (cm)	8.26	8.86	8.17	8.25	8.22	8.62	9.00	0.38
Albumen index	0.09	0.09	0.10	0.10	0.10	0.09	0.09	0.04
Egg yolk colour	1.23 [d]	6.80 [c]	6.76 [c]	9.12 [b]	9.14 [b]	11.72[a]	11.75 [a]	1.07
Haugh Unit (%)	73.03	75.06	74.21	74.38	74.98	73.85	74.68	0.62

a, b, c, d means bearing identical or no superscripts within rows are similar (P >0.05) while those with unidentical superscripts differ significantly (P<0.05) DW = Dressed weight.