UTILIZATION OF ENZYMES SUPPLEMENTED *Gliricidia sepium* (JACQ) LEAF MEAL BY BROILER CHICKENS (*Gallus domesticus* Brizzen)

***Ogungbesan, A.M., Adeleke G.A., Akanji, A.M. and Fasina, O.E.**

Department Of Animal Production, College Of Agricultural Science, Yewa Campus Ayetoro
Olabisi Onabanjo University Ago-Iwoye, Ogun State
**amkgbesan@gmail.com and 08057458575*

ABSTRACT

Ninety six day old-broiler chicks were randomly allocated to four dietary treatments of eight birds per replicate. Four experimental diets were formulated viz: A without Gliricidia sepium leaf meal (GLM), B with GLM at 5% replacement of dietary soybean, C 5% GLM with Roxazyme (G2)® and D 5% GLM with Maxigrain® .During the eight weeks trial , data collected were feed intake, weight gain and body weight changes. Daily feed intake was more significantly (p<0.05) reduced in birds fed control diet. Highest (P<0.05) body weight gain (36.46g/d) and nutrients utilization were observed in birds fed with Maxigrain® (diet D) supplement (p<0.05). Since the best response was recorded in diet D which is the one with GLM and Maxigrain®. Therefore, Gliricidia sepium leaf meal at 5% replacement of dietary soybean with 0.10% Maxigrain can be recommended for broiler production.

Keywords: Broilers, Enzymes, Gliricidia, Nutrients-utilization, Performance-characteristics

INTRODUCTION

The conventional protein feedstuffs for poultry such as soybean, groundnut cake, and fish meal are scarce and expensive because they are competed for by humans as food and other industrial uses. The rising cost of finished feed, which is 70 - 80% of the cost of production (Opara 1996) among others, is a major setback. The prices of such conventional protein feed ingredients such as groundnut cake, soybean meal and fish meal have soared so high that it is becoming uneconomical to use them in poultry feeds (Esonu et al., 2003). Alternative non-conventional feed stuff that is locally available throughout the year, easily accessible, cheap and affordable and those that do not attract competition in terms of consumption between humans and livestock should be sought after. Among these are leaf meals (LM) especially those from tree/browse forage legumes of which *Leucaena leucocephala*, *Gliricidia sepium* etc whose leaves can be processed and used for formulation of balanced ration (Simons and Stewart, 1994). Leaf meals do not only serve as protein source but also provide some necessary vitamins, minerals as well as carotenoids that impart yellowish coloration of broiler skin and shank (Esonu et al., 2003) Fasuyi and Kehinde (2009) investigated the effect of cellulase-glucanase-xylanase combination on the nutritive value of *Telfairia occidentalis* leaf meal in broiler diets while Mmereole (2009) evaluated dietary inclusion of sweet potato (*lpomea batatas*) leaf meal(SPLM) with or without enzyme treatment in broilers diet. Also, Onibi et al.,(2008) and Iheukwumere et al.,(2008) fed broilers with cassava leucaena leaf meals and cassava leaf meal respectively .Among promising and prospective results that these workers came out with include high feed intake, body weight gain feed conversion ratio from leaf meal supplementation comparable to control as observed by Iheukwumere et al. (2008) and Mmereole (2009) also recorded superior results in that containing 20% enzyme treated Sweet potato leaf meal. Hence, LM are gaining acceptance as feed stuff in poultry diet,since they are locally available and considered to be non-conventional feed stuff. (Fasuyi and Kehinde, 2009). The nutrient profile of these leaf meals compare favorably well with some conventional feeding stuffs. Satisfactory performances have been reported of various leaf meal tested in the diet of some classes of poultry birds. *Gliricidia sepium* is a multipurpose tree legume that is second only to *Leucacena leucocephala* in worldwide popularity (Simons and Stewart, 1994). Gliricidia possesses the ability to provide large quantities of high quality forage matter all-year-round as well as the ability to maintain a sustainable environment through nitrogen fixation thus replenishing the soil, though it contains bitter tasting coumarols, cyanogenic glycoside, tannin saponin, and cell wall content that makes it less useful for monogastrics (Kabi and Lukatome, 2013). Exogenous enzyme supplements (classical feed biotechnological method) are now widely used (Wu et al., 2004; Ahmed et al., 2007; Fasuyi and Kehinde, 2009; Duru and Dafwang, 2010 and Ademola et al., 2012)in poultry diets in an attempt to improve nutrient utilization, health and welfare of birds, product quality and to reduce pollution as well as increase the choice and contents of ingredients at cheaper cost which are acceptable for inclusion in diets (Bedford, 2000). There is need therefore to investigate the effect of these unconventional feed resources on the performance characteristics of broiler. The main objective of the study was to determine the effect of Roxazyme and Maxigrain on utilization *Gliricidia sepium* leaf meal by broilers chickens.

Table1: Chemical composition of test ingredients (Gliricidia Leaf Meal)

Gliricidia Leaf Meal	**COMPOSITION (%)**
Moisture	36.48
Crude protein	24.38
Ether extract	1.75
Crude fibre	12.45
Ash	11.58
N.F.E	49.84

MATERIALS AND METHODS

Study Location: This experiment was carried out at the Poultry Unit of the Teaching and Research Farm of College of Agricultural Sciences, Olabisi Onabanjo University, Yewa Campus, Ayetoro, Ogun State. Ayetoro is located in latitude $7^0$15 N Longitude $3^0$3E a deciduous derived savannah zone in Ogun State. Climate sub-humid tropics with an annual rainfall of 963.3mm in 74 days with maximum of 29^0c during the peak of wet season and 34^0c during the dry season; mean annual relative humidity is 81^0C.Ayetoro lies between 90 and 120m above the sea level. The entire area is made up of undulating surface, which is drained majorly by River Rori and River Ayinbo (Ogungbesan et al., 2013)

Processing of test ingredients: Fresh, young *Gliricidia sepium* leaves were harvested, dried under shade for seven days, milled to obtain Gliricidia Leaf Meal (GLM) and incorporated into four broiler diets in which soybean was replaced with Gliricidia Leaf Meal at 0% (control diet) and 5% respectively for the other diets. The diets (Table 1) were formulated to contain approximately 21.45% and 20.50% Crude protein and 2644.36 Kcal/kg and 2567.53 Kcal/kg Metabolizable Energy (ME). The diets (Tables 2 and 3) was formulated to contain approximately 21.59% and 20.65% Crude protein and 2675.36 Kcal/kg and 2553.53 Kcal/kg Metabolizable Energy (ME).

Experimental Birds: A total of 96 day-old broiler chicks (Rock Harnicks) were purchased at UNAB Leventis Farm, Abeokuta. The birds were divided into four treatments of 24 birds per treatment. Each treatment was replicated three times at 8 birds per replicate. The feeding trial lasted for eight weeks. Feed and water were supplied *ad-libitum.* Vaccines against Newcastle disease were administered to the birds immediately after hatching and when they were 3 weeks old respectively. The birds were de-wormed adequately, while antibiotics were also given.

Experimental Diets: Four experimental diets were formulated (Tables 2 and 3). Two of the diets were formulated without enzyme supplementation i.e. Diet A and Diet B while Diet C was supplemented with Roxazyme (G2)® (Each gram of Maxigrain contains 10,000 IU Cellulase, 200 IU Beta-glucanase,10,00IU Xylanase and 2500 FTU Phytate) and Diet D with Maxigrain® (Each gram of Maxigrain contains 8,000 IU Cellulase, 18,000 IU Beta-glucanase, and 26,000 IU Xylanase). The test ingredient (Gliricidia leaf meal) was included at 5% replacement of dietary soybean.

Growth Study: The experimental birds were offered feed and water *ad libitum* in separate feeders in the morning, and afternoon. Birds in each replicate were weighed at the commencement of the experiment and weekly thereafter. Feed consumption record was kept on a weekly basis.

Digestibility Studies: The metabolic study was carried out between the seventh and eighth week of the feeding trial. Three birds per replicate were put in metabolic cages. Droppings from each replicate group of birds were collected on a wooden sheet placed under the cages. The droppings collected were weighed fresh, dried to constant weight at 100% and ground before chemical analysis.

Economic Analysis: The effects of experimental diets on feed cost and economy of feed conversion to body weight were calculated using these formulae:

1. Feed Cost=Total cost of ingredients in ₦ kg^{-1}
2. Feeding cost=Feed intake(kg) *Feed cost(₦ kg^{-1})
3. Cost kg bw^{-1} = Feeding cost(₦ kg^{-1}) / body weight gain (g) where bw is body weight

Table 2: Percentage Composition of experimental starter diets

Ingredients (%)	Diet A (Control)	Diet B With GLM	Diet C GLM with R	Diet D GLM with M
Maize	45	45	45	45
SBM	20	15	15	15
GLM	-	5	5	5
GNC	8	8	8	8
Fish meal	3	3	3	3
Oyster shell	10	10	10	10
Wheat offal	11.25	11.25	11.25	11.25
Bone meal	2	2	2	2
Vit. Premix	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated chemical composition				
Crude protein	21.45	20.50	20.50	20.50
Ether extract	2.64	3.35	3.35	3.35
Crude fiber	6.79	7.08	7.08	7.08
Ash	2.74	3.02	3.02	3.02
Energy (Kcal/kg)	2644.36	2567.53	2567.53	2567.53

GLM = Gliricidia leaf meal, SBM = Soybean meal, GNC = Groundnut cake, R = Roxazyme G2, M = Maxigrain

Chemical and Statistical Analysis

Ground samples of feeds and faeces were analysed for dry matter (DM) by drying samples at 105^0C for 24 h in forced air oven. Ash content was measured after igniting samples in a muffle furnace at 550^0C for 4 h. The crude protein (CP) was determined by Kjeldahl method (AOAC, 1995) Ether extract (EE) was determined by Soxhlet method (AOAC, 1995) The resultant data from analysis were used to calculate the digestibilities and were further subjected to analysis using one-way and significantly different means were separated using least significance difference at 0.05 level of probability using SAS (2002). The general linear model is as defined thus:

$$X_{ij} = \mu + \alpha_i + e_{ij}$$

X_{ij}= individual data generated from the fixed treatment (effects)
μ= Grand population mean
α_i = the fixed treatments effects

e_{ij}= *the error (replicate) term within each treatment.*

Tables 1-3 show test forage, starters, and finishers composition, respectively while in Table 4, final weight and body weight gains of birds reduced with inclusion of Roxazyme G2 (diet C), though there were higher weight gains with Maxigrain (diet D) supplement ($p<0.05$). The observed improvement in the weight gains of the enzyme supplemented diet D (Maxigrain) may be explained by the fact that exogenous enzymes supplement the digestive enzymes of monogastric animals by aiding the breakdown of non-starchy polysaccharides, protein and antinutritional factors thereby increasing their nutritional value (Bedford, 2000). Birds placed on diet D (5% GLM with Maxigrain) were observed to have highest feed intake per day. This may be due to action of enzyme cellulase that breakdown cellulose for more energy and releases locked nutrients (Chot, 2006). There was significant difference in feed conversion ratio of the experimental diets. Improvements in the efficiency of utilization of poultry diets have been reported as a result of enzymes supplementation of feed (Chot, 2006) In all, it appeared that birds on diet D (5% GLM with Maxigrain) had better performance than the control diets which is in consonance with the work of Ogungbesan et al. *(*2014) who obtained the best nutrient utilization and normal blood profiles in Maxigrain supplemented GLM compared that supplemented with Roxazyme (G2)® and sole GLM.

Table 3: Percentage composition of experimental finisher diets

Ingredients (%)	Diets A (Control)	Diets B with GLM	Diets C GLM with R	Diets D GLM with M
Maize	41	41	41	41
SBM	20	15	15	15
GLM	-	5	5	5
GNC	8	8	8	8
Fish meal	3	3	3	3
Oyster shell	10	10	10	10
Wheat offal	15.25	15.25	15.25	15.25
Bone meal	2	2	2	2
Vit. Premix	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated chemical composition				
Crude protein	21.59	20.65	20.65	20.65
Ether extract	2.65	3.36	3.36	3.36
Crude fiber	6.49	6.78	6.78	6.78
Ash	2.78	3.06	3.06	3.06
Energy [Kcal/kg]	2675.36	2553.53	2553.53	2553.53

GLM = Gliricidia leaf meal, SBM = Soybean, GNC = Groundnut cake, R = Roxazyme G2, M = Maxigrain

Characteristics of Broiler Fed on Experimental Diets

Crude protein digestibility (Table 5) was significantly (P<0.05) affected by dietary treatment. Birds on diet D (5% GLM with Maxigrain) had superior digestibility of protein compared to the other diets. Diet A (Control) had the least protein digestibility (69%). Crude fiber digestibility was also significantly (P<0.05) influenced by dietary treatment. These results were in accordance with the findings of (Ademola et al., 2012) who reported an improvement in the nutrient digestibility due to Roxazyme and Maxigrain enzymes addition. Ash digestibility and Lipid digestibility were also significantly(P<0.05) influenced by the dietary treatment (Ademola et al., 2012; Ogungbesan et al., 2013)

Table 4: Effect of Gliricidia sepium Leaf Meal Supplemented with enzymes on the performance characteristics of broiler fed on experimental diets

Parameters	A (0%) (Control)	B (5% GLM)	C (5%) (5% GLM with R)	D (5%) (5%GLM with M)	SEM
Initial body weight (g)	38.65	36.58	38.13	38.46	1.13
Final body weight (g)	1900^{b}	1850^{c}	1740^{d}	2080^{a}	6.52
Body weight changes (g)	1861.35^{b}	1813.42^{c}	1701.87^{d}	2041.54^{a}	5.24
Daily body weight gain (g)	33.24^{b}	32.38^{c}	30.39^{d}	36.46^{a}	1.14
Daily feed intake (g)	101.9^{d}	113.4^{c}	115.85^{b}	116^{a}	1.32
Feed conversion ratio	3.07^{d}	3.50^{b}	3.81^{a}	3.18^{C}	2.38

^{abcd} means within the same row bearing different superscripts are significantly different (p<0.05). SEM = Standard Error of Mean,

Table 5: Effect of Gliricidia sepium Leaf Meal Supplemented with enzyme on the nutrient digestibility of the experimental diets

Parameter	A (Control)	B (5% GLM)	C (5% GLM with R)	D (5% GLM with M)	SEM
Crude protein (%)	69^{d}	72^{c}	75^{b}	78^{a}	0.88
Lipid (%)	92^{b}	91^{b}	92^{b}	94^{a}	1.00
Fiber (%)	55^{c}	52^{d}	62^{b}	64^{a}	1.00
Ash (%)	60^{b}	60^{b}	61^{a}	63^{a}	1.00
Dry matter	78^{c}	72^{d}	79^{b}	80^{a}	0.67

Means within the same row bearing different superscripts are significantly different (p<0.05). SEM = Standard Error of Mean

Table 6: Economic analyses of feeding broiler on experimental diet supplemented with Gliricidia Sepium leaf meal, enzyme Maxigrain and Roxazyme

Treatment	Cost of feeding	Cost per kg body weight	Cost per kg of feed
1	8315.04	250.15	81.60
2	8845.20	273.17	78.00
3	9082.64	298.87	78.40
4	9071.20	248.80	78.20

Cost per kilograms feed, cost per kilograms weight gain of Broiler bird fed different dietary treatment

Table 6 shows the cost effectiveness of the different dietary treatment. Diet 1 was the most expensive in terms of cost per kg. Addition of GLM reduced the cost of feed per kg by 4.10 %. Feed cost per kg reduced with increasing concentration of GLM from diet A to diet D by ₦3.40 kobo. However, the cost of Roxazyme increased the cost per kilogram of diet containing it. Nonetheless, supplementing the diet D with Maxigrain per kilogramme of diet resulted in cheaper cost per kilogramme weight gain compared to control diet by ₦3.40 kobo. This outcome in diets D, favours inclusion of the enzyme Maxigrain, (Ademola et al.,2012; Ogungbesan et al., 2013) since the desire of every investor is to maximize profit and productivity at the least cost. Hence, Maxigrain has practical advantages (Maxigrain® is blend of the most relevant digestive enzymes to serve the purpose of optimizing the cost and performance of birds. The following are active ingredients in Maxigrain® enzyme and their effect on target substrate as specified by the manufacturer ;α-amylase: Hydrolyzes glycosidic bonds from starchy material liberating metabolizable sugar, Xylanase: Acts on residues of arabinoxylans and mannans, β-Glucanase: Hydrolyzes beta glucans,;Exo-Cellulase: Hydrolyzes glycosidic bonds to liberate metabolizable sugar,; Pectinas: Hydrolyzes pectic acid,;Protease: Acts on proteins to liberate peptides and amino acids, Phytase: Hydrolyzes phytic acid to release phosphorus, Lipase: Complements indigenous lipases to digest extra fat added to the feed. Hence, benefits of Maxigrain® enzyme, optimizing the use of non-conventional feed ingredients, improving weight gain, improve litter quality and dropping consistency, improving feed conversion ratio (FCR), improves egg production and shell quality and reduces levels of DCP incorporation in the feed substantially and is strongly recommended for use in broiler diets. Per adventure, there is apparent lack of response to enzyme supplementation, reasons advanced by (Acamovic, 2001) include the following; the likelihood/possibility of the diet being fed be of extremely good quality and allow the animals to perform close to their genetic potential that enzyme has the incorrect main specificity (amylases, pectinases, β-glucanases, arabinoxylases, cellulases, hemicellulases, acidproteases, alkalineproteases, phytases, esterases, lipases) and or attendant supplementary activity for the substrate, denaturation of the enzyme before the diet is consumed, or supplementation of the diet with wrong enzyme, variation within an ingredients in the concentration or activity of proteinaceous antinutrients to the enzyme, variation in the quality of feed ingredients, animal stage of growth /maturity. It must be emphasized, however according to Bedford (1995), that for commercial use, exogenous enzymes must be able to survive the rigours of feed processing (Temperature, Pressure, and Moisture) and the in-hospitable environment of the digestive tract. Not only do these enzymes have to survive the fluctuations of pH and proreolytic attack by enzymes, but they also have to operate in these conditions at a meaningful rate in order to accomplish the necessary degrees of digestion of the intended substrate but lastly, application of enzymes allow the animal to plant phosphorus and depend less on inorganic P thereby reducing P environmental pollution (Bedford,1995)

CONCLUSION AND RECCOMENDATION

Based upon the findings in this study, it is recommended that 5% *Gliricidia sepium* leaf meal and 0.10% Maxigrain can be incorporated into broiler diets respectively. Gliricidia being a multipurpose legume species in the sense that it is an environmentally friendly species because it is involved wind breaking, erosion control, and global warming control. Further research is needed to get a more cost effective way of using GLM at various levels with various level of inclusion of enzymes

Treatment Keys: CBFN--Carbofuran; KAYV/MeOH----*Khaya ivorensis* methanol extract; KAYV/DCM----*Khaya ivorensis* Dichloromethane extract; KAYV/ODR----*Khaya ivorensis* plant materials incorporated directly into the soil as soil admix.

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