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Haematology,Serum chemistry, and biochemistry of layinhens(*Gallus domesticus*)fed Maxigrain®supplemented *Gliricidiasepium* (Jacq)leaf meal

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Abstract: *Sixty(60) laying hens (Rhode Island Red strains)were used in monitoring blood profile as influenced byGliricidia leaf meal (GLM) supplemented with Maxigrain® enzyme The birds were randomly allotted to five dietary treatments of 12 birds per treatment; each treatment was replicated into 4 groups with 3 birds per replicate. Five experimental diets were formulated; the test ingredient being Gliricidiasepium. Four of the diets were formulated with enzyme supplementation i.e. diets B, C, D, E while diet A was without enzyme supplementation. Gliricidiasepium leaf meal was included at 5%, 7.5% and 10% replacement of dietary soyabean meal in diets C, D and E respectively. The feeding trial lasted for twelve weeks after which blood variables were monitored. using One-way ANOVA,diet C had more(P<0.05)Pcv ,Hb,Rbc,Wbc , Rbc and minerals than control,while Neutrophils and lymphocytes were similar(P>0.05).Similarly C, diet also had higher(P<0.05)Glucose and Protein but lower(P<0.05)in other metabolites than control.ThereforeGLM(5%)withMaxigrain® can be used with assurance of high animal welfare and food safety.*
Key words:*,Gliricidia,Heamatology ,Layers Maxigrain® Serology*

I. Introduction

The limitation of poultry production in Nigeria has hinged particularly on the cost of feed production. Feed cost accounted for about 70-80% of the total cost of production due to the competitiveness of the conventional protein feed sources between humans and poultry. In fact, this singular problem is conspicuously responsible for the widening animal protein intake shortage because animal products are produced at costs out of reach of the populace. There is the need to produce at an affordable price to the consumers and also the farmers through the search and use of cheaper feed ingredients that are always available and have no competition with man's dietary demands i.e. non-conventional sources of feeds like*Leucaenaleucocephala* and*Gliricidiasepium[1]*so as to meet 0.83g/kg per day protein requirement for man. . Leaf meals are gaining acceptance as feed stuffs in poultry diet as due to its availability and its similar nutrient content and are considered to be un-conventional feeds. Satisfactory performances despite the inherent limitations for monogasters(cell walls and plant secondary metabolites like coumarols ,oestrogenicisoflavonestannin,etc although withantinutritional properties ,are indispensable co-evolutionary principles[2]) have been reported of various leaf meals tested in the diet of some classes of poultry birds[3].Exogenous enzyme(among which we have maxigrain-a complex enzyme with multiple function) supplements are used widely in poultry diets in an attempt to improve nutrient utilization, health and welfare of birds, product quality and to reduce pollution as well as to increase the choice and content of ingredients which are acceptable for inclusion in diets [4]. The role of enzymes as feed additive in poultry diets is well established. The advent and use of commercial feed enzymes in livestock feeding has opened new horizon for the use of hitherto waste feedstuff without detrimental effect on poultry performance. . There is therefore the need to investigate the effect of these unconventional feed resources(with various allelochemical and deleterious principles) on the physiological status of the animals especially the hematology and serum biochemistry. Haematology and serum biochemistry assay of livestock suggests the physiological disposition of the animals to and their nutrition.[5]The objective of the study was to evaluate the effect of Maxigrain® supplementation on haematological and serum chemical with biochemical characteristics in layers fed *Gliricidiasepium* leaf meal. It is thus expected that this study would provide a basis for recommendation of the supplementation of *Gliricidiasepium* leaf meal in layers diet.

II. Materials And Method

2.1 The Site of the study

This experiment was carried out at the poultry unit of the Teaching and Research Farm of the College of Agricultural sciences, OlabisiOnabanjo University, Yewa Campus, Ayetoro, Ogun State. Ayetoro is 35km North West of Abeokuta, located on latitude $70^0$12' N Longitude $30^0$3'E; a deciduous derived savannah zone in Ogun State. Climate sub-humid tropics with a gravelling ultisol soil and 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%. 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[6].

2.2 Processing of test ingredient

Fresh, young *Gliricidiasepium* leaves were harvested from pasture and range unit of the College. The long stalks were then removed to reduce fibrousness before air drying. Air drying in shade was done to reduce the moisture content of fresh leaves, to prevent fungal growth and for easy milling. Drying was completed within few days of good sunshine. The dried Gliricidia leaves was then milled to obtain Gliricidia Leaf Meal (GLM) and incorporated into five layers' diet in which soyabean was replaced with Gliricidia Leaf Meal.

2.3 Management of experimental birds

A total of 60 point of lay (16 weeks) laying birds was purchased from a reputable farm at 16 weeks of age. The birds were allotted randomly into five treatments at 12 birds per treatment. Each treatment was replicated three times at 4 birds per replicate. The experiment lasted for 12 weeks. Feed and water were given *ad-libitum.* The birds were dewormed and vaccinated appropriately. Body weight of each bird was taken at the beginning of the experiment and at 2 weeks intermittently. The parameters monitored were feed intake, Hen day production, egg weight, feed intake and feed conversion efficiency or utilization, body weight changes.

2.4 Collection of Blood Sample

At the end of the 12^{th} week feeding trials, three birds per treatment weighing close to pen average were bled trough the jugular vein to determine the value of some haematological parameters such as Packed Cell Volume (PCV),White Blood Cell (WBC), Glucose (GLU), Haemoglobin level (HB). Vials that were pre-treated with ethylene diamine tetra-acetic acid (EDTA) as anti-coagulant was used to collect 3ml of blood samples for haematological analysis to facilitate the separation of the serum. Also, blood samples for serum biochemical analysis were collected into plain vacutainers (without coagulant) for serum separation. Serum was obtained by centrifugation.

2.5 Analysis of Blood Samples

PCV was determined by microhaematocrit method, Haemoglobin concentration was measured spectrophotometrically by cyanomethaemoglobin method, W B C andRBC counts were determined using the Neubauerhaemocytometermethod. [7]. Serum total protein was determined by biuret method[8].Glucose was determined by O-toluidine method, Globulin was determined by colorimetric techniques as described by [9]. The minerals wasdetermined by [10]. Urea was by Urease method. Serum Alkaline phosphatase (S.Alp) wasestimated using the Para- Nitrophyl phosphate (Pnpp) System. Glutamate-oxalo-acetictransmmase (Got)(AST) was assayed by monitoring the concentration of oxalo acetate by hydrazone formed with 2,4,denitrophenyl–hydrazine while Glutamic pyruvictransaminase (Gpt)(ALT) was done by monitoring pyruvate hydrazine formed with 2, 4,dinitro- phenylhydrazine.Creatinine (Crea) was determined by folin-wu filtrate method without the use ofLloud'sreagent[11].Cholesterol was by direct method[12] and test diet was done using [13]

2.6 Mathematically/statistical Analysis.

Resultant data from chemo-metric of the samples ,after arc sine transformation where necessary , were further subjected to ANOVA for one way or completely randomized design using individual animals as replicate using the general linear model (GLM) procedure as packaged in [14]. the general linear model is as defined thus:

$Xij = \mu + \alpha i + eij$

μ = grand population mean

αij = individual generated from the fixed treatment (Diets A-E)

αi = the fixed treatment (Diets A-E)effects.

eij = the error (replicate) term within each treatment.

Table 1; Chemical Composition Of Test Ingredient (GLM)

Composition(%)	GLM
Crude protein	24.38
Ether extract	1.75
Crude fibre	12.45
Ash	18.07

NFE	43.36

Table 2: Percentage Comositipon of Experimental layers Diets

Ingredients (%)	Diet A 0%(Control)	Diet B 0% with M	Diet C 5%with M	Diet D 7.5% with M	Diet E 10% with M
Maize	40.00	40.00	40.00	40.00	40.00
Soybean meal	20.00	20.00	15.00	12.50	10.00
Gliricidia leaf meal	-	-	5.00	7.50	10.00
Palm kernel cake	10.00	10.00	10.00	10.00	10.00
Wheat offal	14.25	14.25	14.25	14.25	14.25
Fish meal	3.00	3.00	3.00	3.00	3.00
Oyster shell	8.00	8.00	8.00	8.00	8.00
Bone meal	4.00	4.00	4.00	4.00	4.00
Vit-premix*	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
TOTAL	100.00	100.00	100.00	100.00	100.00
Calculated Chemical Composition					
Crude protein	18.94	18.94	17.96	17.47	16.98
Ether extract	7.06	7.06	7.36	7.50	7.66
Crude fibre	7.06	7.06	7.36	7.50	7.66
Ash	2.87	2.87	3.00	3.07	3.13
Energy[KCAL/KG]	2655.3	2655.3	2534.2	2473.7	2413.2

M= Maxigrain®

Table 3 Effect of Gliricidia leaf meal supplemented with Maxigrain® on Haematologicalparametrs of layers

Parameters	Diet A 0%(Control)	Diet B 0% with M	Diet C 5%with M	Diet D 7.5 % with M	Diet E 10% with M	SEM	LOS
Pcv, (%)	50.0^{b}	50.0^{b}	52.5^{a}	52.5^{a}	42.5^{c}	3.6	*
Hb (g/100ml)	14.3	13.8	14.8	14.2	13.8	1.2	ns
Wbc (x10^{6}/mm^{3})	5000^{c}	5350^{ab}	5250^{b}	6100^{a}	5000^{c}	48	*
Rbc(2.9x10^{12}m^{3})	55500^{e}	68000^{b}	61000^{c}	73500^{a}	59500^{d}	156	*
Neutrophil(%)	62.5	65.0	65.0	66.5	65.0	2.7	ns
Lymphocyte(%)	35.0	35.0	35.0	32.5	35.0	0.9	ns

^{abcde}means within the same row bearing different superscripts are significantly different ($p<0.05$)
SEM= Standard Error of Mean, LOS= Level of Significance, NS= Not Significant

Table 4; Effect of Gliricidia leaf meal supplemented with Maxigrain® on Serum chemistry of layers

Parameters	Diet A 0%(Control)	Diet B 0% with M	Diet C 5%with M	Diet D 7.5 % with M	Diet E 10% with M	SEM	LOS
P(Mg/dl)	6.9^{b}	6.5^{b}	8.5^{a}	5.2^{c}	4.6^{d}	0.6	*
Ca (Mg/dl)	9.8^{b}	10.5^{b}	11.4^{a}	10.8^{a}	7.3^{c}	1.2	*

^{abcd}means within the same row bearing different superscripts are significantly different ($p<0.05$)
P=Phosphorus,Ca=Calcium
SEM= Standard Error of Mean, LOS= Level of Significance, NS= Not Significant

Table 5 Effect of Gliricidia leaf meal supplemented with Maxigrain®on Serum Biochemistry of layers

Parameters	Diet A 0%(Control)	Diet B 0% with M	Diet C 5%with M	Diet D 7.5 % with M	Diet E 10% with M	SEM	LOS
Glucose(Mg/dl)	169.6^{c}	174.7^{b}	176.6^{b}	261.6^{a}	162.7^{c}	18	*
Protein(g/l)	57.2^{b}	51.1^{c}	60.6^{a}	51.2^{c}	50.6^{c}	8.9	*
Urea(Mg/dl)	23.0^{ab}	22.5^{b}	22.2^{b}	24.0^{a}	21.7^{b}	1.5	*
Creatinine Mg/dl	1.1	1.4	1.3	1.4	1.4	0.3	ns
CholesterolMg/dl	185.4^{a} 159.7^{d}	146.5^{a} 175.7^{b}	168.2^{c} 13	*			
ALT(IU/L)	102.8^{c}	140.0^{a}	97.4^{d}	116.2^{b}	122.5^{a}	8.0	*
AST(IU/L)	46.3	47.3	43.8	44.6	45.6	3.6	ns
ALP(IU/L)	89.8^{d}	101.9^{c}	101.7^{c}	106.1^{b}	120.7^{a}	6.5	*

^{abcde}means within the same row bearing different superscripts are significantly different ($p<0.05$)
SEM= Standard Error of Mean, LOS= Level of Significance, NS= Not Significant

III. Results And Discussion

The chemo-assay of the test ingredient and the composition of the experimental diet as presented in Tables 1 and 2 respectively. The Pcv (Heamatocrit ,%) values as shown in Table 3 ranged from 42.5(diet E) to 52.5 (diets Cand D) which means that the enzyme inclusion interaction at that GLM level liberated enough nutrients for haematocrit synthesis at the same time preventingantinutrients from inhibiting nutrients release , absorption, and utilization [15].In the same vein Hb(g/100ml) ranged from 138(B and E)this also confirms release and utilization from ingredients by the enzyme.[16].The Wbc($x10^6/mm^3$)was from 5000(A and E) to 6100(D).This is an infection or related conditions monitory parameters,the irregular trend is difficult to arrogate to the treatments imposed .Diet A(55500)had the lowest($P<0.05$)and D(73500) highest($P<0.05$) Rbc($2.9x10^{12}m^3$) which could mean that GLM inclusion imparted other blood forming ingredients apart from protein in the diet.Neutrophils and Lymphocyte(%) which are infcetions indicators were not significantly different for one-another.Serum Phosphorus(mg/dl)was highest in C(8.5)as depicted in Table 4 and lowest in E (4.6).This shows that GLM has minerals furnishing potentials as does control diet[17]and that enzyme could liberate high minerals at that GLM-enzyme combination(5),The same reason could be adduced to Ca (mg/dl) trend which was from 7.5 (E) to 11.4 (C).This is apt for laying birds particularly the strong and thick egg shell especially in developing countries where logistics (terminals) from producers to consumers are not organize.Lastly,Table 5 contains Serum biochemical parameters, of which Glucose (mg/dl) a carbohydrate (energy furnishing) metabolite was highest($P<0.05$) in D(261.2)and lowest in E(162.7).Contrary to Glucose trend, Protein(g/l) was highest ($P<.05$) in C (60.6) and as usual,lowest ($P<0.05$) in E. The GLM-ENZ combination was exhibited interms of its superiority over the control as by [5].Urea (mg/dl) a controversial (inpoultry protein metabolism) product of protein observed breakdown in liver, with its controversy centering on its nomenclature, some school of thought believe it should be called uric acid [18]but there could be acidosis if the medium is acidic[19].Uric acid is best applied after leaving the digestive and circulatory system for the excretory system[20]which its abnormally high serum contents depicts a malfunction of kidney clearance from blood.The trend seems irregular from diet point of view.Creatinine(mg/dl)is similar to urea in terms origin and diagnostics purposes and were similar ($P<0.05$)in all the treatments which indicate less or non hepatomyo-cytolytic and nephrocytolytic properties of the diets .Cholesterol(mg/dl) was indefinitely highest ($P<0.05$) in A(185.4) and lowest ($P<0.05$) in C (146.5), is one of the most important parameters of public health and food safety importance .Though,not discerned into LDLP(low-density lipo-protien) and HDLP(high-density lipo-protien) the fact still remains that even the control was higher than the rest.ALT(IU/L)(Alanine amino transferase)or SGPT(Serum glutamate pyruvic transaminases) is an enzyme whoseabmormally high serum content depicts extensive hepatolytic/hepatocytolytic conditions and moderate to low injury or damage to the heart hence used specifically for liver test[11] whose highest was observed in E (122.5) and lowest ($P<0.05$) in C (97.4) .Concerning Serum glutamic-oxalo acetic transaminasesor AST(Aspertate-amino transferases) ,a diagnostic aid in viral hepatitis and myocardial infarction, that is liberated prominently in cardiotoxicity or heart injury and to an extent in moderate or low injury to kidney and liver,was highest ($P<0.05$) in B(47.3) and also lowest($P<0.05$) in C(43.8) which denotes non toxicity of the GLM-ENZ to heart ,kidney, or liver.Serum alkaline phosphate (IU/L) was some how linear in that it increased from A(89.9) to E(120.7) which depict a slight ,[21],hepatotoxic/hepatocytolytic properties in that it is used in diagnosis of liverinjury or damage.

IV. Conclusion and Recommendation

From the various parameters monitored ,the inclusion, the inclusion GLM particularly at 5% level of inclusion with Maxigrain will cause no discomfort or dideasiness to the animals,furthermore,and most importantly the combination is of high health importance because of its low cholesterol content which is a cardiac friendly development in addition to the cheapcarotene that is present in majority of herbage feedstuff .Hence Eyes and Earth eill be taken care of , while enjoying the poultry animal protein.Therefore technology that will support browes legume establishment and utilization. So that other benefits like pulp, fuel(charcoal),environmental purification and global warming control will be exploited to the fullest.

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