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ORIGINAL RESEARCH ARTICLE

Serology of laying chickens fed enzyme supplemented browse legume leaf meal

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ABSTRACT

Sixty (60) laying hens (Rhode Island Red strains) were used in monitoring blood profile as influenced by Gliricidia 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 Gliricidia sepium. Four of the diets were formulated with enzyme supplementation i.e. diets B, C, D, E while diet A was without enzyme supplementation. Gliricidia sepium leaf meal was included at 5%, 7.5% and 10% replacement of dietary soya bean meal in diets C, D and E respectively. The feeding trial lasted for twelve weeks after which blood indices were monitored using completely randomised design .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) among all the treatments .Also, diet C had higher(P<0.05) Glucose and Protein than the control but all other metabolites were lower (P<0.05) in diet C than control. Therefore GLM (5%) with Maxigrain® can be used with satisfactory result in terms animal performance and welfare.

Keywords: Gliricidia, Haematology, Layers, Serum-profile, Maxigrain®

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 (lge *et al*., 2006). 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 *Leucaena leucocephala* and *Gliricidia sepium* (Simons and Stewart,1994) 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 to the conventional feeds and are considered to be un-conventional feeds. Satisfactory performances despite the inherent limitations for Monogastric animals (cell walls and plant secondary metabolites like coumarols ,oestrogenic isoflavones tannin,etc(although with antinutritional properties) are indispensable co-evolutionary principles (Ogungbesan,2011) have been reported of various leaf meals tested in the diet of some classes of poultry birds (Ige *et al*.,2006).Exogenous enzymes (among which we have Maxigrain-a complex enzyme with multiple function) supplements are used widely in poultry diets in an attempt to improve nutrients utilization, health and welfare of birds, products quality and to reduce pollution as well

as to increase the choice and content of ingredients which are acceptable for inclusion in diets (Acamovic,2001). The role of e**nzymes 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 haematology and serum biochemistry. Haematology and serum biochemistry assay of livestock suggests the physiological disposition of the animals to environment and their nutrition (Ogungbesan *et al*., 2009).The objective of the study was to evaluate the effect of Maxigrain® supplementation on haematological and serum biochemistry in layers fed *Gliricidia sepium* leaf meal. It is thus expected that this study would provide a basis for recommendation of the supplementation of *Gliricidia sepium* leaf meal in layers diet.

MATERIALS AND METHODS

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, Olabisi Onabanjo University, Yewa Campus, Ayetoro, Ogun State. Ayetoro is 35km North West of Abeokuta. It is located on Latitude7^0 15^N to longitude 3^0 3^E, Altitude is 90-120m above sea level, Temperature 28. 9^0c, Rainfall, 1945mm, Relative Humidity 72.81 %, Evaporation 1806.9m, soil type- oxicpaleustalf, soil texture: sandy loam and vegetation: sub-humid forest mosaic savanna type; Elements (Cmo^1kg^{-1}) Na. 50, K. 22, Ca. 95, Mg. 86, H^+ 0.13, C.E.C. 2.67, Ph (H_20) 5.55, P($MgKg^1$) 6.86, Zn ($MgKg^1$) 6.75, OC. (%).79, OM (%) 1.19, N (%).079, Sand (%) 75.16, Clay (%) 15.57, Silt (%) 9.25). 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 ingredient**:** Fresh, young *Gliricidia sepium* leaves were harvested from pasture and range unit of the College. The long stalks were then removed to reduce fibre content 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.

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. Collection of Blood Sample**:** At the end of the 12^{th} week feeding trials, three birds per treatment weighing close to hen average were bled trough the jugular vein to determine the value of some blood 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. Also, blood samples for serum biochemical analysis were collected into plain vacutainer bottles (without anticoagulant) for serum separation. Serum was obtained by centrifugation.

Table 1: Chemical composition of Glyricidia meal

Composition(%)	GLM
Crude protein	24.38
Ether extract	1.75
Crude fibre	12.45
Ash	18.07
NFE	43.36

Table 2: Percentage Composition of Experimental layers Diets

Ingredients (%)	DietA 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	100	100	100	100
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]	2655.30	2655.30	2534.30	2473.70	2413.20

M= Maxigrain®

Analysis of Blood Samples:

The PCV was determined by microhaematocrit method, Haemoglobin concentration was measured spectrophotometrically by cyanomethaemoglobin method,WBC and RBC counts were determined using the Neubauer haemocytometer method. (Eseivo and Sarror, 1992). Serum total protein was determined by biuret method (Reinhold, 1953).Glucose was determined by O-toluidine method, Globulin was determined by colorimetric techniques as described by (Raj and Holder, 1983). The electrolyte were determined by (Boehringeran, 1979). Urea was by Urease method. Serum Alkaline phosphatase (S.Alp) was estimated using the Para- Nitrophyl phosphate (Pnpp) System. Aspartate aminotransferase (AST) was assayed by monitoring the concentration of oxalo acetate by hydrazone formed with 2, 4,denitrophenyl–hydrazine while Alanine aminotransferase (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 of Lloud'sreagent (Ogungbesan *et al*., 2009).Cholesterol was by direct method (Toro and Ackerman,1975) .The test diets were analysed using (A.O.A.C. 1995)

Statistical analysis

Resultant data from chemo-metric of the blood and serum samples, after arc sine transformation where necessary, were further subjected completely randomized design using individual animals as replicate using the general linear model (GLM) procedure (SAS 2002).

RESULTS AND DISCUSSION

The gross composition of the test ingredient and the experimental diets as presented in Tables 1 and 2, respectively. The haematological variables are important indices that reflect the physiological state of the individual animal. The ability to interpret the blood profile in normal and diseased condition is among objectives of haematological studies. The blood contains myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and nutritional status of animals. Hence, World Health Organisation has recommended the use of blood and biochemical parameters in medical nutritional assessment (Provan *et al*.,2004). Haematological parameters most commonly used for assessment in nutritional studies include PCV, RBC, HBC, MCHC, MCV and clotting time (Adeyemi *et al*., 2000). The PCV or Heamatocrit (Ht, Hct, crit) in % (is also called EVF erythrocyte volume fraction is the percentage (%)of red blood cells in blood is also involved in the transport oxygen and nutrients. Although its increased shows a better transportation and thus results in an increased primary and secondary polycythemia, abnormally high level signify systemic dehydration (Etim, *et al*., 2014) values as

Table 3 Effect of Gliricidia leaf meal supplemented with Maxigrain® on Haematological parameters 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	50.00^{b}	50.00^{b}	52.50^{a}	52.50^{a}	42.50^{c}	3.60	*
Rtc	100.00	100.00	105.00	105.00	85.00		
Hb (mmol/L)	2.22	2.12	2.29	2.20	2.12	0.19	NS
Rtc	100.00	97.00	103.00	99.00	97.00		
Wbc (7.8 x10^{6}/L)	5000.00^{c}	5350.00^{ab}	5250.00 ^{b}	6100.00^{a}	5000.00^{c}	48.00	
Rtc	100.00	107.00	105.00	122.00	100.00		
Rbc (5.4 x10^{12}/L)	55500.00^{e}	68000.00^{b}	61000.00^{c}	73500.00^{a}	59500.00^{d}	156.00	*
Rtc	100.00	123.00	110.00	132.00	107.00		
Neutrophil (%)	62.50	65.0 0	65.0 0	66.50	65.00	2.7 0	Ns
Rtc	100 .00	104.00	104 .00	106.00	104.00		
Lymphocytes (%)	2.07	2.07	2.07	1.91	2.07	0.05	Ns
Rtc	100.00	100.00	100.00	93.00	100.00		

^{abcde} :Means within the same row bearing different superscripts are significantly different P(<0.05).SEM=Standarnd error of means.LOS=Level of significance, Ns=Not Significance Rtc=Relative to control

shown in Table 3 ranged from 42.50(diet E) to 52.50 (diets C and D) which suggests that the enzyme inclusion interaction of GLM at these led to an interactive synergistics level that released enough nutrients for haematocrit synthesis at the same time preventing antinutrients from inhibiting nutrients release (Fasuyi and Kehinde ,2009).

In the same way, Hb which is the iron-containing oxygen –transport metalloprotein in the red blood cells of all vertebrates with the exception of the fish family channicthyldae as well as tissues of invertebrates. Haemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Sidell and O′ Brien, 2006). Haemoglobin (mmol/L) ranged from 2.12 (B and E) to 2.29(C) this also confirms release and utilization from ingredients by the enzyme.(Ogungbesan *et al*., 2013) .The white blood cell and its differential counts major functions are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response.Abnormally higher (leucocytosis) and lower (leucopenia) are two undesirable conditions in that, the former is an indication of damages destruction of tissues and infections, While the latter a signal of a situation in which the circulation and marginal pool of W.B.C. is small and consequently reduced phagocytosis in case of infection (Bell *et al*. 1972). White blood cell (7.8x10^{6}/L) was from 5000.00(A and E) to 6100.00(D).This is an infection or related conditions monitory parameters, the irregular trend is difficult to arrogate to the treatments imposed in that control with tested diet had the same with 10%GLM which suggests the less impact of GLM inclusion with enzyme on the WBC values.

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(mmol/L)	6.90^{b}	6.50^{b}	8.50^{a}	5.20^{c}	4.60^{d}	0.60	*
Rtc	100.00	94.00	123.00	75.00	67.00		
Ca (mmol/L)	9.80^{b}	10.50^{b}	11.40^{a}	10.80^{a}	7.30^{c}	1.20	*
Rtc	100.00	107.00	116.00	110.00	74.00		

^{abcde}: Means within the same row bearing different superscripts are significantly different (P<0.05). SEM=Standard error of means. LOS=Level of significance, Ns=Not Significance Rtc=Relative to control

Diet A (55500.00) had the least significant value of RBC (P<0.05) and D (73500.00) highest (P<0.05) significant value of RBC ($5.4x10^{12}$/L) which could suggest that GLM inclusion improve blood formation and availability of other ingredients apart from protein in the diet. Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration. According to Etim *et al.,* (2014) red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs. The Neutrophils (%) among all the treatments showed no statistically significant (P>0.05) difference likewise the Lymphocyte (%).These two leucocytes differential counts are infections' indicators. Serum Phosphorus(mmol/L)was highest in C(8.50)as depicted in Table 4 and lowest in E (4.60).This shows that with the supplementation of GLM with has increased minerals furnishing potentials of minerals like phosphorus and also reducing the antinutritional factors like phytate even in quantity more than that of the control there by leading to digestion ,absorption and circulation in the blood for various biological functions (Roberts and Choct,2006) as does control diet (Ige *et al*.,2006) and that enzyme could liberate high minerals at that GLM-enzyme combination(Fasuyi and Kehinde ,2009). The same reason could be adduced to Ca (mmol/L) trend which was from 7.50 (E) to 11.40 (C) (Roberts and Choct,2006) .This is apt for laying birds particularly because calcium is required for strong and thick egg shell formation to be able to withstand transport logistics (terminals) from producers to consumers.(Oluyemi and Roberts, 2000) Lastly, Table 5 contains Serum biochemical parameters, of which Glucose (mmol/l) a carbohydrate (energy furnishing) metabolite was highest (P<0.05) in D (145.97) and lowest in E (90.77).Contrary to Glucose trend, Protein (g/L) was highest (P<.05) in C (60.60) and as usual, lowest (P<0.05) in E. The GLM-ENZ combination was exhibited in terms of its superiority over the control in that more protein could have been result from the action of the enzymes on the cell wall thereby releasing more nutrients as reflected by the values present in the serum,a phenomenon that has been reported by Fasuyi and Kehinde (2009). .Uric acid (mmol/L) in poultry protein metabolism is a product of protein observed breakdown in liver, (Wang *et al*., 2006) which its abnormally high serum contents depicts a malfunction of kidney clearance from blood (Yang *et al*., 2009).

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(mmol/L)	94.64^{c}	97.48^{b}	98.54^{b}	145.97^{a}	90.77^{c}	10.04	*
Rtc	100 .00	103.00	104.00	154.00	96.00		
Protein(g/L)	57.20^{b}	51.10^{c}	60.60^{a}	51.20^{c}	50.60^{c}	8.90	*
Rtc	100 .00	89.00	106.00	90 .00	88.00		
Uricacid(mmol/L)	1.38^{ab}	1.35^{b}	1.33^{b}	1.44^{a}	1.30^{b}	0.09	*
Rtc	100 .00	98.00	97.00	104.00	94.00		
Creatinine(µmol/L)	97.43	124.00	115.14	124.00	124.00	26.57	Ns
Rtc	100.00	127 .00	119.00	127.00	127.00		
Cholesterol (mmol/L)	4.82^{a}	4.15^{d}	3.81^{e}	4.57^{b}	4.37^{c}	0.34	*
Rtc	100 .00	86.00	79.00	95.00	91.00		
ALT(IU/L)	102.80^{c}	140.00^{a}	97.40^{c}	116.20^{b}	122.50^{a}	8.00	*
Rtc	100.00	136 .00	95.00	113.00	119.00		
AST(IU/L)	46.30	47.30	43.80	44.60	45.60	3.60	Ns
Rtc	100 .00	102 .00	95 .00	96.00	98.00		
ALP(IU/L)	89.80^{d}	101.90^{c}	101.70^{c}	106.10^{b}	120.70^{a}	6.50	*
Rtc	100 .00	113.00	113.00	118.00	134.00		

^{abcd}: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 Rtc=Relative to control

The trend seems irregular from diets point of view which suggests that the diets neither led to increase or decrease value and hence malfunctioning of the kidney. Creatinine (mmol/L) is similar to uric acid 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 in other words since the values observed in the control diet which is conventional is statistically not different (P>0.05) from other diets, it suggests that the other tested diets are not injurious to both the liver and the kidney which are among the vital organs in the animals (Ogungbesan *et al.,* 2013) .Cholesterol (mmol/L) was statistically highest (P<0.05) in A(4.82) which is the control diet and lowest (P<0.05) in C (3.81) which is one of the tested diet, is one of the most important parameters of public health and food safety. Though, not differentiated into LDLP (low-density lipo-protien) and HDLP(high-density lipo-protien) since the control was statistically highest P<0.05) among the other tested diets, this is showing , among others, the advantages of leaf meal inclusion particularly with enzymes supplementation . ALT (Alanine amino transferase, IU/L) or is an enzyme whose abnormally high serum content depicts extensive hepatolytic/hepatocytolytic conditions and moderate to low injury or damage to the heart hence used specifically for liver test (Ogungbesan *et al*., 2009) . ALT was significantly highest (P<0.05) in diet E (122.50) and lowest significantly (P<0.05) in C (97.40) which is still pointing to the safe use of the leaf meal to that level.

Concerning Serum 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 similar (P>0.05) across the treatments though B had 47.30,followed by with 46.30, then E having 45.60, and also lowest (P<0.05) in C (43.80) after D (44.60) which denotes non toxicity of the GLM-ENZ to heart ,kidney, or liver. Serum alkaline phosphate (IU/L) was somehow linear in that it increased from A(89.90) to E(120.70) which depict a slight, hepatotoxic/hepatocytolytic properties in that it is used in diagnosis of liver injury or damage (Anbarasu,*et al*., 2002). Factors although not monitored that can influence blood profile include, age, sex,genotype, breed or strain, sampling techniques, environmental, non-environmental, stress, hormonal changes and testing methodology. Others are oestrus cycle, pregnancy and parturition, genetics, method of breeding, breeds of animal, housing, feeding, fasting, extreme climatic conditions, stress, exercises, transport, castration and diseases. (Etim, *et al*., 2014). Inclusion of leaf meal especially that of legumes will also confer other advantages like phyto–ostrogen which will also increase efficiently of utilization from of nutrients (Ogungbesan *et al.,* 2014), anti – helminthic properties which reduce the incidence (Lisonbee *et al*., 2009) of helminthiasis particularly the nemathelminths. Lastly, leaf meals (*Giliricidia sepium* inclusive) has inherent fibrous content that can simulate less nutritious diet and or nutrients deprivation used to force moult layers (Landers, *et al.,* 2005).

CONCLUSION AND RECOMMENDATION

From the various parameters monitored, the inclusion Gliricidia leaf meal particularly at 5% level of inclusion with Maxigrain will cause no discomfort or disorder to the animals, furthermore, and most importantly the inclusion of enzyme is of high health importance because of lower cholesterol content which is a cardiac friendly development, while enjoying the poultry animal protein. Therefore, technology that will support browses legume establishment and utilization. So that other benefits like pulp, fuel (charcoal), environmental purification and global warming control will be exploited to the fullest.

CONFLICT OF INTEREST

Authors declare that no conflict of interest exists.

REFERENCES

A.O.A.C 1995 Association of official analytical chemist official method of analysis 15th edition, Washington D.C

Acamovic,TA 2001 . Commercial application of enzyme technology for poultry production *World Poult. Sci. J.,* 57. 226-242

Adeyemi, O.A., Fashina, O.E. and Balogun, M.O. 2000. Utilization of full-fat Jatropha seed in broiler diet: Effect on haematological parameters and blood chemistry. In: *Proceedings of 25th Annual Conference of Nigerian Society for Animal Production*

(NSAP) 19th –23rd, March, 2000, Umudike,Nigeria, Pp. 108 – 109.

Anbarasu, C., Naraya, Duha, KSharina,. And N. Uma, 2002 .. Blood biochemical profile and rumen fermentation pattern of goats fed leaf mixture or conventional cakes as dietary protein supplements. *Asia- Australasia J. of Animal Science* 15 (5) 665-670

Bell, G.A., Davidson, J.H. and Emslie-Smith, Donald (1972). Textbook of physiology and biochemistry. (ELBS) Tetiot palace Edinburgh Pp. 448.

Boehringeran MnheimGimbh Diagnostica 1979 6800 Mannheim 31,Germany Cat No1249

Esievo, K.A.N and D.J Sarror. 1992 Haematological assaya;A laboratory manual in veterinary clinical Pathology 1st edA.B.U. Zaria Kaduna Faculty of Veterinary Medicine 243Pp

Etim,NseAbasi N., Williams Mary E.,Akpabio Uduak and Offiong, Edem A,E. 2014 Haematological Parameters and Factors Affecting Their Values. *Agricultural Sciences* 2(1) :37-46

Fasuyi, A.O and O.A Kehinde , 2009 Effect of cellulase-glucanase-xylanase combination on the nutritive value of *Telfaria occidentalis* leaf meal in broiler diets. *Journal of cell and Animal Biology* 3(11); 188-195

Ige, A.O, A.A, Odunsi, J.A.Akinlade, L.O. Ojedapo, S.A.Ameen, O.A.Aderinola and, T.A. Rafiu. 2006 Gliricidia Leaf meal in Layer's Diet: Effect on Performance, Nutrient Digestibility and Economy of Production. *Journal of Animal Veterinary Advances 5(6): 483-486(1)*

Landers, K.L., Howard, Z.R., Woodward, C.L., Birthold, S.G. and Ricke, S.C 2005 Potential of alfalfa as an alternative moult induction diet for laying hens: egg quality and consumer acceptability. *Biors. Tech*. 96; 907-911

Lisonbee , Larry D., J. J. Villalbai, Fred. D Provenza, and Jeffrey O. Hall, 2009 Tannins and self-medication: Implication for sustainable parasite control in herbivores. *Behav. Prcs*. 82:184-189

Ogungbesan, A. M. Akanji A. M., Akeem A. L.and Elizabeth, M. 2009 Effects of Tephrosia *bracteolata* (Gull et perr) Based Diets on some blood parameters of West Africa dwarf goats .*Epizootiology& Animal Health In West Africa* 553-65

Ogungbesan, A,M 2011 Secondary plant metabolites utilization in west African dwarf does fed combined levels of *Andropogon gayanus* (kunzth)and Gliricidia sepium(Jacq)with Cassava offal based concentrate *Journal of Environmental Issues and Agriculture in Developing Countries,*3,(2): 1-9

Ogungbesan ,A.M .Aluko ,F.A, Adeleke , G.A and Akanji ,A.M 2013 The Effect of Enzymes on Blood Constituents and Minerals Utilization in Broilers Fed *Gliricidia sepium* (Jacq) *Epizootiology and Animal Health in West African* 9 (In press)

Ogungbesan, A.M,. Lamidi,A.,Fasina,O.E.,and Eniolorunda,O.O.2013.*Leucaena pallida's* (berth) seedling vigour indices as influenced by different dormancy breaking presowing treatments. *Journal of Enviromental Extension* 11:20-26

Ogungbesan, A .M**.** Fasina, O.E. Lamidi, A. and Fajemisin, A,N. 2014 Macro-elements utilization in W.A.D. does fed combined levels of *Andropogon gayanus* (Kunthz) and *Gliricidia sepium* (Jacq) with cassava offal based concentrate .*African Journal of Livestock Extension* 14 ; 65 -71

Oluyemi,J.A and Roberts, F.A 2000 Poultry production in warm wet tropics. Spectrum books Ibadan. Pp. 228

Provan,D. Singer,C.R.J. Baglin,T and Lilleyman,J. 2004 Oxford handbook of clinical haemetology 2nd ed. Oxford University Press.Pp732

Raj, R and Holder, M .1983 Aspartate amino-transferase.In methods of enzymatic analysis 3rdedn, Bergmeyer . H.U.J and Grasel,, M.L.(Eds) WenhieimVelaghinePp416-433

Reinhold, J.G. 1953 . Manual determination of serum total protein, Albumin, and globulin fractions by the biuret method .In:Reiner, M (ed) standard methods of clinical Chemistry N.Y. Aca. Press Pp. 88.

Roberts, J.R and Choct, M.(2006) Effects of commercial enzymes preparation on egg and egg shell quality in laying hens. *British Poultry Science* 47(4):501-510

Sidell, B.D. and O'Brien, K.M. 2006 when bad things happen to good fish: the loss of haemoglobin and myoglobin expression in Antarctic ice fishes. *The Journal of Experimental Biology*,209:1791-1802

Simon A. J and J.L. Stewart 1994 *Gliricidia sepium* – a multipurpose tree legume. In:

Forage Tree Legumes in Tropical Agriculture (R.C Gutteridge and H.M Shelton, eds)Walling Ford, UK CABI Pg30-48

Statistical Analytical System SAS/STAT; 2002 Guide for personal computers, S.A.S Inst.IncNew York

Toro,.and A .Ackerman 197 5 Practical Clinical ChemistryI[st] EdBoston U.S.A Bal. Little Brown and Company, Pg 16-18

Wang, R.J. S.X. Fui, C.H, Miao, and D.Y Feng 2006 Effects of different Mycotoxin Adsorbents on Performance, Meat Characteristics and Blood Profiles of Avian broilers Fed Mold Contaminated Corn. *Asia-austrlasia J .Anim .Sc* 19(1) 72-79

Yang,Y.NGuo,J.,Yoon,S.Y.,Jin,Z.,Choi,J.Y.,Pia o,X.S,Kim,B.W.Ohh,.,WangS.Jand,B.JChae. 2009 Early energy and protein reduction; effect on growth, blood profiles and expression of genes related to protein and fat metabolism in broilers. *British journal of poultry science.*50 (2) : 218-227.