Nigerian J. Anim. Sci. 2018, 20 (1): 52-60

Antioxidant status and serology of laying pullets fed diets supplemented with mistletoe leaf meal

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***Target Audience:** Poultry farmers, Nutritionist and physiologist*

Abstract

The study was conducted to examine the potential of African mistletoe (Viscum album L.) as alternative feed additives for laying pullets. Fresh African mistletoe leaf were harvested from cocoa trees and air dried to constant weight. The leaves were grounded and were designated as mistletoe leaf meal (MLM). Sixty (60) eighteen weeks old ISA Brown pullets were randomly allocated to four dietary treatments when egg production was 4% in a completely randomized design. The birds were fed layer ration and dietary mistletoe supplement as treatments T1 (basal diet + 0% MLM), T2 (basal diet + 2% MLM), T3 (basal diet + 4%MLM) and T4 (basal diet + 6%MLM) during eight week feeding trial. At the end of the feeding trial, blood was collected through the jugular vein into a sample bottle for serum oxidative status assay; malondialdehyde (mMDA/mgprotein), total antioxidant activity (mmol/litre), glutathione peroxidase (GPx, µgGSH/min/mgprotein), superoxide dismutase (SOD, U/min/mg protein) and catalase (nmH_2O_2/min/mg protein) and serum biochemical assay; total protein and its fractions, cholesterol and its fractions, alanine amino transferase (ALT), aspartate amino transferase (AST). The result obtained revealed that lipid peroxidation in laying pullets were significantly ($p<0.05$) lowered by mistletoe supplementation while total antioxidant activity of laying pullets significantly ($p<0.05$) increased with mistletoe inclusion. The result revealed that 6% mistletoe supplementation significantly ($p<0.05$) enhanced catalase and glutathione peroxidase activity. Pullets fed mistletoe leaf meal had significantly ($p<0.05$) lower serum cholesterol and low density lipoprotein compared to the control. Serum triglyceride and high density lipoprotein were not significantly ($p<0.05$) influenced by mistletoe. The ALT and AST of birds fed mistletoe leaf meal compared favourably with the control. It can be concluded that mistletoe inclusion in laying pullets diet enhanced antioxidant profile, does not pose organ toxicity and tends to confer hypocholesterolemic response on pullets.

Keywords: *Antioxidant activity; Serum cholesterol; Lipid peroxides; Mistletoe; Pullets*

Description of Problem

Alternative therapy to antibiotics, synthetic growth promoters and drugs usage in animal agriculture are gaining global recognition, with increase in consumer consciousness of safety of animal products. Plants with medicinal properties are been investigated as remedies to synthetic/inorganic material usage in agriculture. Plant extracts are ingredients of many commercial diet preparations currently used in animal

production. They provide antioxidant (1), antimicrobial (2), immunity development (3) and growth promoting effects (4). In general, herbs and other plant extracts can help improve feed intake, digestive enzymes, and reinforce immunity (5).

Medicinal plants have been a readily available source of drugs since ancient times and even today almost 50% of the new drugs have been patterned after phytochemicals (6). Recognizing the medicinal significance of indigenous plants, World Health Organization (WHO), in its 1997 guideline, stated that "effective locally available plants can be used as substitutes for drugs" (6). In comparison to commonly used conventional forages, mistletoe contains low protein, moderate fiber, and is high in minerals; therefore it can provide alternative mineral and forage sources for ruminant feeding (7, 8, 9). The main constituents of mistletoe are lectins (mistletoe Lectins I, II, III), viscotoxins, polysaccharides, cyclitols, flavonoids, phenyl propane derivatives, triterpenoids like amyrin, betulinic acid, oleanolic acid, phytosterols, amino acids, alkaloids, cyclic peptides, histamine, acetylcholine, and 9.3% protein (10). Previous studies have demonstrated that extracts from this plant possess pharmacological properties having immunomodulatory, anti-inflammatory, cardiovascular, and antimicrobial effects (7, 11,12). Nwaegerue (13) observed a glucose lowering effect in normal and diabetic rats using leaf extracts of *V. album* (7). Shi (14) found that some compositions of Mistletoe (Mistletoe alkali) can act effectively *in vitro* as antioxidants and peroxyl radical scavengers.

Oxidative stress, defined as an imbalance between oxidants and antioxidants in favor of oxidants, leads to multiple biochemical changes in animal and human organism, which are causative factors of several chronic diseases, such as cardiovascular diseases, mutagenesis and cancer, several neurodegenerative disorders and aging process (6). Endogeneous antioxidants in medicinal herbs may play an important role in antioxidative defense against oxidative damage, possibly protecting the biological functions of cells (14). There is increasing interest in the protective and biological function of natural antioxidants contained in herbs, which are candidates for the prevention of oxidative damage. There is dearth of information on effects of this plant on serum of laying pullets. Therefore, this experiment was designed to evaluate the effect of supplementation of varying African mistletoe leaf on serum biochemistry and oxidative status of pullets.

Material and Methods

The study was carried out at the Poultry unit, Department of Agricultural Technology of the Federal polytechnic Ado Ekiti, Ekiti State. Fresh African mistletoe leaves were harvested from cocoa trees and shade dried to constant weight. The dried leaves were ground and designated as mistletoe leaf meal (MLM). Sixty (60) eighteen weeks old ISA Brown pullets were used in the study. The pullets were housed in battery cage and were randomly allocated to four dietary treatments when egg production was 4% in a completely randomized design. The birds were fed layer ration and dietary mistletoe supplement as treatments T1 (basal diet + 0% MLM), T2 (basal diet + 2% MLM), T3 (basal diet + 4%MLM) and T4 (basal diet + 6%MLM), respectively during eight week feeding trial. All diets were formulated to meet or exceed the nutrient

requirements of laying pullets (15). At the end of the feeding trial, blood was collected through the jugular vein into a sample bottle; serum was separated by centrifugation and stored at -20^{0}C before analysis. Serum biochemical assay was carried out using Randox commercial assay kits and its procedures. Determination of serum total antioxidant capacity activities was carried out according to (16). Its principle is based on a standardized solution of Iron-Ethylene diamine tetraacetic acid Fe–EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction, leading to the formation of hydroxyl radicals (OH). These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS) (17, 18, 19). Antioxidants from the added sample of fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of colour development defined as the antioxidant activity.

Superoxide dismutase (SOD) is estimated by the method of Marklund and Marklund (20) adopted as follows by Soon and Tan (21): To 2.1 ml of 50 mM buffer, 0.02 ml of enzyme source and 0.86 ml of distilled water. The reaction is initiated with 0.02 ml of 10 mM pyrogallol and change in absorbance monitored at 420 nm. One unit of SOD is defined as that amount of enzyme required to inhibit the auto-oxidation of pyrogallol by 50% in standard assay system of 3 ml. The specific activity is expressed as units/min/mg protein.

Glutathione peroxidase activity is estimated as described by Rotruck *et al.* (22) and Ellman's (23). Briefly, to 0.5 ml, 0.4 M buffer pH 7.0), 0.2 ml enzyme source, 0.2 ml 2 mM reduced glutathione (GSH), 0.1 ml 0.2 mM H_2O_2 were added and incubated at room temperature for 10 min along with a control tube containing all reagents except enzyme source. The reaction was arrested by adding 0.5 ml of 10% TCA, centrifuged at 4000 rpm for 5 min and the GSH content in 0.5 ml of supernatant was estimated. The activity expressed as µg of GSH consumed/min/mg protein.

Catalase activity is estimated by Beers and Sizer (24) method. The assay system contains 1.9 ml, 0.05 M buffer pH 7.0 and 1.0 ml 0.059 M H_2O_2. The reaction is initiated by addition of 0.1 ml enzyme source. The decrease in absorbance is monitored at 1 min interval for 5 min at 240 nm and activity is expressed as nmoles of H_2O_2 decomposed/min/mg protein. Serum lipid peroxidation was determined using TBARS assay according to (25)

Statistical Analysis

Data obtained in this study was subjected to one way ANOVA to detect significant effects with a confidence level of 95% and the means were separated using Duncan multiple range test.

Result and Discussions

Serum biochemistry can reflect the condition of an organism and the changes happening to it under the influence of internal and external factors (26). The result as shown in Table 2, revealed that mistletoe inclusion significantly ($p<0.05$) influenced serum total protein of pullets across the treatment. Pullets fed diets with 6% inclusion of MLM had significantly ($p<0.05$) highest serum total protein. However, serum globulin and albumin were not influenced by mistletoe leaf meal

inclusion. Mistletoe inclusion tends to induce hypocholesterolemic response on pullets, this is revealed by significantly ($p<0.05$) lower serum cholesterol and low density lipoprotein in pullets fed mistletoe leaf meal-based diets (T_2 - T_4)

Table 1: Composition of Basal Diet

Ingredients	Composition (%)
Maize	44.5
Wheat offal	9.40
Palm kernel cake (PKC.)	13.8
Groundnut cake (GNC.)	7.50
Fish meal	5.70
Blood meal	1.90
Bone meal	2.00
Salt	0.25
Premix	0.25
Lysine	0.25
Methionine	0.25
Total	**100.0**
Calculated Nutrient Analysis (%):	
Dry matter	78.17
Crude protein	15.98
Metabolisable energy (ME kcal/kgDM)	2430.2
Ether extract	3.83
Crude fibre	5.16
Lysine	0.94
Methionine	0.51
Calcium	4.21
Phosphorus	0.44

This is in agreement with earlier findings (27) who reported that administration of crude moringa extract lowered serum cholesterol in rabbits. This result also agrees with the work of Ghasi *et al.* (28) who reported the hypocholesterolemic effect of crude extract of moringa leaves in the serum of Wister rats fed high fat diet. The mechanism to lower cholesterol may be by lowering plasma concentrations of Low Density Lipoprotein (28). This result is contrary to the report of Ben *et al*., (29) that hypercholesterolemia is induced by crude extract of mistletoe leaf in wistar rats. However, triglyceride and high density lipoprotein apparently increased with mistletoe inclusion. Also the report of (29) that increase in plasma level of high density lipoproteins (HDL) and decrease in low density lipoprotein (LDL) of wistar rats administered crude extract of mistletoe leaf. There exist a negative relationship between LDL and HDL and this probably suggests why the HDL increases, the LDL decreases (29). The fall in serum cholesterol level of pullets suggest that

mistletoe could be used to produce animal products with reduced cholesterol content.

The AST of birds fed mistletoe leaf meal had statistically lower values compared favorably to the control, while similar values were obtained in ALT of the birds across the treatments. The observations of no changes in liver enzymes in comparison to control, may indicate that mistletoe had no negative effect on liver functions. This is contrary to reports of Hossain *et al*., (30) that mistletoe increased activity of liver enzymes in broilers. This result is consistent with that reported by Ohiri (31) and Ben *et al.,* (29) which suggested that the extract had a high safety margin and may not contain any cardio- or neuro-toxic agents. This probably explains why there is a widespread use of this extract for local or traditional treatment of diseases and for preventive purposes without adverse effects reported (29).

Table 2: Serum Biochemistry of laying pullets fed mistletoe leaf meal supplemented diets

Parameters	T1 (0%MLM)	T2 (2%MLM)	T3 (4%MLM)	T4 (6%MLM)
Total protein g/l	63.81^{ab}	66.78^{ab}	49.98^{b}	72.24^{a}
	3.52	0.14		
Globulin g/dl	4.00	4.71	3.26	4.65
	0.30	0.30		
Albumin g/dl	2.38	1.97	2.19	2.12
	0.13	0.72		
Cholesterol mmol/l	9.35^{a}	6.04^{b}	5.27^{b}	4.43^{b}
	0.50	0.00		
Triglyceride mmol/l	1.38	1.33	1.39	1.47
	0.06	0.86		
Low density lipoprotein mmol/l	7.33^{a}	4.19^{ab}	4.70^{b}	3.19^{b}
	0.54	0.04		
High density lipoprotein mmol/l	0.43	0.78	0.79	1.01
	0.09	0.19		
Aspartate amino transferase	41.69^{a}	41.45^{ab}	39.83^{c}	40.11^{bc}
	0.27	0.19		
Alanine amino transferase	17.96	22.54	20.65	20.13
	1.58	0.79		

abc: means in the same row with different superscripts are significantly (P<0.05) different. SEM: Standard Error of Mean; P value: probability value.

Table 3: Oxidative status of laying pullet fed mistletoe leaf meal supplemented diets

Parameters	T1 (0%)	T2 (2%)	T3 (4%)	T4(6%)	SEM	P value
Total Antioxidant Activity (mmol/litre)	0.87^{c}	1.62^{bc}	1.81^{b}	2.75^{a}	0.18	0.001
Lipid Peroxidation $x10^{-3}$(TBARS/mg protein)	54.21^{a}	37.64^{b}	30.87^{b}	30.19^{b}	2.91	0.01
Catalase(nm H_2O_2 / min/mg protein)	12.60^{b}	12.45^{b}	11.38^{b}	18.75^{a}	1.08	0.04
Superoxide Dismutase (U/min/mg protein)	0.06	0.12	0.12	0.12	0.01	0.38
Glutathione Peroxidase (µgGSH/min/mg protein)	2.93^{b}	3.85^{ab}	4.39^{ab}	5.81^{a}	0.41	0.04

abc: means in the same row with different superscripts are significantly (P<0.05) different. SEM: Standard Error of Mean; P value: probability value

High oxidative stress usually breaks down immune system, precipitates radicals as well as severe diseases and this must be prevented (32). Even though, the body has developed a variety of ways to deal with damaging free radicals, antioxidants from dietary sources also play important role in their control, thus limiting cellular damage (32).The result obtained in this study revealed that dietary mistletoe supplement had significant effect on antioxidant and peroxide formation in laying pullets as shown in Table 2 and 3. Total antioxidant activity of laying pullets significantly ($p<0.05$) increased with mistletoe inclusion. This showed that mistletoe endogenous antioxidant had enhancive effect on laying pullets' total antioxidant activity. This could be by stimulating the animal's enzymatic antioxidant system (GPx, SOD, Catalase) or non-enzymatic antioxidants (mistletoe alkali) present in mistletoe. This is in line with the claims of Ogechukwu (32) that antioxidation is one of the mechanisms of action of mistletoe.

Lipid peroxidations in laying pullets were significantly ($p<0.05$) lowered by mistletoe supplementation. This could be attributed to the antioxidant status of the birds as influenced by the treatment administered. There is an inverse relationship between lipid peroxidation and antioxidant, increase in the formal leads to oxidative stress while increase in the later protects against free radical and peroxides. This is in line with the report of Ogechukwu (32) that antioxidants from dietary sources also play important role in the control of oxidative stress, thus limiting cellular damage. Also mistletoe alkali (a component of mistletoe) acts as inhibitor of lipid peroxidation in rats (14). Antioxidant enzyme activity assayed showed that Superoxide dismutase which is a first line defense against free radicals were not significantly ($p>0.05$) affected by mistletoe supplementation, though apparent increase were observed in mistletoe treated groups. Catalase and glutathione peroxidase were involved in the mitigation of hydrogen peroxide accumulation (26). The result revealed that 6% mistletoe supplementation significantly ($p<0.05$) enhanced hydrogen peroxide scavenging in laying pullets (via catalase and glutathione peroxidase activity). This is similar to the report that mistletoe alkali not only scavenged OH directly but also inhibited OH generation in rats and mistletoe alkali could be a potential herbal medicine for improving GPX and SOD activity (14).

Modulation of the immune system and optimizing oxidative processes in humans and animals with the aid of natural products represents a field of drug development-based research witnessing unprecedented upsurge in recent times (33). Immune system is intricately interwoven with oxidative processes in the body.

Conclusion and Applications

1. Mistletoe inclusions in laying pullets' diet enhance antioxidant profile and inhibit oxidative stress.
2. Farmers in the management of oxidative stress and its related diseases should adopt it.

Acknowledgement

The authors wish to thank Oluwasinmi Modupe Oluwayemisi, Omolafe Adewale Ezekiel, Agunbiade Damilola Benjamen, Oladokun Theresa Modupe, Jegede Adedoyin, Aderounmu Oyindamola, Ajidahun Olamilekan, Olanrewaju Olayinka Tirzo, Ajimoti

Esther and Akinlolu Olamilekan for their efforts and resources in the management of experimental animals and experimental procedure.

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