**Herbal modification of broiler diet: Implication for haematological indices, antioxidant activities on meat, meat analysis, carcass and internal organs of broiler chickens.**

**ABSTRACT**

This study assessed the effects of *Vernonia amygdalina* leaf meal (VALM), *Moringa oleifera* leaf meal (MOLM) and the mixture of VALM and MOLM at 1:1 dietary supplementation on haematological, antioxidant activities on meat of broilers, meat analysis and carcass characteristics and internal organs of broiler chickens fed diets supplemented with herbs. A basal diet divided into four portions, designated diet 1 (control diet) and diets 2,3 and 4 supplemented with 0.2% VALM, 0.2% MOLM and VALM +MOLM composite mix 1:1, respectively. One hundred and forty four one-day broiler chickens were randomly assigned to four experimental diets (36 birds per diet; 12 birds per replicate) using a Completely Randomized Design. At the finisher phase, the Haemoglobin count (Hb) of birds fed diets 3 and 4 were significantly (P<0.05) better than those birds fed diets 1 and 2. The white blood cell of birds fed diet2 was higher (P<0.05) than those fed other treated diets though birds fed control diet had the highest WBC. The lymphocytes of birds fed diet 2 were higher (P<0.05) compared to those on other diets. The catalase concentration in meat of bird fed diets 3 and 4 were (P>0.05) similar but significantly (P<0.05) higher than the values recorded for birds fed diets 1 and 2. The glutathione peroxidase concentration in the meat of the birds fed diet 4 was significantly (P<0.05) higher than the values recorded for glutathione peroxidase concentration in the meat of birds fed diets 1, 2 and 3. The superoxide dismutase concentration in the meat of birds fed diets 3 and 4 were similar (P>0.05) but significantly (P<0.05) higher than the values recorded for the meat of birds fed diets 1 and 2. The meat cholesterol and lipid peroxidation concentration recorded in the birds fed diets 2, 3 and 4 were significantly (P<0.05) lower than the values recorded for birds on control diets. The final liveweights and dressed weight of the birds fed diets 4 were significantly (P<0.05) better and higher than the birds fed diets 1, 2 and 3. The eviscerated weight of the birds fed diet 1 was significantly (P<0.05) higher than those fed diets 2, 3 and 4. The VALM, MOLM and the mixture of VALM and MOLM at 1:1 dietary supplementation reduced the cholesterol and lipid peroxidation of broiler meat. The overall health status were also improved by the herbal supplements in this study.

Keywords : Herbs, broiler, antioxidant activities, haematology, meat analysis.

Introduction

Poultry production is the most popular of all animal production enterprises, it comprises an important pillar of food security improvement as well as socio-cultural and economic developments in most countries [1]. Broiler production is a source of income, it is a good source of protein and have quick returns of investment. Poultry industry in the developing countries is facing some challenges. These challenges include high feed to gain ratio and increase in the cost of feed because of high prices of feed of ingredients [2].

Numerous attempts have been made to overcome the challenges; one of them is the use of feed additives. Feed additives are ingredients added to the poultry diets to enhance production efficiency, improve health and reduce mortality [3]. Plant based feed additives also known as phytogenic have been advocated to be included in broiler chickens feeds as growth promoting feed additives because of their abundance in our natural environment and the fact that they do not have residual effect [4]. Their non-residual effect is because, the active ingredients of phytogenic feed additives are absorbed in the intestine of enterocytes and are quickly metabolized by the body. Phytogenic can improve feed consumption, feed conversion, feed digestibility and weight of broiler chickens [5].

*Vernonia amygdalina* leaf meal (VALM) contains bioactive compounds vernolide and vernodalol both compounds exhibit a significant bactericidal activity against gram positive organisms [6]. Bitter leaf meal (*Vernonia amygdalina*) is a valuable plant with antimicrobial activity. It is a staple vegetable leaf used to prepare soup especially in the south eastern Nigeria. Some principal chemical constituents found in bitter leaf are steroid glycoside and Vernonioside B1 which possesses potent antiparasitic, anti tumor and bactericidal properties.

[7] reported that VALM contained 15.67% crude protein, 11.53% crude fibre and 6.95% ether extract. It has been reported that bitter leaf meal used in poultry production was able to increase feed conversion efficiency of broiler birds without affecting their haematological profile [8]. VALM may provide anti-oxidant benefits [9].

*Moringa oleifera* leaf meal (MOLM) is widely available in many tropical countries. It is also a good source of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids [10]. The use of *Moringa oleifera* as a feed additive in poultry nutrition necessitates through investigation into its nutritional value, as well its impact on haematological parameters as a measure of nutritional and medicinal benefits of the leaves in broiler chicks [11]. The underlying effects of the bioactive compounds in *Moringa oleifera* leaves not clear. They are believed to induce prebiotic effects, bacterial and immune-stimulant activities [12] resulting in increased productivity of broiler chickens, similar effects have been observed in the presence of antibiotic growth promoters [13].

There is a concern about antibiotic growth promoters in poultry nutrition which have resulted into efforts being made to use different alternative growth promoting medicinal plants. This is because of the emergence of antibiotic resistant pathogens and its residual effects in meat and meat products which constitute adverse effect to consumers [14]. Bans on the use of synthetic antibiotics as feed additives have accelerated and led to investigations of natural alternative additives in animal production [15].This study is aimed to access the effect of dietary supplementation of VALM, MOLM and mixture of VALM and MOLM (their combinations) on the haematological parameters, antioxidant activities on meat, meat analysis and carcass quality and internal organs of broiler chickens.

**Materials and methods**

**Ethical approval, preparation of VALM and MOLM:** This experiment was carried out according to the specifications and guidelines of Animal Production protocol approved by the Research and Ethics Committee of the Department of Agricultural Technology, The Federal Polytechnic, Ado, Ekiti State, Nigeria. The test ingredients *Vernonia amygdalina* leaf meal (VALM) and *Moringa oleifera* leaf meal (MOLM) were prepared by harvesting fresh and matured VALM and MOLM before flowering. The fresh leaves were subjected to air-drying in an open cleaned concrete floor space until moisture content became constant at 13%. The air dried leaves were later milled using a commercial feed milling machine and then sieved through a (2mm) siever. The leaves were analysed for proximate composition according to (AOAC, 2016). Basal diets were formulated for starter (0-28days of age) and finisher (29-56days of age) according to [17] recommendation.

**Location and duration of the study:** The experiment was carried out at the poultry unit of the Department of Agricultural Technology, Teaching and Research Farm, The Federal Polytechnic, Ado-Ekiti. The study lasted for 8 weeks.

**Experimental diets and management:** Four experimental diets were formulated and designated at treatments1,2,3 and 4 such that diet 1 was the basal diet without supplementation, diets 2,3 and 4 were supplemented with 0.2% *Vernonia amygdalina* leaf meal (VALM), 0.2% of *Moringa oleifera* leaf meal (MOLM) and 0.2% mixture of VALM and MOLM i.e (1:1), respectively for the starter and finisher diets.

**Table 1: Composition of experimental diet (g/100g) for broiler starter and finisher**

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| --- | --- | --- |
| **Ingredients** | **Starter** | **Finisher** |
| Maize | 51.00 | 65.00 |
| Soybean cake | 23.00 | 15.00 |
| Groundnut cake | 17.00 | 11.00 |
| Fish meal | 2.00 | 2.00 |
| Bone meal | 4.00 | 4.00 |
| Limestone | 2.00 | 2.00 |
| Broiler premix | 0.25 | 0.20 |
| Methionine | 0.25 | 0.30 |
| Lysine | 0.25 | 0.20 |
| Common salt | 0.25 | 0.30 |
| Total | 100.00 | 100.00 |
| Calculated composition |  |  |
| Metabolizable energy (kcal/kg) | 2938.00 | 2984.70 |
| Crude protein (g/100g) | 24.68 | 20.31 |
| Crude fibre (g/100g)  Average calcium | 2.56  2.35 | 2.85  1.99 |
| Average phosphorus | 0.84 | 0.65 |
| Lysine | 1.33 | 1.27 |
| Methionine | 0.60 | 0.58 |

**Experimental layout:** The trial was done using completely randomized design (CRD). The experiment involved one hundred and forty four day old Abor-acre commercial breed were randomly distributed into four dietary treatments with 12 birds per replicate. Each treatment was replicated under experimental diets and management. The birds were given necessary vaccinations. Water and feed were given *ad-libitum* during the experimental periods. The research lasted for four weeks (finisher phase), prior to the arrival of the birds from hatchery, the brooding house was washed and disinfected, the pens were pre-heated few hours before the arrival of the birds with 200 watt bulb.

**Parameters measured:**

**Haematological parameters**

**Blood collection:** At the end of the four weeks (finisher phase), blood samples collection was done in the morning after the birds were starved overnight in order to attain a stable haematological evaluation. Three birds were randomly selected from each treatment. Blood samples were collected from the wing vein using a 5ml syringe and placed in sterilised bottles containing ethylene-diamine tetra-acetic acid (EDTA) anticoagulant for the determination of the haematological values [18]. The samples were cooled at 40C using ice packs and transferred to the medical laboratory. The following haematological parameters were determined.

Packed cell volume (PCV): The packed cell volume was determined by the microhaematochrit method [19].

Haemoglobin concentration (HBC): The haemoglobin concentration of the blood samples was determined by the cyanomethaemoglobin method [20].

White blood cell count: Haemocytometer method [19] was used.

Red blood cell count (RBC): Haemocytometer method [19] was used.

Mean corpuscular values: Mean corpuscular haemoglobin concentration = HBC(g/dl) x 100/pcv.

Mean corpuscular volume (MCV) = PCV% x 10/RBC counts (million)

Mean corpuscular haemoglobin = HBC (g/dl) x 100/RBC counts (million)

Mean corpuscular haemoglobin concentration = HBC(g/dl) x 100/PCV

**Antioxidant activities in meat and meat analysis:** About 100g of the meat was excised from the breast meat for determination of the level of the meat cholesterol [21], lipid peroxidation [22], catalase activity [23] glutathione peroxidase activity and superoxide dismutase [24]**.**

**Organ evaluation**: At the end of the experiment three birds were randomly selected from each treatment. They were weighed and slaughtered in accordance with the provisions of the Ethical Committee on the use of animals and human for biomedical research of The Federal Polytechnic, Ado Ekiti at the department of Agricultural Technology (Animal Production Technology Option). Following slaughtering, the carcass were scalded at 750Cin water for about 30seconds before defeathering. The dressed chickens were eviscerated and the measurements of the carcass traits were taken before dissecting out the organs. Weights of organs were determined using a manual and electronic weighing scale. All carcasses and the internal organs were expressed as percentage of liveweight.

**Experimental design** : The experiment was executed using Completely Randomized Design (CRD). The experimental model of Completely Randomized Design:

XiJ = µ + Ti + ∑Ij, were:

µ = Population mean

Ti = Treatment effect

XiJ = experimental error

I = number of treatment

j = number of replicates

**Statistical analysis:** Analysis of variance using SPSS software was used to detect significant treatment effects. Duncan’s Multiple Range Test was used to separate treatment means.

**Results**

The effect of herbal supplements on haematological indices of broiler chickens are presented in Table 2.The haemoglobin concentration of birds fed control diet and VALM supplemented diet were similar (P<0.05) lower than values recorded for birds fed MOLM and mixture of VALM and MOLM supplemented diets. The white blood cells of birds fed VALM and mixture of VALM and MOLM supplemented diets were similar (P>0.05) but significantly (P<0.05) higher than the WBC values recorded for birds fed MOLM supplemented diet. The highest WBC values was recorded for birds fed control diet at 4616.67 ×109/L while the lowest values was recorded for birds fed MOLM supplemented diets 2000.00×109/L . The lymphocytes values recorded for birds fed control diets, VALM and MOLM supplemented diets were similar (P>0.05) but significantly (P<0.05) higher than the value recorded for birds fed mixture of VALM and MOLM supplemented diets. There were no significant difference (p>0.05) on PCV, granulocytes, monocytes, MCHC, MCV, MCH and Rb. The Hb, WBC and lymphocytes of birds were significantly (p<0.05) influenced by the experimental diets.

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| **Table 2.** Haematological indices of broiler chickens fed diets supplemented with herbs | | | | | | |
| **Parameters** | **Treatment groups** | | | | **SEM** | ***p* value** |
| **1** | **2** | **3** | **4** |
| Packed cell volume (%) | 31.00 | 30.00 | 32.33 | 30.33 | 0.68 | 0.70 |
| Haemoglobin count (g/l) | 8.83c | 9.23c | 11.09a | 9.87b | 0.26 | 0.01 |
| WBC (×109/L)  Granulocytes ,×109/L  Lymphocytes (×109/L)  Monocytes (×109/L)  MCHC (g/dl)  MCH (g/dl)  MCV (g/dl)  RBC (×1012/l) | 4616.67ª  0.13  2.71ª  0.12  33.03  42.23  123.77  2.23 | 2966.67b  0.14  2.72ª  0.12  32.90  42.67  125.40  2.33 | 2000.00c  0.13  2.70ª  0.14  33.13  42.50  124.07  2.30 | 2833.33b  0.13  2.52b  0.14  32.97  42.77  123.90  2.36 | 291.97  0.01  0.03  0.01  0.06  0.28  0.40  0.06 | 0.01  0.98  0.01  0.62  0.60  0.94  0.58  0.91 |

*a,b,cMeans in the same row with different letters show significant differences (p<0.05) among dietary treatments*

*\*\*WBC= White blood cell; MCHC=Mean corpuscular haemoglobin concentration; MCH= Mean corpuscular haemoglobin ; MCV= Mean concentration volume; RBC= Red blood cell*.

The effect of herbal supplements on antioxidant activities on meat of broilers are presented in Table 3. The glutathione peroxidase and superoxide dismutase of the meat of broilers on VALM, MOLM and mixture of VALM and MOLM were significantly (P<0.05) higher than the birds fed control diet.

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| **Table 3.**Effect of herbal supplements on antioxidant activities on meat of broilers | | | | | | |
| **Parameters** | **Treatment groups** | | | | **+SEM** | ***p-* value** |
| **1** | **2** | **3** | **4** |
| Catalase (mg/ml) | 102.97b | 104.66ab | 105.35a | 105.03a | 0.37 | 0.08 |
| GPx (mg/ml) | 125.28d | 164.79c | 168.55b | 169.70a | 5.56 | 0.01 |
| SOD (%) | 0.92b | 1.05a | 1.06a | 1.06a | 0.02 | 0.01 |

*a, ,b,cMeans in the same row with different letters show significant differences (p<0.05) among dietary treatments: GPx- Glutathione peroxidase; SOD-Superoxide dismutase*.

The effect of herbal supplements on meat analysis of broilers are presented in Table 4. The cholesterol values of birds on treated groups (VALM, MOLM and mixture of VALM and MOLM) were lower than the values recorded for birds on control diet. The highest cholesterol value was recorded for birds fed control diet at 83.31mg/dl while the least value was recorded for birds fed mixture of VALM and MOLM at 47.50mg/dl. The lipid peroxidation values of birds fed VALM and MOLM were similar (P>0.05) but significantly higher (P<0.05) than the values recorded for birds fed mixture of VALM and MOLM. The highest value lipid peroxidation value was recorded for birds fed control die at 3.71mg/ MDA and the least value was recorded for birds fed mixture of VALM and MOLM at 1.37mg/MDA.

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| **Table 4.** Effect of herbal supplements on meat analysis of broilers | | | | | | |
| **Parameters** | **Treatment groups** | | | | **+SEM** | ***p* –value** |
| **1** | **2** | **3** | **4** |
| Cholesterol (mg/dl) | 83.31a | 54.62b | 52.46c | 47.50d | 4.22 | 0.01 |
| Lipid peroxidation (mg/MDA) | 3.71a | 1.46b | 1.44b | 1.37c | 0.30 | 0.02 |

*a, ,b,cMeans in the same row with different letters show significant differences (p<0.05) among dietary treatments*

The effect of herbal supplements on carcass characteristics and internal organs of broiler chickens are presented in Table 5. The carcass characteristics and internal organs of broiler chickens fed diets supplemented with herbs is presented in table 4. The final liveweight, dressed weight and eviscerated weight of the birds fed control diet and mixture of VALM and MOLM supplemented diets were higher (p<0.05) when compared to the VALM and MOLM supplemented diets. The internal organs were not significantly (p>0.05) affected by experimental diets.

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| **Table 5.** Carcass characteristics and internal organs of broiler chickens fed diets supplemented with herbs | | | | | | |
| **Parameters** | **Treatment groups** | | | | **SEM** | ***p* value** |
| **1** | **2** | **3** | **4** |
| Final liveweight (g) | 2755.43a | 2646.76c | 2689.42b | 2734.48a | 48.76 | 0.01 |
| Dressed weight (%) | 93.75b | 94.06b | 91.88c | 94.76a | 0.40 | 0.02 |
| Eviscerated weight (%)  Liver (%)  Heart (%)  Lung (%)  Kidney (%)  Bile (%)  Gizzard (%)  Spleen  Proventiculus (%) | 78.71a  2.72  0.57  0.44  0.62  0.11  2.15  0.11  0.53 | 75.01b  2.70  0.58  0.43  0.63  0.12  2.13  0.12  0.52 | 76.01b  2.70  0.57  0.40  0.61  0.11  2.13  0.10  0.52 | 75.26b  2.69  0.57  0.47  0.65  0.12  2.12  0.11  0.50 | 0.53  0.07  0.01  0.01  0.01  0.02  0.01  0.01  0.01 | 0.01  0.21  0.31  0.26  0.28  0.25  0.19  0.17  0.12 |

*a, ,b,cMeans in the same row with different letters show significant differences (p<0.05) among dietary treatments*

Discussion

There is a relationship between nutritional deficiency and changes in the blood constituents. The haematological indices are among the known good indicators of the physiological status of the animals [25]. In this study, the stable erythrogram constituents values recorded in the birds recorded the various dietary treatments indicate that the dietary phytogenic feed supplements did not have harmful interference on the haematopoiesis in the experimental birds. The variations recorded in the white blood cell values and its differential (lymphocytes) may indicate the immune-modulatory effects of the phytogenic supplements in the experimental birds as reported by [26]. The white blood cells play a significant role in the fighting against an infection, defend the body by phagocytosis and to produce, transport as well as distribute antibodies in immune response [27]. However, the rise in lymphocytes of the birds fed with the mixture of VALM and MOLM could trigger a complex but beneficial immune-modulatory response in the birds. The phytochemicals such as flavonoids has been show to possess immune stimulatory properties by improving the activities of lymphocytes [28].The use of herbal supplements as an antioxidant for reducing the effects of oxidative stress is high [29]. The notable rise in glutathione peroxidase and superoxide dismutase activities in the meat of the experimental birds fed VALM, MOLM and mixture VALM and MOLM disclosed the antioxidant properties of VALM, MOLM and mixture of VALM and MOLM. The result is opined with the report of [30] who noted high antioxidant activities in rabbits fed mucuna leaf meal supplemented diets. The increase in antioxidant activities recorded in the birds fed the VALM, MOLM and mixture VALM and MOLM supplemented diets in this work may be due to the polyphenolic contents (flavonoids or phenolic acids) of the herbs [31]. The herbal active ingredients generate powerful antioxidant effects that hunt the radicals or give rise to the catalase, glutathione peroxidase and superoxide dismutase activities. Oxidative process during shelf-life of meat can reduce its nutritional and sensory values [32].The decreased in meat cholesterol level in this study is of advantage to the consumers because they are now conscious of the type and quality of the meat they consume. Animal products low in cholesterol is good for health especially for those people who have heart related issues [30]. The outcome of this study revealed the low cholesterol effect of herbal supplements. The noted low cholesterol effect may be as a result of activities of the bioactive compounds in VALM and MOLM by preventing cholesterol absorption in the intestine due to the likeness in the structure of phytosterol and cholesterol [33]. This result is in tandem with the report of many researchers who reported a notable reduction in cholesterol content of broiler chickens fed herbal supplements. Moreso, the notable decrease in lipid peroxidation reported in the meat of the broiler chickens fed herbal supplemented diets compared to control diet could be a result of the increase in amount of the antioxidant enzymes in the meat of birds in the research. Lipid peroxidation is one of the high cause of meat deterioration causing off-flavour and oxidized compounds that are dangerous to people’s health [34]. Several studies reported the ability of herbal supplements to lower the lipid peroxidation in meat due to in-built or natural bioactive compounds and enzyme activity [35].The improved final live-weight, dressed weight and eviscerated weight of the birds fed mixture of VALM and MOLM supplemented diets in this study agreed with report of [36] who noted that supplementation of the broiler chickens diet with phytogenic supplements such as thyme, lemon balm and cinnamon improved the carcass weight. This suggest that the phytogenic supplements used in this study have phytoconstituents or bioactive compounds, (e.g hydoxycinnamic acid derivatives of the phenylalanine) that can modulate animal metabolism in a similar pattern with β-adrenergic agonist compound (36). These plant based compounds have a similar structure with the catecholamines (the natural animal hormones). They could interact with β-adrenergic receptor agonists to modulate animal metabolism by increasing the lipolysis and protein synthesis and by decreasing the lipogenesis [37]. The similarity in the growth response of these animals’ internal organs to the phytogenic supplementation in this study suggests the support of the supplements to the normal functioning of the birds’ internal organs.

**CONCLUSION**

The VALM and MOLM have phytochemicals of health benefits and possess antioxidants properties. The 0.2% VALM, 0.2%MOLM and the mixture of VALM and MOLM at 1:1 dietary supplementation reduced the cholesterol and lipid peroxidation of broiler meat. The phytogenic supplementation did not have a harmful interference on the haematopoiesis and the carcass characteristics and internal organs of the experimental birds.

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