

THE EFFECT OF VARYING LEVELS OF *Vernonia amagdalina* Del. LEAF ON THE HEMATOLOGICAL PARAMETERS OF BROILER BIRDS

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ABSTRACT

This study was carried out to investigate the effect of various levels of *Vernonia amagdalina* on the hematological parameters of one hundred and ninety eight (198) arbor acre broiler birds. The experiment lasted for 6 weeks and was divided into two (2) phases, 0-3 weeks and 4-6 weeks. The birds were randomly selected and sub-divided into 6 dietary treatments with 3 replicates, each replicate contained eleven (11) birds in a complete randomized design in a deep litter system. The experimental treatments include a control diet which was without any form of antibiotics, treatment1 (T1), another treatment diet contained an antibiotic named collistin, treatment 2 (T2), the remaining four diets include different levels of powdered form of bitter leaf at 0.1, 0.5, 2.5 and 12.5% augmentation of dry matter weight of feed stock, respectively. There was no significant difference between the test results obtained from various treatments ($P > 0.05$). There was also no significant difference between the two phases ($P > 0.05$). For the packed cell volume and hemoglobin 0.1% inclusion of *V. amygdalina* had the highest figure for the first phase (0-3 weeks) but 2.5% inclusion of *V. amygdalina* has the highest figure for the second phase (4-6 weeks). For the red and white blood cell 0.1% inclusion of *V. amygdalina* and 2.5% inclusion of *V. amygdalina* have the highest figure for the first phase (0-3 weeks) respectively while 2.5% inclusion of *V. amygdalina* and T2 for the second phase (4-6 weeks). The result shows that inclusion of *V. amygdalina* at the rate of 0.1%, 0.5, 2.5, 12.5% in their feed had no adverse effect on the hematological parameters measured and can be used as an alternative for antibiotics in broiler production.

KEYWORDS: *Vernonia amagdalina*, Collistin, Hemoglobin, Antibiotics, Augmentation, *ad libitum*

INTRODUCTION

One of the profitable agro-industries which can effectively tackle the problems of unemployment in the rural and urban areas is the poultry sector.

Poultry industry can be adopted under a wide range of climatic conditions and can generally be combined conveniently with other farm enterprises. Despite the low growth in

the poultry sector for the past two decades, a huge gap exists between availability and requirement of poultry products. Feed items account for up to 75% of total cost of production of livestock, the energy fraction of the major ingredients is one of the highest contributory factors to the rising animal feed cost in Nigeria (Nwokoro, 2015). It is a common knowledge that feed constitute the greatest and most costly input in any livestock farm; especially poultry. Thus, any significant reduction in the cost of feeds will significantly reduce the overall cost of production and increase the profit margin of the farm (Owen and Amakiri, 2011).

The problem has been worsened due to the increasing competition between humans, industries and livestock for these energy feedstuffs. Due to high cost of the conventional protein ingredients like fish meal, groundnut cake and soybean, the trust of nutritional research is now towards identifying non-conventional sources that are locally available with low human demands (Owen *et al.*, 2009).

Also using antibiotics in animal diets to prevent animal diseases and to improve productivity in modern animal husbandry has been a common practice for decades (Cheng *et al.*, 2014). However, their use in feeds caused increase in development of resistant bacteria (Aarestrup, 1999), which could be transferred from animals to humans (Stanton, 2013). The European Centre for Disease Prevention and Control (ECDC, 2015) states that antibiotic resistance continues to be a serious public health threat worldwide. (Lin, 2014) reported that the growth-promoting effect of antibiotics was

correlated with the decreased activity of bile salt hydrolase, an intestinal bacteria-produced-enzyme that exerts a negative impact on host fat digestion and utilization. Many plant extracts, herbs and spices are used as alternatives to antibiotics due to antimicrobial properties and growth promoters effect in poultry (Akyildiz and Denli, 2016). Thus, the aim of this study is to investigate the effect of *V. amygdalina* as alternative to antibiotic on the hematological parameters of abor acre broiler birds.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Animal Unit of Benson Idahosa University Teaching and Research Farms, Ugbor Village Benin City, Edo State. The area lies within the rainforest vegetation belt of Nigeria and is characterized by two district seasons which run from November to March for wet and April to October for dry each year. The Latitude of the study area is 06°17'01.6"N and Longitude 005°36'10.6"E on an elevation of 73m above sea level.

Management of Birds and Experimental Design

A total of one hundred and ninety eight day old broiler birds were used for the experiment. The birds were randomly selected and sub-divided into 6 dietary treatments with 3 replicates, each replicate contained eleven (11) birds in a complete randomized design. The experiment was conducted in six (6) weeks and was divided into two (2) phases of 3 weeks. The experimental treatments includes a diet with control

which was without any form of antibiotics, another treatment diet contained an antibiotic named collistin, the remaining four diets include different levels of the leaves of *V. Amygdalina* at 0.1, 0.5, 2.5 and 12.5% augmentation of dry matter weight of feed stock, respectively. The leaves were prepared by sun drying before milling into powder form.

The birds were fed twice daily in the morning and in the evening, water was administered *ad libitum* throughout the period of this experiment. Table 1 shows the composition of experimental diets. Heat source was provided through charcoal pots and light bulbs. Upon arrival the birds were administered with anti-stress and glucose through their water after randomization had taken place to select the cages in which they would be put in. The birds were treated in cages in an open-sided wire mesh constructed poultry house to allow for adequate ventilation. The medications, vaccinations and other routine management practices were adequately followed according to the manufacturer instructions. The birds were raised in a cool, quiet and clean atmosphere during the period of the 6 weeks.

Materials Used

The materials used during the course of the experiment include the following: Battery cages, wood shavings, concrete blocks, charcoal pots, baskets, storage drums, cartons, feeders, drinkers, 100 watts electric bulbs, weighing scales, environmental thermometer, electric micro-scale, weighing balance, buckets, broom, packer, shovels, cutlass, knives, 5ml syringes, 10ml syringes, surgical blade, EDTA bottles, test tubes, distilled water, aluminium fuel, paper tape, knives, ruler, face masks, hand gloves, charcoal pot, lantern, drugs, vaccines and disinfectant.

Experimental Diet and Treatment

Six experimental diets were prepared for the course of six weeks; diet one and two were the control diets. Diet I has no antibiotics, diet II is a control diet with collistin, diet III, diet IV, diet V and diet VI include different levels of *V. Amygdalina* at 0.1%, 0.5%, 2.5% and 12.5% respectively. Vaccination and medication were given at appropriate times throughout the whole period of the experiment.

Table 1: Experimental diet composition for arbor acre broiler birds for 0- 6 weeks

INGREDIENTS	Experimental diet composition (%)					
	Diets without <i>Vernonia Amygdalina</i>		Diets with <i>Vernonia Amygdalina</i>			
	DIET 1 (Control, no antibiotic)	DIET 2 (Control, with collistin)	DIET 3 (0.1% VA)	DIET 4 (0.5% VA)	DIET 5 (2.5% VA)	DIET 6 (12.5% VA)
Maize	45	45	45	45	44.4	38.6
Soybean Meal	29.4	29.4	29.6	29.7	28.5	22.6
Rice bran	9.9	9.9	9.7	9.3	8.3	7.4
Fishmeal	6	6	5.9	5.8	6.5	9.1
<i>Vernonia Amygdalina</i>	0	0	0.1	0.5	2.5	12.5
Palm Oil	5	5	5	5	5.1	6.1
Limestone	1.5	1.5	1.5	1.5	1.7	1
Dicalcium Phosphate	2	2	2	2	2	2
NaCL	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin Premix	0.5	0.5	0.5	0.5	0.5	0.5
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Lysine	0.2	0.2	0.2	0.2	0.2	0.2
Antibiotic	-ve	+ve	-ve	-ve	-ve	-ve
Total	100	100	100	100	100	100
Calculated nutrient levels:						
ME Kcal/Kg	3025.31	3025.31	3025.5	3025.54	3025.72	3025.45
CP (g/kg)	220.15	220.15	220.29	220.21	220.65	220.43
Ca (g/kg)	10.64	10.64	10.62	10.64	10.28	10.94
Available P (g/kg)	9.76	9.76	9.72	9.66	9.3	9.28

*Supplied per kg of diet – Vit A. 5000iu; VitD 3 800iu; Vit E 12mg; Vit B6 1.5mg; Pantothenic acid 5mg; Biotin 0.02; Vit B120.0mg; Folic acid 0.3mg; Choline Chloride 150mg, Manganese 60mg; Iron 10mg; Zinc 15mg, Copper 0.8mg; Iodine 0.4mg; Cobalt 0.08mg; Selenium 0.04mg and Anti-oxidant 40mg.

DATA COLLECTION

Collection of Blood Samples

Collection of blood samples was done in two phases, the first phase was at the end of 3rd week and the second phase was done at the end of the 6th week. The birds from each dietary replicate were randomly sampled to determine hematological responses. 5 ml of blood was taken from the jugular vein of randomly selected birds per replicate. 2.5 ml of sampled blood was put into labeled blood sample bottles containing anticoagulant (Ethyl Diamine-Tetra-Acetate powder (EDTA)) to determine hematological parameters. Parameters analyzed include; Packed Cell Volume (PCV)(%), Hemoglobin (g/l), Red Blood Cells (RBC)(10⁶ ul), Mean Corpuscular Volume (MCV) (fl), Mean Corpuscular Hemoglobin (MCH) (pg), Mean Corpuscular Hemoglobin Concentration (MCHC (g/dl)) White Blood Cell (WBC)(10³ ul), Neutrophils (%), Monocytes (%), Eosinophil (%) and Basophils (%) according to the procedure of Howlett and Jamie (2008).

Statistical Analysis

The data obtained from the hematological parameters were subjected to the analysis of variance (ANOVA) using the statistical package Statistical Product and Service Solutions (SPSS).

RESULTS

The results of the hematological parameters of broilers birds fed varying levels of *V.amygdalina* based diet for 0-3 weeks is shown in table 2. The result shows there is no significant difference between the test results obtained from various formulations ($P > 0.05$).

Table 3 shows the results of the hematological parameters of broiler birds fed varying levels of *V. amygdalina* based diet for 4-6 weeks. The result shows the null hypothesis which means there is no significant difference between test results obtained from various formulations ($P > 0.05$).

Table 2: The hematological parameters of broilers birds fed varying levels of *V.amygdalina* based diet for 0-3 weeks

TREATMEN TS	PCV (%)	HB (g/dl)	RBC (x10 ¹² /l)	MCHC (g/dl)	MCV (fl)	MCH (pg)	WBC (x10 ⁹ /l)	NEUT (%)	LYMPH (%)	EOSI (%)	MONO (%)	BASO (%)
1	33.3	11	3.87	32.6	86.1	27.6	7.63	29.33	65	1.67	3	1
2	23.3	7.87	2.7	33.7	85	28.9	4.73	32.67	64.67	0.33	2.33	0
3	33.67	11.6	3.9	33.2	86.07	28.57	6.6	36.67	60.33	0.67	1.33	1
4	27.33	9.2	3.2	33.9	85.57	28.97	5	36.67	60	1	1.67	0.67
5	26.67	8.8	3.1	33.2	85.43	18.97	7.97	32.67	63	1.67	3.33	0.67
6	31	10.17	3.6	32.77	86.1	28.2	6.2	33.3	62	1.67	2.67	0.33
Mean	29.21	9.77	3.40	33.23	85.71	26.87	8.42	33.55	62.5	1.17	2.39	0.61

Key: PVC = Packed Cell Volume, HB = Heamoglobin, RBC = Red Blood Cell, MCV = Mean Corpusular Volume, MCH = Mean Corpuscular Heamoglobin, MCHC = Mean Corpuscular Heamoglobin Concentration, WBC = White Blood Cell, Neut = Neutrophil, Eosi = Eosinophil, Baso = Basophil, Mono = Monocyte

Table 3: The hematological parameters of broilers birds fed varying levels of *V.amygdalina* based diet for 4-6 weeks

TREAT MENTS	PCV (%)	HB (g/dl)	RBC (x10 ¹² /l)	MCHC (g/dl)	MCV (fl)	MCH (pg)	WBC (x10 ⁹ /l)	NEUT (%)	LYMPH (%)	EOSI (%)	MONO (%)	BASO (%)
1	28	9.3	3.3	33.67	84.83	28.5	6.53	38.33	57.67	1.67	2	0.33
2	26.67	8.33	3.13	33	84.97	28.07	6.83	40.33	57	2	1	0.67
3	26.33	8.63	3.13	32.83	83.23	27.33	6.07	40.33	57.33	1	0	1.33
4	24.33	8.2	2.93	33.2	83.3	27.67	6.27	38.33	58.67	1.33	1.67	0
5	28.67	9.57	3.37	33.47	84	76.7	5.13	38.67	58	1	1.33	1
6	26.67	8.93	3.13	35.17	60.47	28.3	4.7	43	56	1.33	3	0
Mean	26.78	8.82	3.165	33.56	80.13	36.10	7.63	39.83	57.45	1.39	1.5	0.56

Key: PVC = Packed Cell Volume, HB = Heamoglobin, RBC = Red Blood Cell, MCV = Mean Corpusular Volume, MCH = Mean Corpuscular Heamoglobin, MCHC = Mean Corpuscular Heamoglobin Concentration, WBC = White Blood Cell, Neut = Neutrophil, Eosi = Eosinophil, Baso = Basophil, Mono = Monocyte

DISCUSSION

The results from the hematological parameters of broiler birds fed varying levels of *Vernonia amygdalina* for 0-3 weeks shown in table 2 shows that there is a decrease in the packed cell levels volume of the various treatments compared to that of the control, with the exception of treatment 3 (T3), which has higher PCV than the control at 33.67, but it falls within the normal range for healthy birds (24.9 - 45.20) according to (Mitruka and Railshey, 1977, Archetti *et al.*, 2008). For hemoglobin treatment 3 (T3) has the highest inclusion, but the other treatment showed a reduction in the hemoglobin compared to the control treatment (T1) but all the values fall within the normal range of healthy birds (7.40 - 13.10). The red blood cells show a decrease in the *V. amygdalina* treatments compared with the control with the exception of treatment 3 (T3) which has the highest red blood cell values at 3.9.

Owen and Amakiri (2011) made similar observations. The white blood cell treatment 5 (T5) has the highest inclusion with 7.97 is slightly higher than the control treatment (T1) at 7.63. According to Isaac *et al.* (2013), packed cell volume is involved in the transport of oxygen absorbed nutrients. Increased packed cell volume shows a better transportation and thus prevents anaemia (Coles, 1986). This result shows a decrease except in T3 which increases. The lymphocytes decreased in the varying levels of *V. amygdalina* from the control treatment (T1). The mean corpuscular hemoglobin concentration increases in the varying levels compared to the control treatment

(T1), with treatment 4 (T4) having the highest inclusion which is also the case for mean corpuscular hemoglobin with the exception of T5 which reduces.

The Neutrophils, monocytes, Eosinophils and Basophils all have values that fall within the normal range for healthy birds according to (Mitruka and Railshey, 1977; Archetti *et al.*, 2008). The low values in monocytes and basophils agreed with the statement that the monocytes and basophils are present in small to moderate numbers in the blood system. For Eosinophil, treatment 1 (T1), treatment 5 (T5) and treatment 6 (T6) have the highest value and eosinophil is known to protect against infection. This shows birds on this treatment were able to fight against infections.

The results of the hematological parameters of broiler birds fed varying levels of *V. amygdalina* for 4-6 weeks shown in table 3 shows that treatment 5 (T5) has the highest level of PCV. For hemoglobin treatment 5 (T5) has the highest volume, the other values decrease compared to treatment 1(T1) the control, but all values fall within the normal range for healthy birds (Mitruka and Railshey, 1977, Archetti *et al.*, 2008). For red blood cells, there is a decrease in the values from the control treatment (T1) with the exception of treatment 5 (T5) which is slightly higher than the control treatment. In the white blood cell the treatment with collistin (T2) has the highest inclusion.

There is a decrease in the Mean corpuscular hemoglobin concentration of the varying levels of *V. amygdalina* if compared to the control in treatment 1 with the exception of T5 which is slightly higher. In the mean corpuscular

volume there is also a decrease in the treatments with *V. amygdalina* from the control T1, but, the treatment containing collistin, treatment 2 (T2) is higher than the control with 84.97.

Neutrophils, monocytes, Eosinophils and Basophils are all within the normal range. It was reported by Frandson (1986) that the number of Neutrophils increases rapidly when acute infection is present.

CONCLUSION

Based on the study, it can be concluded that inclusion of *V. amygdalina* at the rate of 0.1%, 0.5%, 2.5%, 12.5% in the feed of arbor acre broiler birds had no adverse effect on the haematological parameters measured. Hence, the addition of this natural herb at 0.1%, 0.5%, 2.5%, 12.5% can be used in broiler chickens production without any deleterious effect on the haematological parameters of the birds. The result also shows that *V. amygdalina* can be used as a substitute for antibiotics in poultry production.

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