HAEMATOLOGICAL PROPERTIES OF BROILER ADMINISTERED WITH AQUEOUS EXTRACT OF TRIUMFETTA CORDIFOLIA LEAF

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ABSTRACT

The effect of Aqueous Extract of Triumfetta cordifolia Leaf on the Haematological Properties of Broiler was investigated in this study. A total of 96 Abor-acre chicks were randomly allocated to 4 treatment (3 replicates each) using RCBD. Results were subjected to statistical analysis using SPSS version 25, and the significant difference across treatments were determined using Duncan multiple range test at (P<0.05). Results at day 28 showed that WBC, Neutrophil, MCV were (P<0.05) affected across the treatments when Aqueous Extract of Triumfetta cordifolia Leaf were administered to the broilers. At day 42, results showed significance (P<0.05) between various treatment groups for WBC, Lymphocytes, Haemoglobin and PCV. Among all treatments group, treatment 2 was highest (P<0.05) across the haematological parameters in both Day 28 and Day 42 and hence 10%w/v concentration of the aqueous extract of Triumfetta cordifolia Leaf is recommended to attai better haematological profile of broilers.

KEYWORDS

Abor-acre, Haematological Properties, Treatments, Replicates, Statistical analysis

1. INTRODUCTION

According to [1] and [2] poultry production remains the wide spread of all livestock enterprises. The poultry production does constitute an important pillar of food security, improvement as well as the socio-cultural and economic development in most countries. According to [3], poultry is one of the most profitable agro-industries, that can effectively tackle unemployment problems in most rural and urban areas.

The various types of poultry birds include Chickens, Mallard Ducks, Muscovy Ducks, Geese, Guinea Fowls, Turkeys, Quails, Pigeons, Pheasants, and Ostriches. The most common type amongst these birds are the chickens [4]. Chickens raised for table egg production is known as layers while those raised for meat purpose are known as broilers [5].

Among other livestock, broilers are considered to be the most efficient in feed convention in the world [6]. Broilers have been selectively bred due to certain desirable traits that they possess such as growing larger breast muscles, which are been sold as white meat. Broiler production is a good source of income and quick returns on investment. However, proper care and quick detection of disease conditions is vital for a successful production [7]. Blood assessment is one major way to diagnose disease in blood or body organs of livestock [8].

Haematological profile is the parameter used to determine the health status of animals as it plays a vital role in managing the body physiology of livestock during auction [9]. Blood which is composed of blood cells such as erythrocyte, leukocyte, and thrombocyte circulating in a fluid

called blood plasma functions in the transportation, regulatory and the defence functions in the body system [10]. Blood, is a vital circulatory tissue that is composed of cells that are suspended in a fluid intercellular substance (plasma) which function in maintaining homeostasis [11].

According to [12], blood plays a role in transportation, regulation, and defence functions. [13] stated that blood also plays a complex role in maintaining a sound physiological process so as to attain optimum animal productivity. Age, disease, surrounding temperature, physical activity, genes, sex, and geographical condition are known to be the contributing factors to blood profile in animals.

Haematological parameters are influenced by factors such as age, gender, feed, drugs, toxic compounds, diseases and parasite infestation [15]; [16]. According to [17] certain medicinal plants are usually used as feed supplements for chickens, they are also being used for medicinal purposes thereby becoming involved in a Cascade of their physiological reactions, that in turn lead to the alteration of their haematological parameters. Numerous researches have shown a positive effect of plant extracts being used as a solution in haematological parameters of broilers [18]. The results of haematological parameters could serve as the baseline information for comparison in conditions such as physiology, nutrient deficiency, and the health status of farm animals [14]. Generally, the results of haematology are usually used to assess the health status of an animal [19].

Haematological parameters are good indicators of the physiological status of animals and their changes are important in assessing the response of such animals to various physiological and nutritional situations [19]. The Changes in the haematological parameters in chicken can be used to ascertain stress in animals due to nutritional, environmental, and pathological factors [20].

However, there is no research done to assess the haematological properties of broilers administered with aqueous extract of Triumfetta cordifolia leaf. Therefore, this study is aimed to determine the effect of aqueous extract of Triumfetta cordifolia leaf on haematological properties of broilers.

2. MATERIALS AND METHODS

2.1. Experimental Site

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm, Faculty of Agriculture, Niger Delta University, Wilberforce Island, Bayelsa State. The state is geographically located within latitude 4°15′N, 5°23′S and longitude 5°22′W and 6°45′E. It shares boundaries with Delta State on the north, Rivers State on the east and the Atlantic Ocean on the west and south. The state located in the heaviest rainfall area of Nigeria. The area is a riverine area; the vegetation consists mainly of forest swamps. The poultry house is made up of a wall of about 3ft to 4ft from the ground with wire gauze covering the open sides of the wall to prevent predators and to allow proper ventilation into the poultry house. The pens to be used were repaired and cleaned properly.

2.2. Duration of the Study

The duration of this experiment is a period of six weeks with two-weeks initial period of acclimatization.

2.3. Plant Collection and Authentication

Fresh leaf of Triumfetta cordifolia was collected from the premises of Yenuzue-Gene, Yenagoa, Bayelsa State. The plant was authenticated by the Department of Crop Science, Faculty of Agriculture, Niger Delta University, Wilberforce Island, Bayelsa State.

2.4. Pre- and Post- Experimental Management

The brooding pen was cleaned and disinfected two weeks prior to the arrival of the experimental birds and the equipment used were properly cleaned and tested to see if they were in a good condition to carry out the experiment. The brooding pen was also pre- heated with stove and high (200Watts) energy bulbs a day to the arrival of the birds to keep the pen warm. A temperature ranging from 35^0C – 38^0C was maintained at the brooding stage while that of the finisher stage was between 28^0C – 30^0C.

For the first two weeks, the birds were allowed to acclimatize with their new environment after which the various treatments were administered. Litter materials used during the experiment were changed after every two weeks. The birds were supplied a minimum of twenty hours of light daily. Throughout the experimental period, feed and water were given to the bird on ad-libitum basis. During the first four weeks of the experimental period, the birds were fed broiler starter mash and broiler finisher from week five. At four weeks there was a partial replacement of starter feed with the finisher feed which kept on increasing until the fifth week when it was totally replaced with the finisher feed. This was done to reduce stress and also to get the birds acclimatize to the new feed.

2.5. Procurement of Experimental Birds

A total number of ninety-six day-old broiler chicks of Abor-Acre commercial breed were used in the experiment. Ninety-six birds were assigned randomly to four treatments designated T_1, T_2, T_3, and T_4 with three replicates per treatment containing 8 birds each. The birds were purchased from Chi Farms LTD, No. 1 TGI Close, Ilupeju-Industrial Estate, Apapa-Oworonshoki Expressway, Lagos, Nigeria. On arrival, the birds were administered Glucose-D.

2.6. Experimental Layout

The experimental layout used during this study was a Randomized Complete Block Design (RCBD) in which the birds were randomly selected and allocated to four treatments designated T_1, T_2, T_3, and T4. Before allocating the birds to the various treatments, their initial body weight was taking. Each treatment groups were further sub-divided to three replicates per treatment, each replicate had seven birds. The T_1 served as the control, 0% while T_2, T_3, and T_4 contained 10% w/v, 20% w/v and 30% w/v concentration of Aqueous Extract of Triumfetta cordifolia Leaf respectively [21].

Table 1. Experimental Layout Using Randomize Complete Block Design (RCBD)

TOTAL OF NINETY-SIX BIRDS			
T_1R_1 0%	T_2R_1 10% w/v	T_3R_1 20% w/v	T_4R_1 30% w/v
T_1R_2 0%	T_2R_2 10% w/v	T_3R_2 20% w/v	T_4R_2 30% w/v
T_1R_3 0%	T_2R_3 10% w/v	T_3R_3 20% w/v	T_4R_3 30% w/v

, where: T- Treatment; R- Replicate; w/v- weight per volume

2.7. Preparation of Leaf Extract

The fresh leaves of Triumfetta cordifolia was washed with clean water at room temperature for several times to remove sand, dust and other contaminants, and then chopped with sterilized knife. The aqueous extract was prepared according to the method described by [22]. The plant extract was prepared at a concentration of 10% w/v by weighing 400g of the chopped leaf of Triumfetta cordifolia, 20% w/v by weighing 800g of the chopped leaf of Triumfetta cordifolia, 30% w/v by weighing 1200g of the chopped leaf of Triumfetta cordifolia. The chopped and weighed leaves of Triumfetta cordifolia was blended using an electric blender and then soaked in a plastic bucket containing 4litres of water at room temperature for 24hrs. The solution was then filtered using a muslin cloth to get the aqueous extract. The extract was weighed and stored in a clean plastic container until use.

$$\text{Percentage Yield (\%)} = \frac{\text{weight of extract obtained}}{\text{Total weight of sample}} \text{ x } 100$$

2.8. Procedure of Data Collection

2.8.1. Blood Collection and Handling

Personal protective equipments (PPE) were worn. Birds were restrained on a work table and blood of about 2ml was collected from the bronchial vein of each selected bird with the aid of a 5ml syringe and transferred into a vacuum tube. Blood tubes were inverted gently between four and six times and then set in a tube holder. The blood tubes were then placed in a centrifuge at room temperature and were spun at 2000 g for 15 minute, with rapid acceleration to about 2000 g in 20 second. After centrifugation the supernatant above the Ficoll/80X NATURAL CYTOTOXICITY TO MURINE TUMOURS Isopaque interface was discarded [23].

2.9. Haematological Parameters

At week 4 and at the end of experiment, blood samples were collected from birds randomly selected from each replicate for haematology analysis.

2.9.1. Determination of Full Blood Count (FBC)

"Principle: Automated analyzer is used for assessment of full blood count using whole blood or pre-diluted mode. The whole blood sample were aspirated from the sample probe into the sample rotor valve; 6 ul of blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.994 ml of diluent. At the same time, 1.0 ml of WBC/HGB lyse

is added to prepare 1.500 dilution sample. When the solution is made to react in this status for approximately 10 seconds, RBC is haemolyzed and platelets shrink with WBC membrane held as they are. At the same time, haemoglobin is converted into red coloured methaemoglobin".

"Of the diluted/haemolyzed sample in the WBC transducer chamber, approximately 1.0ml is transferred to the HGB flow cell. 500ul of the sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method. In the HGB flow cell, a 555nm wavelength beam irradiated from the light-emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of diluent alone that was measured before the addition of the sample, thereby calculating HGB (haemoglobin value)".

"Procedure: The blood samples were mixed at least 10mins on the blood mixer before the analysis. The machine power switch was turned on and self-check was conducted. The machine was now ready for use when Ready (ready for analysis) appeared. Samples and control samples were then introduced into the instrument through the probe. After about 5-minutes the results (Haemoglobin (HGB), Haematocrit/packed cell volume (PCV), White cell count, Platelets, RBC, MCV, MCH, MCHC, Neutrophil, Lymphocyte, Monocytes, and Eosinophil) showed on the screen of the machine. After recording the results, the SOP for putting off the machine" was followed by [23].

2.10. Statistical Analysis

Two-way analysis of variance (ANOVA) was performed using SPSS Data Editor Version 25 to test for the significant differences in main and interactive effect of the treatments. The data collected were subjected to Duncan multiple range test at 5% level of significance [24].
The following models were used to analyze the quantitative data.

$Yij = \mu+Ai+eij$

Where: Yij = Individual Observation of haematological properties of broiler, μ = Overall mean, Ai = The effect of the aqueous extract of Triumfetta cordifolia Leaf on Haematological Properties of broiler, eij = Error term.

3. RESULTS

3.1. Main Effect of Graded doses of Aqueous Extract of Triumfetta cordifolia Leaf on Haematological Parameters of Broilers

The main effect of graded doses of aqueous extract of T. cordifolia leaf on haematological parameters is shown in Table 2 below. According to the results, at Day 28 WBC, Neutrophil, and MCV had significantly different ($P<0.05$) across treatments groups while the rest of the haematological parameters were not significantly affected ($P>0.05$) by the aqueous extract of T. cordifolia leaf. Results also revealed that at Day 42, WBC, Lymphocytes, Haemoglobin, and PCV haematological parameters were significantly ($P<0.05$) affected by the aqueous extract of T. cordifolia leaf. While Neutrophil, Monocyte, Eosinophil, RBC, MCV, MCH, MCHC and Platelet had no significantly difference ($P>0.05$).

According to the results at Day 28, higher ($P<0.05$) value of WBC was observed in birds administered 10%w/v concentration of the aqueous extract, but significantly lower in those administered 30%w/v. Neutrophil had the highest significant ($P<0.05$) difference at treatment 2

(10%) while treatment 3 (20%w/v) had the least significant difference (P<0.05). MCV had the highest (P<0.05) value at treatment 1 (control group, 0%w/v) and treatment 3 (20%w/v) while treatment 2 (10%) had the least significant difference (P<0.05).

Results at day 42 revealed that, WBC had the highest (P<0.05) value at treatment 2 (10%w/v) while treatment 4 (30%w/v) had the least significant difference (P<0.05). Lymphocytes had the highest significant (P<0.05) difference at treatment 4 (30%w/v) while treatment 2 (10%w/v) had the least significant difference (P<0.05). Haemoglobin had the highest significant (P<0.05) difference at treatment 2 (10%w/v) while treatment 4 (30%w/v) had the least significant difference. PCV had the highest (P<0.05) at treatment 2 (10%w/v) while treatment 4 (30%w/v) had the least significant difference (P<0.05).

3.2. Interactive Effect of graded doses of Aqueous Extract of T. cordifolia Leaf on Hematological Parameters

The interactive effect of graded doses of aqueous extract of *T. cordifolia* leaf on haematological parameters of broilers is shown in Table 4.2. The results showed that WBC, Neutrophil and Lymphocytes were significantly different (P<0.05) while Monocyte, Eosinophil, RBC, Haemoglobin, PCV, MCV, MCH, MCHC and Platelet were not significantly different (P>0.05). According to the results WBC had the highest (P<0.05) at treatment 4 (30%w/v) while treatment 1 and 2 (0%w/v and 10%w/v respectively) had the least significant difference (P<0.05). Neutrophil had the highest significant (P<0.05) difference while at treatment 2 (10%w/v) had treatment 1 and 3 (0% and 20%w/v respectively) the least significant difference (P<0.05). Also, Lymphocytes had the highest (P<0.05) at treatment 3 (20%w/v) while treatment 2 (10%w/v) had the least significant difference (P<0.05).

4. DISCUSSION

This study examined the Effect of Aqueous Extract of Triumfetta cordifolia Leaves on Haematological parameters of broilers birds. White Blood Cell (WBC), Packed cell volume (PCV), haemoglobin and mean corpuscular haemoglobin (MCH) are major parameters for evaluating circulatory red blood cells and are essential in the detection of anaemia [25]. According to [26], the overall conditions of poultry birds is influenced by the number of red blood cells. Thus, the numerical increases in PCV, haemoglobin and RBC counts (Day 28 and 42) of the birds administered various concentrations of the aqueous extract are an indication that the oxygen-carrying capacity of the blood was enhanced.

The findings of this study is agreement with the report of [27], who stated that high PCV count indicates either an increase in the number of red blood cells or a reduction in circulating plasma volume. According to [28], PCV values greater than 56% indicates dehydration in most birds. However, all PCV values in the present study were within the reference ranges for broilers stated in [29] and [30] (22% – 35% and 25% – 45% respectively).

According to [31], Hb, MCH and MCHC are important blood parameters whose values are used to determine the presence and severity of anaemia, and a decrease in their levels is said to be an indication of the birds being exposed and are poorly dealing with stress. According to the results at Day 28 no significant differences were observed in both haemoglobin and MCH values but at day 42,Haemoglobin values were significantly different across the treatments groups. However, treatment 2 (10%w/v) had a significantly lower MCV value (129.2fL) at Day 28 as compared to the other treatment groups and the control group which ranged between 132.5fL and 138.87fL. At Day 42, Treatment 4 (30%w/v) recorded the least MCV value (128.7fL) compared to other

groups which had values ranging from 132.87fL to 138.67fL It can be denoted that only treatment 2 (10%w/v) generally improved the ability of the birds to withstand stress.

It has also been reported that low MCHC value less than 29.0 g/dl can be attributed to iron deficiency [32]. In the present study no treatment group had an MCHC value lower than 29.00g/dl. However, at Day 42 MCHC values across treatments were slightly higher than 29.0g/dl but were within the recommended ranges of 24.00g/dl – 31.00g/dl [33].

The interactive effect of graded doses of aqueous extract of T. cordifolia leaf on haematological parameters of broilers is shown in Table 2. The results showed that WBC, Neutrophil and Lymphocytes were significantly different ($P<0.05$) while Monocyte, Eosinophil, RBC, Haemoglobin, PCV, MCV, MCH, MCHC and Platelet were not significantly different ($P>0.05$). WBC had the highest ($P<0.05$) at treatment 1 and 2 (0%w/v and 10%w/v respectively). However, treatment 4 (30%w/v) recorded the least significant difference ($P<0.05$). Neutrophil had the highest significant difference ($P<0.05$) at treatment 2 (10%w/v) while treatment 1 and 3 (0% and 20%w/v respectively) had the least significant difference ($P<0.05$). Furthermore, Lymphocytes had the highest ($P<0.05$) at treatment 3 (20%w/v) while treatment 2 (10%w/v) had the least significant difference ($P<0.05$).

5. CONCLUSION AND RECOMMENDATION

The results from this study shows that the administration of aqueous extract of Triumfetta cordifolia Leaf to the broiler birds had significant effect on WBC, Neutrophil, and MCV haematological properties at Day 28, while WBC, Lymphocytes, Haemoglobin, PCV were significantly affected at Day 42. However, birds on 10%w/v concentration of the aqueous extract had the highest values in most of the haematological parameters in both Day 28 and Day 42. Significant age effect was observed for WBC, Lymphocytes, Eosinophil, RBC, PCV and Platelets as all recorded increased values with advancement of age. However, the rest parameters (Neutrophil, Monocytes, Haemoglobin, MCV, MCH and MCHC) were all inversely affected with advancement of age. The results of this study can be useful and contributes baseline haematology values for management of broilers.

According to the results, 10%w/v concentration of the aqueous extract of Triumfetta cordifolia Leaf is recommended for better haematological profile of broilers birds, as the highest values of haematological parameters was observed for birds in this treatment group. Furthermore, with the dearth of information on the use of aqueous extract of Triumfetta cordifolia Leaf for the improvement of haematological properties of broilers, more studies should be carried out in order for comparison to be made and eventually getting a baseline effect of the aqueous extract of Triumfetta cordifolia Leaf on broilers. Studies should also be carried out using different breeds of broilers.

Table 2. Main Effect of graded doses of Aqueous Extract of *Triumfettacordifolia* Leaf on Haematological Parameters

	Day 28					Day 42				
Parameters	0% w/v	10% w/v	20% w/v	30% w/v	SEM	0% w/v	10% w/v	20% w/v	30% w/v	SEM
WBC (10^9/L)	36.27±8.47^{ab}	38.2±4.03^{a}	27.6±4.65^{bc}	23.27±1.63^{c}	2.26	207.3±2.76^{ab}	214.23±2.24^{a}	205.1±4.89^{ab}	193.53±19.34^{b}	3.36
Neutrophil (%)	13.67±2.08^{b}	24±8.54^{a}	13.67±2.89^{b}	22.00±1.00^{ab}	1.83	1.67±0.58	2±0	2±0	1.33±0.58	0.13
Lymphocytes (%)	77.33±6.81	64.67±17.39	82±5.2	72±3	3.1	90.33±0.58^{ab}	88.67±0.58^{b}	90±1^{ab}	92.33±3.06^{a}	0.57
Monocyte (%)	8.33±5.51	10.33±9.24	4.00±2.65	5.00±2	1.58	5.00±1	5.00±1	3.67±1.15	3.33±2.08	0.41
Eosinophil (%)	0.67±0.58	1±0	0.33±0.58	1±0	0.13	3±1	4.33±1.15	4.33±2.08	3±1	0.4
RBC (10^{12}/L)	1.97±0.15	2.17±0.06	1.97±0.15	2.27±0.21	0.05	2.1±0.1	2.2±0.1	2.07±0.12	1.93±0.23	0.05
Haemoglobin (g/dL)	9.67±0.93	10.07±0.32	9.77±0.67	10.83±0.93	0.26	8.57±0.45^{ab}	9.13±0.42^{a}	8.27±0.25^{ab}	7.5±0.95^{b}	0.23
PCV (%)	27±1.73	28±1	27.6±1.44	29.67±2.89	0.56	28.67±1.53^{ab}	31±0^{a}	27.67±0.58^{ab}	25±3.46^{b}	0.8
MCV (fl)	136.07±3.1^{a}	129.2±6.75^{c}	138.87±3.4^{a}	132.5±6.37^{b}	1.68	134.33±6.07	138.67±4.15	132.87±7.45	128.7±2.4	1.7
MCH (pg)	48.83±0.71	46.37±1.59	49.1±0.46	47.87±1.12	1.42	39.97±0.32	40.33±0.61	39.33±2.65	38.17±0.23	0.42
MCHC (g/dL)	35.87±1.1	35.9±0.62	35.37±1.05	36.17±0.93	0.25	29.8±1.11	29.13±1.23	29.63±0.93	29.67±0.38	0.25
Platelet	5±2.65	7.33±3.06	3±2.65	4±2.65	0.83	13.33±7.57	13.33±12.74	7±3	10.67±2.52	2.04

^{abc}means with different superscript on each row are significantly different (P<0.05), $^{Mean \pm standard\ error}$, WBC = White Blood Cell, RBC = Red Blood Cell, PCV = Packed Cell Volume, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCH = Mean Corpuscular Haemoglobin Concentration, SEM = Standard Error of Mean

Table 3. Interactive Effect of graded doses of Aqueous Extract of Triumfetta cordifolia Leaf on Haematological Parameters

Parameters	**0% w/v**	**10% w/v**	**20% w/v**	**30% w/v**	**SEM**
WBC	121.78±93.85^{a}	126.22±96.46^{a}	116.35±97.31^{ab}	108.4±94.06^{b}	2.3
NEUTROPHIL	7.67±6.71^{b}	13±13.21^{a}	7.83±6.65^{b}	11.67±11.34^{ab}	1.35
LYMPHOCYTES	83.83±8.33^{ab}	76.67±17.14^{b}	86±5.51^{a}	82.17±11.46^{ab}	2.87
Monocyte	6.67±3.98	7.67±6.56	3.83±1.83	4.17±2.04	1.67
Eosinophil	1.83±1.47	2.67±1.97	2.33±2.58	2±1.26	0.42
RBC	2.03±0.14	2.18±0.08	2.02±0.13	2.1±0.27	0.06
Haemoglobin	9.12±0.89	9.6±0.61	9.02±0.94	9.17±2.01	0.28
PCV	27.83±1.72	29.5±1.76	27.63±0.98	27.33±3.83	0.78
MCV	135.2±4.42	133.93±7.21	135.87±6.14	130.6±4.78	
MCH	44.4±4.88	43.35±3.48	44.22±5.61	43.02±5.36	0.5
MCHC	32.83±3.47	32.52±3.81	32.5±3.26	32.92±3.62	0.39
Platelet	9.17±6.82	10.33±8.91	5±3.35	7.33±4.32	2.35

^{abc}means with different superscript on each row are significantly different (P<0.05), $^{Mean \pm standard\ error}$

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