



Haematology and Serum Biochemical Changes associated with the Consumption of *Jatropha tanjorensis* Leaf in Broilers

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Abstract

The study aimed at determining the effect of *Jatropha tanjorensis* leaf meal on the haematology and serum biochemistry of broiler birds. A total of 132 unsexed two weeks old broiler chicks were randomly divided into four groups of thirty-three broilers each, with 11 birds per replicate. The four experimental diets were formulated to contain 0, 1.5, 3.0, and 3.5% dietary supplementation of *Jatropha tanjorensis* leaf meal, respectively. The groups were randomly assigned to the four experiment diets (*T1*, *T2*, *T3* and *T4*) at 3 replicates for 10 weeks in a completely randomized design. At the start of the experiment, the haemoglobin ranged from 6.82 to 7.40g/dl, packed cell volume (PCV) ranged from 24.30 to 28.56%, red blood cell (RBC) $2.46-2.63 \times 10^6 \text{mm}^3$, White blood cell (WBC) $33.20-34.01 \times 10^3 \text{mm}^3$, Mean Corpuscular Volume (MCV) 81.00-86.05fl, while mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration ranges from 24.11-23.24pg and 17.98-19.01g/dl, respectively. At the end of the experiment, haemoglobin, PCV, RBC, WBC and MCV showed significant improvement ($p < 0.05$) across the treatment groups, while MCH and MCHC significantly reduced ($p < 0.05$). At the start of the experiment, total protein ranges from 3.62 to 4.20g/dl, albumin 2.12 to 2.89g/dl, globulin 1.04 to 1.65g/dl, cholesterol and glucose ranged from 83.67 to 86.59mg/dl and 161.67 to 162.05mg/dl, respectively. At the end of the trial, total protein, albumen, and globulin values were significantly ($p < 0.05$) higher in *T3* and *T4*. Cholesterol and glucose was significantly reduced ($p < 0.05$) from *T1* to *T4*. The study revealed that the supplementation of *Jatropha tanjorensis* leaf meal in broiler diets had no deleterious effect on these haematological and serum biochemical parameters of broilers and could be supplemented in broiler diets up to 3.5%.

Keywords: Haematology, Serum Biochemistry, *Jatropha tanjorensis*, Immuno-modulatory, Broiler Chicken

Introduction

The global interest in phytochemicals has led to increased investigation of different plants than before. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in pharmaceutical and drug research. Plant materials, plant products, and byproducts have continued to play an important role in the maintenance of human and animal health since antiquity (Ekuma, *et al.*, 2017). Currently, several plants and herbs are being used in part or whole to treat many diseases, and enhance blood profile and animal physiology (Uchewa, *et al.*, 2018). Active component compounds and the beneficial effects of these plant materials are now being investigated, extracted, and developed into drugs or used as growth promoters in animal production (Oguike *et al.*, 2019). Some synthetic chemical compounds and strategies aimed at reducing the cost of animal

production have impacted negatively on blood profile. As such, phytochemicals, leaf meals, and herbal products have received increased attention as they have acquired more acceptability among consumers as a natural product (Oguike, *et al.*, 2019; Jiwuba, *et al.*, 2020; Amaduruonye, *et al.*, 2020). One of these phytochemical plants that need to be investigated and harnessed is *Jatropha tanjorensis*.

Jatropha tanjorensis is a flowering plant belonging to the family of *Euphorbiaceae*. (Ebena *et al.*, 2019; Malik *et al.*, 2022). It is commonly called hospital too far, catholic vegetable; ugu-oyibo, iyana-Ipaja and lapalapa. It is a multipurpose plant, cultivated for medicinal applications and as a food condiment. It is primarily used in most rural communities for fencing, and secondarily as a source of edible leafy vegetable and as a medicinal plant (Ocholor, *et al.*, 2018, Ebenyi, *et al.*,

2021). Virtually every part of the plant is beneficial, depending on the part being used. The leaves are cooked and eaten as a vegetable (Ebena, *et al.*, 2019). The leaf extract is consumed as a blood tonic to improve blood profiles and blood volume (Chigozie *et al.*, 2018). The stem sap is haemostatic through enhancement in platelet coagulation mechanisms (Ansari, *et al.*, 2020). The root decoction is used as a toothpaste for treating bleeding gums, toothache, and several skin and venereal diseases (Chibuogwu, *et al.*, 2021; Ebenyi, *et al.*, 2021). In Nigeria, the different plant parts of *Jatropha tanjorensis* have been utilized in rural communities for the treatment of several ailments, ranging from anaemia, malaria, hypertension, cardiovascular disorder, diabetes, urinary tract, and sexually transmitted infections (Ebana, *et al.*, 2019). Due to the vast traditional use of *Jatropha*, many have consumed it indiscriminately without minding toxicity and safe limits.

The use of local or traditional herbs for medicinal purposes and animal nutrition has heightened over the years. In Nigeria, the utilization of various plants, leaves, stem, and root in animal nutrition is on the increase due to the strong belief that these herbs can enhance animal health and physiology. As such, the depth of information on the toxicity of these plants needs to be ascertained, as most plants used in monogastric nutrition can affect the blood profile. Therefore, this study aimed to evaluate the impact of *Jatropha tanjorensis* on the blood profile and the safety of its consumption and utilization in animal physiology.

Materials and Methods

Experimental Site

This research was conducted at the Poultry Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State. The area is located in the South-Eastern part of Nigeria on latitude 5°27' north, longitude 7° 32' East, an altitude of 123m above sea level with an annual rainfall of 2177mm, temperature of 22°C – 36°C, and relative humidity of 50 – 90%. It is situated within the humid rainforest zone of West Africa, characterized by a long duration of rainy season (March-October) and a short period of the dry season (November - February). Climatic data were collected from the Meteorological Center of the National Root Crop Research Institute, Umudike, Abia State (NRCRI, 2010).

Experimental Animals and Management

A total of One hundred and fifty (150) unsexed Ross 308 broiler strain day-old chicks were procured for the study. The chicks were brood for 2 weeks. Thereafter, one hundred and thirty-two (132) birds were sampled and randomly assigned to four treatment groups and replicated three times with 11 birds per replicate in a completely randomized design (CRD) feeding trial that lasted for 10 weeks. Prior to the arrival of the birds, the brooder house was washed, disinfected, dried and the floor of the brooder house was covered with layers of

wood shaving as litter materials evenly spread on the floor. The feeders and drinkers were washed and dried properly. Kerosene stove, electric bulbs, and lanterns were provided as heat source during brooding. On arrival, the birds were vaccinated against New Castle Disease (NCD I/O), and also given glucose and multivitamin as anti-stress preparations to recover from the stress of transportation. Adequate heat and light were provided with the aid of kerosene stoves and electric bulbs respectively. They were managed in deep litter pens throughout the experimental period and fed a concentrate diet and water *ad libitum*. Routine management practices were carried out appropriately. The fieldwork lasted for 12 weeks.

Experimental Diets

Sourcing and processing of *Jatropha tanjorensis* leaf

The fresh *Jatropha tanjorensis* leaves were harvested from Michael Okpara University demonstration farms, Umudike, Olokoro and its environs. After harvesting, the stalks of the *Jatropha tanjorensis* were removed and the leaves were chopped into small sizes to facilitate air-drying. The *Jatropha tanjorensis* leaves were air-dried at room temperature for 7 days. After drying, the leaves of *Jatropha tanjorensis* were ground to produce *Jatropha tanjorensis* leaf meal using a hammer mill (CF-158 Hammer Muhle 2,2 Kw 380 V-cissonius) brand. The *Jatropha tanjorensis* leaf meal was used as the test ingredient for the experiment. The composition of the experimental starter and finisher diet is presented in Tables 1 and 2 respectively.

Experimental Design

The experiment was a Completely Randomized Design (CRD) of 4 treatments and replicated 3 times with 11 birds per replicate. The treatments were designated: T₁, T₂, T₃, and T₄. T₁ served as the control. Thirty-three (33) birds were randomly allocated to each treatment. The birds in different treatments were fed the same concentrate diets and supplemented with varying levels of *Jatropha tanjorensis* leaf meal at 0% (T₁), 1.5% (T₂), 3.0% (T₃), and 3.5% (T₄) respectively. Treatment 1 with no *Jatropha tanjorensis* leaf meal served as the control. The experimental model is as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} = individual observation on the broiler characteristics.

μ = overall mean

T_i = treatment effect

e_{ij} = Experimental error assumed to be independently, identically, and normally distributed with zero means and constant variances.

Data Collection

Blood Collection

Four (4) birds were randomly sampled from each replicate at two-weeks old after brooding and at the end of the experiment for blood collection and analysis. Four (4) blood samples were collected from each replicate for haematology and serum biochemistry. A 10 ml sterile

syringe fitted with a sterile hypodermic needle was used to collect 10ml of blood intravenously from the web of the wing for haematology and serum biochemistry. Thereafter, 5ml were immediately transferred to sample bottles containing Ethylene diamine tetra-acetic acid (EDTA), an anti-coagulant to prevent blood clotting, and used for hematological analyses. The remaining 5 ml were transferred to EDTA-free sample bottles and used for serum biochemistry.

Haematology

The blood samples collected were analyzed within 2 hours of collection. The EDTA sample bottles were shaken gently to prevent clotting. The following hematological indices were determined: haemoglobin, packed cell volume, red blood cell, and white blood cell, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. The Haemoglobin (Hb) concentration was determined using a spectrophotometer through the Cyanomethanoglobin method as described by Feldman *et al.*, (2000) and Bain *et al.* (2017). Packed cell volume (PCV) was determined by the micro-hematocrit method as described by Kahn, *et al.* (2010). Red blood cell (RBC) and white blood cell (WBC) counts were determined using Neubauer-hemocytometer method as described by Bain *et al.* (2017). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated as described by Douglas and Wardrop, (2010). The MCV, MCHC, and MCH were calculated thus:

$$\text{Mean Corpuscular Haemoglobin (MCH)} = \frac{\text{Hb} \times 10}{\text{RBC}}$$

$$\text{Mean Corpuscular Volume (MCV)} = \frac{\text{PCV} \times 10}{\text{RBC}}$$

$$\text{Mean Corpuscular Haemoglobin Conc. (MCHC)} = \frac{\text{Hb} \times 100}{\text{PCV}}$$

Serum Biochemistry

The bottles of the coagulated blood samples were subjected to standard methods of serum separation and the harvested sera used for evaluation of serum parameters. The standard flame photometry was used to determine the serum parameters. The following blood chemistry indices were analyzed colorimetrically and spectrophotometrically according to standard clinical biochemistry procedures as described by Kahn, *et al.*, (2010): total protein, albumin, globulin, cholesterol, serum urea, and Glucose. The blood samples for Hematology and Serum biochemistry were analyzed at the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

Statistical Analysis

Data collected on the different parameters were subjected to Analysis of Variance (ANOVA) using a

software package (SPSS version 23). Significant means were separated using Duncan's Multiple Range Test at 5% level of significance (Duncan, 1955). All statistical analyses were in accordance with the methods of Steel and Torrie (1980).

Results and Discussion

The Haematology of broiler birds administered *Jatropha tanjorensis* leaf meal is presented in Table 3. At the start of the experiment, the haemoglobin ranged from 6.82 to 7.40g/dl, packed cell volume (PCV) ranged from 24.30 to 28.56%, red blood cell (RBC) $2.46\text{--}2.63 \times 10^6 \text{mm}^3$, White blood cell (WBC) $33.20\text{--}34.01 \times 10^3 \text{mm}^3$, Mean Corpuscular Volume (MCV) 81.00-86.05fl, while mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration ranges from 24.11-23.24pg and 17.98-19.01g/dl, respectively. At the end of the experiment, the haemoglobin, packed cell volume, red blood cell, white blood cell, and the mean corpuscular volume significantly ($p < 0.05$) increased following the administration of *Jatropha tanjorensis* leaf meal on the broiler birds in T_2 , T_3 and T_4 ; while the mean corpuscular haemoglobin and the mean corpuscular haemoglobin concentration significantly ($P < 0.05$) reduced in T_2 , T_3 , and T_4 compared with the control. These haematological parameters are within the normal range for healthy Ros 308 broiler chickens as recommended by Al-Nedawi, (2018). This result showed that *Jatropha tanjorensis* leaf meal stimulated the formulation of the red blood cells, increased the percentage of cells in the blood, enhanced the oxygen-carrying capacity of the blood, and as well as the erythropoietic potentials of the bone marrow of the broiler birds. This significant improvement observed on the blood profile following the administration of *Jatropha tanjorensis* leaf meal could be attributed to the nutritional and beneficial phytochemical potential of the test ingredient, as *Jatropha tanjorensis* leaf has been shown to be rich in iron, protein, other nutrients and many macro and micro mineral elements (Falodun, *et al.*, 2013; Omigie *et al.*, 2020). The appreciable amount of iron, protein and other mineral elements present in *J. tanjorensis* leaf may have increased the amount of iron and other nutrient availability for erythropoiesis, thereby enhancing the production of red blood cells, haemoglobin and the PCV of the broiler birds. It may also have been possible that some of the chemical constituents of *Jatropha tanjorensis* leaf have an erythropoietin-like effect on the bone marrow, leading to the increase in the rate of erythropoiesis and a resultant increase in packed cell volume, red blood cells, and hemoglobin concentration. Furthermore, these bioactive phytochemical agents found in *Jatropha tanjorensis* leaves may have stimulated the kidney to release erythropoietic factors that convert blood protein to erythropoietin, thus, stimulating the production of red blood cells, thereby improving the haematological parameters. The serum biochemistry of broiler birds administered *Jatropha tanjorensis* leaf meal is presented in Table 4.

At the start of the experiment, total protein ranges from

3.62 to 4.20g/dl, albumin 2.12 to 2.89g/dl, globulin 1.04 to 1.65g/dl, cholesterol and glucose ranged from 83.67 to 86.59mg/dl and 161.67 to 162.05mg/dl, respectively. At the end of the trial, administration of *Jatropha tanjorensis* leaf meal did not significantly ($p>0.05$) affected the serum urea. The serum total protein, albumin and globulin in T₃ and T₄ improved significantly ($p<0.05$); while the serum cholesterol and glucose significantly reduced ($p<0.05$) following the administration of the test ingredient compared with the control group. These observations indicated that *Jatropha tanjorensis* leaves could enhance immunity by stimulating the production of the globulin/immunoglobulin and the antibodies in the serum. The serum total protein also increased, showing that *J. tanjorensis* leaves increased the production of serum protein thus increasing the blood formation by enhancing the mechanism of action of the protein-erythropoietin biochemical partway. Furthermore, the result also showed that *Jatropha tanjorensis* leaves reduced the cholesterol and the sugar level in the blood of the broiler birds compared with the control group. In human, high blood cholesterol and high blood sugar is associated with heart disease and diabetes respectively. This inferred that *Jatropha tanjorensis* leaves can be used to regulate cholesterol and sugar levels in the blood. These findings collaborated with the review reports of Ansari, *et al*, (2020) that leaf extract of *Jatropha tanjorensis* caused a significant reduction in serum glucose, total lipids, and cholesterol parameters in rats.

Conclusion

Based on the results and observations from this study, it is concluded that administration of *Jatropha tanjorensis* leaf meal at 1.5%, 3.0%, and 3.5% in broiler diets significantly improved the red blood cell, white blood cell, packed cell volume, haemoglobin and mean corpuscular volume; while the mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration reduced. Also, *Jatropha tanjorensis* leaf meal at 3.0% and 3.5% improved the serum total protein, albumin, and globulin; while at 1.5%, 3.0%, and 3.5% reduced the serum cholesterol and blood glucose level of the broiler birds. From the results obtained, it is recommended that *Jatropha tanjorensis* leaves at 1.5%, 3.0% and 3.5% in broiler diets can be used to improve the blood profile of broiler birds and also to regulate blood sugar and cholesterol level. Therefore, the utilization of *Jatropha tanjorensis* in broiler production can have a wider applications in livestock production and in animal agriculture at large.

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Table 1: Composition of Broiler Starter experimental diets

Ingredients (%)	T ₁ (0.0%)	T ₂ (1.5%)	T ₃ (3.0%)	T ₄ (3.5%)
Maize	46.00	46.00	46.00	46.00
Soya bean meal	26.00	26.00	26.00	26.00
Palm kernel cake	7.00	7.00	7.00	7.00
Fish meal	2.00	2.00	2.00	2.00
Blood meal	4.00	4.00	4.00	4.00
Wheat offal	10.00	10.00	10.00	10.00
Bone meal	4.00	4.00	4.00	4.00
Common salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.10	0.10	0.10
Methionine	0.25	0.10	0.10	0.10
Vit/mineral Premix*	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
<i>Jatropha tanjorensis</i> leaf meal	0%	1.5%	3.0%	3.5%
Crude protein (%)	23.12	23.12	23.12	23.12
Energy (kcal/kg)	2801.00	2801.00	2801.00	2801.00

*Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride, 300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg

Table 2: Composition of Broiler Finisher experimental diets

Ingredients (%)	T ₁ (0.0%)	T ₂ (1.5%)	T ₃ (3.0%)	T ₄ (3.5%)
Maize	60.00	60.00	60.00	60.00
Soya bean meal	18.00	18.00	18.00	18.00
Palm kernel cake	5.00	5.00	5.00	5.00
Fish meal	2.00	2.00	2.00	2.00
Blood meal	2.00	2.00	2.00	2.00
Wheat offal	8.00	8.00	8.00	8.00
Bone meal	4.00	4.00	4.00	4.00
Common salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vit/mineral Premix*	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
<i>Jatropha tanjorensis</i> leaf meal	0%	1.5%	3.0%	3.50%
Crude protein (%)	19.00	19.00	19.00	19.00
Energy (kcal/kg)	2920.00	2920.00	2920.00	2920.00

Table 3: Haematology of broiler birds administered *Jatropha tanjorensis* leaf meal

Parameter	Normal Range	T ₁ (0.0%)	T ₂ (1.5%)	T ₃ (3.0%)	T ₄ (3.5%)	SEM
At the start of the experiment						
Haemoglobin (g/dl)	4.08-16.40	6.82	7.02	7.04	7.40	0.10
Packed Cell Volume (%)	21.20-47.60	28.56	24.30	25.23	26.05	0.56
Red blood cell (x10 ⁶ mm ³)	2.30-3.90	2.46	2.61	2.60	2.63	0.57
White blood cell (x10 ³ mm ³)	30.40-56.00	33.80	33.54	34.01	33.20	0.30
Mean Corpuscular Volume (fl)	48.90-187.50	81.00	84.50	86.05	83.00	0.20
MCH (pg)	12.25-63.13	24.11	23.24	22.94	22.80	0.39
MCHC (g/dl)	15.86-35.31	18.50	17.98	18.05	19.01	0.52
At the end of the experiment						
Haemoglobin (g/dl)	4.08-16.40	10.20 ^a	10.70 ^{ab}	11.00 ^b	11.06 ^b	0.15
Packed Cell Volume (%)	21.2-47.00	24.50 ^a	27.50 ^b	28.50 ^{bc}	29.02 ^c	0.74
Red blood cell (x10 ⁶ mm ³)	2.30-3.90	2.74 ^a	3.08 ^b	3.09 ^b	3.02 ^b	0.77
White blood cell (x10 ³ mm ³)	30.40-56.00	19.00 ^a	19.75 ^a	20.78 ^b	22.05 ^c	0.28
Mean Corpuscular Volume (fl)	48.90-187.50	92.98 ^a	94.19 ^c	93.44 ^b	94.42 ^c	0.18
MCH (pg)	12.25-63.13	37.30 ^b	35.57 ^{ab}	34.92 ^a	35.68 ^{ab}	0.44
MCHC (g/dl)	15.86-35.31	31.84 ^b	29.07 ^a	28.60 ^a	28.20 ^a	0.56

abc: Means with different superscripts along rows are significantly different (p < 0.05). SEM= Standard error of means. MCH=Mean Corpuscular Haemoglobin; MCHC=Mean Corpuscular Haemoglobin Concentration. The normal range according to Al-Nedawi (2018)

Table 4: Serum biochemistry of broiler birds administered *Jatropha tanjorensis* leaf meal

Parameter	Normal Range	T ₁ (0.0%)	T ₂ (1.5%)	T ₃ (3.0%)	T ₄ (3.5%)	SEM
At the start of the experiment						
Total protein (g/dl)	3.10-5.72	4.05	3.93	4.20	3.62	0.10
Albumin (g/dl)	2.01-4.62	2.40	2.89	2.80	2.12	0.06
Globulin (g/dl)	-	1.65	1.04	1.40	1.50	0.08
Cholesterol (mg/dl)	55.79-214.70	84.01	83.67	86.59	85.25	1.80
Urea (mg/dl)	0.50-6.00	3.80	3.65	4.10	3.81	0.50
Glucose (mg/dl)	125.00-200.00	161.67	162.00	162.05	161.80	8.60
At the end of the experiment						
Total protein (g/dl)	3.10-5.72	4.15 ^a	4.30 ^a	4.69 ^b	4.71 ^b	0.09
Albumin (g/dl)	2.01-4.62	2.62 ^a	2.66 ^a	2.91 ^b	2.90 ^b	0.05
Globulin (g/dl)	-	1.53 ^a	1.64 ^{ab}	1.78 ^b	1.81 ^b	0.04
Cholesterol (mg/dl)	55.79-214.70	107.89 ^c	104.57 ^b	102.76 ^{ab}	98.63 ^a	1.78
Urea (mg/dl)	0.50-6.00	4.11	4.91	4.74	4.82	0.46
Glucose (mg/dl)	125.00-200.00	192.80 ^c	176.00 ^b	173.60 ^{ab}	167.00 ^a	9.09

abc: Means with different superscripts along rows are significantly different (p < 0.05). SEM= Standard error of means. Normal range according to Banerjee (1998) and Abdi-Hachesoo et al. (2011)