



Haematology and Serum Biochemistry Evaluations of Broiler Chickens Inoculated with *Salmonella* Enteritidis and Treated with *Phyllanthus amarus* Leaf extract

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ABSTRACT: A 21-day experiment was conducted to study the effects on haematology and serum biochemistry of broiler chickens inoculated with *Salmonella* Enteritidis (SE) and treated with methanol extract of *Phyllanthus amarus* leaf (PALM). A total of 60 Abor-Acre unsexed 5 week old broiler chickens were randomly allotted to four treatments including T₁ = Ordinary water (control), T₂ = SE inoculated (10⁷ CFU, PO), T₃ = SE inoculated + PALM (150 mg/kg) and T₄ = SE inoculated + Enrofloxacin (10 mg/kg). Each treatment was replicated thrice (n = 5) and the birds allotted to treatments in a completely randomized design. SE was inoculated at 5 weeks of age to T₂ – T₄. One hour prior to inoculation, T₃ and T₄ received PALM and enrofloxacin respectively which continued for another 4 days (ie 5 days in all). At the end of 3 weeks, one bird/replicate from T₁ – T₄ was randomly selected and 4 mL of blood aseptically drawn for haematological and biochemical analyses. Data collected were subjected to analysis of variance and means compared with Duncan's Multiple Range Test. The haematological and biochemical values were not affected (p<0.05) except the monocytes where T₁ and T₂ were different (p>0.05) from T₃ and T₄ and ALP that significantly differed (p>0.05) from others. Meanwhile, PALM stabilized the parameters, particularly the liver enzymes in the event of negative effects due to SE inoculation. It can therefore be concluded that PALM can be used to stabilize haematological and biochemical values in the event of negative alteration in quantities mainly due to microbial assault in broiler chickens.

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Attempt has recently been taken to reduce the cost of feed, including the incorporation of agro-industrial by-products in broiler diets as an energy source (Sugiharto and Rajitkar, 2019). However, some limitations may exist when using the agro-industrial by-products as the ingredients in broiler rations. The high and low contents of fibre and protein in the by-products may limit the digestibility and thus inclusion level of such by-products (Sugiharto *et al.*, 2018). It is known that some foliage contain a number of bioactive compounds that are beneficial for the health of chickens (Rama-Rao *et al.*, 2019). These compounds include vitamins, phenolic acids, flavonoids, isothiocyanates, tannins as well as saponins (Vergara-Jimenez *et al.*, 2017). The use of medicinal plants in

animal production has increased research interests as a potential substitute for antibiotics (Lillehoj *et al.*, 2018). In this regard, the use of leaf meal in rations may not only reduce the cost of feeds, but also elicit the health-promoting effect on broiler chickens (Sugiharto *et al.*, 2019). *Phyllanthus amarus* is widely distributed in all tropical and subtropical regions of the planet (Edeoga *et al.*, 2006). In Nigeria, it is called “Oyomokeisoamank edem” in Efik, “Iyin Olobe” in Yoruba and “Ebebenizo” in Bini (Etta, 2008). *P. amarus* has various groups of compounds such as alkaloids, flavanoids, hydrolysable tannins, major lignans and polyphenols (Peters *et al.*, 2015). Lignans like phyllanthin and hypophyllanthin, flavonoids like quercetin were isolated from the leaves of *P. amarus*

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(Meena *et al.*, 2018). *Phyllanthus amarus* is a plant of the family Euphorbiaceae and has approximately 800 species which are found in tropical and subtropical countries of the world (Tahseen and Mishra, 2013). The name '*Phyllanthus*' means "leaf and flower" and named so because of its appearance where flower, fruit and leaf appears fused (Kumar *et al.*, 2011). Its effect in excretory system is due to its antiurolithic property and is used in the treatment of kidney/gallstones, other kidney related problems, appendix inflammation and prostate problems (Sen and Batra, 2013; Ushie *et al.*, 2013). *P. amarus* like other tropical tree leaves contains some bioactive compounds which may affect nutrient utilization, haematological and serum biochemical parameters in animals (Ogbuewu *et al.*, 2010). The anti-nutritional compounds contained in *P. amarus* include oxalate, phytate, hydrogen cyanide, nitrate and tannin (Gafar *et al.*, 2012). Herbal medicines are believed to be safer than synthetic medicine because phytochemicals in the plant extract target its biochemical pathways (Sandigawad, 2015). Evaluation of haematological parameters are not only used to determine the extent of deleterious effect of herbal extracts, but also to explain functions of plant extracts or their products on the blood of animals (Bin-Jaliah *et al.*, 2014). Therefore, the present study examined the effect of haematology and serum biochemistry of broiler chickens inoculated with *Salmonella* Enteritidis and treated with *Phyllanthus amarus* leaf extract

MATERIALS AND METHOD

Experimental site and Ethical consideration: The experiment was carried out at the student's project site of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. Ibadan is located approximately on longitude 3° 5' to 4° 36' E and latitude 7° 23' to 7° 55' N (Oladele and Oladimeji, 2011). Ibadan has a tropical wet and dry climate, with a lengthy wet season. It has mean total rainfall of 9,233.60 mm, mean maximum and minimum temperatures of 39.82 °C and 22.5 °C respectively (Egbinola and Amobichukwu, 2013) and relative humidity of 74.55%. Ethical conditions governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea (1997). The experimental protocol was approved by the institutions ethical committee for the use of animals for experiment.

Collection and extraction of *Phyllanthus amarus* and sourcing of *Salmonella enteritidis*: The test plant used for this project is *Phyllanthus amarus* (Stone Breaker). The plant was identified by the school's botanists. The leaves were collected from the school's Botanical Garden. The harvested fresh leaves were washed,

rinsed with distilled water and air dried under shade until they were crispy to touch, while still retained their green colour. The leaves were ground with domestic electric grinding machine (Sonik^R Model SB-464) to produce *P. amarus* leaf meal (PALM). The PALM was subjected to 80% methanol extract maceration technique by putting one hundred grams (100 g) of the PALM in 500 mL of 80% methanol at room temperature for 72 h while shaking intermittently with rotary shaker. The extract was filtered through a muslin cloth and Whatman No. 1 filter paper and funnel. The extract was placed in a beaker and evaporated by placing it inside the water bath at 45 °C for 3 days to obtain a thick concentration. The resulting dry hydroalcoholic extract was weighed. *Salmonella* Enteritidis was obtained from Fish and Wild Life Laboratory, Faculty of Veterinary Medicine, University of Ibadan.

Birds, management and design: Sixty (60) day-old unsexed Arbor acre broiler chicks were used for the study. Prior to the arrival of the birds, the pens were cleaned and washed with detergent solutions. Disinfection of the pen was done using saponated cresol (Lysol^R), and the pen was left stock-free for one week and the floor litter laid to 5cm³ with wood shavings. On arrival of the chicks, anti-stress solution (mixture of water, glucose and multivitamin) was served as well as normal feed (Starter Top Feed^R, 22% CP, 2800 kcal/kg) and borehole water *ad libitum*. Routine vaccinations (Newcastle disease vaccine (NDV) i/o, Lasota and Infectious bursal disease (IBD) were administered accordingly during the two weeks of acclimatization. IBD vaccine was repeated on day fourteen. After acclimatization, the birds were allocated to four treatments in a completely randomized design. They were replicated thrice with 5 birds per replicate. Measured quantity of Starter Top Feed^R (0 – 4 wks old) and Finisher Top feed^R (5 wks and above, 19% CP, 3200 kcal/kg) were given by 7 am and 5 pm daily whereas clean borehole water was supplied *ad libitum* throughout the experimental duration of 21 days under standard environmental conditions (a 12 h/12 h light/dark cycle). The care and handling of the chickens were in accordance with internationally accepted guidelines for use of animals (Vogel *et al.*, 2002). Also, coccidiostat was given when the birds showed sign of coccidiosis at week four. The experimental dosing and groupings were as stated hereunder:-T₁ - Ordinary water (control), T₂ - *Salmonella* Enteritidis inoculated (10⁷ CFU, PO), T₃ - *Salmonella* Enteritidis inoculated + *P. amarus* (150 mg/kg), T₄ - *Salmonella* Enteritidis inoculated + Enrofloxacin (10 mg/kg).

Salmonella Enteritidis (10⁷ CFU, PO) was inoculated at 5 wks of age to T₂ – T₄. One hour prior to

inoculation, T₃ and T₄ received *P. amarus* (150 mg/kg) and Enrofloxacin (10 mg/kg) respectively. There was continued administration of *P. amarus* and Enrofloxacin for another 4 days (ie 5 days in all).

Data collection and laboratory analyses: a) Haematology and serum biochemistry: In the end of 3 wks of study, one bird/replicate from T₁ – T₄ was randomly selected and 4mL of blood aseptically (methylated spirit swab) collected using a new sterile syringe and needle, 2 mL was deposited in well labeled plain sample bottles as well as another 2 mL EDTA sample bottles for serum biochemical and haematological analyses respectively. Pack cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), white blood cell (WBC) and absolute counts of neutrophils, lymphocytes, monocytes and eosinophils which were all computed according to standard techniques as reported by Mafuvadze and Erlwanger (2007), mean corpuscular volume (MCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration were computed according to Jain (1986). The serum total protein, Albumin and Globulin were computed according to (Doumas and Briggs, 1972). Alkaline phosphatase, Alanine aminotransferase and alkaline phosphatase were determined according to Reitman and Frankel (1957), glucose were computed according to the procedure of Toro and Ackermann (1975).

Statistical analyses: All data obtained were subjected to analysis of variance (ANOVA) using a Statistical Package for Social Sciences (SPSS) version 20.0. Significantly different means (p<0.05) were separated using Duncan's New Multiple Range Test (DNMRT) as described by Obi (2002).

RESULTS AND DISCUSSION

The haematology of broiler chickens inoculated with Salmonella Enteritidis and treated with Phyllanthus amarus leaf extract: Table 1 shows the haematological values of broiler chickens inoculated with *Salmonella* Enteritidis and treated with PALM and enrofloxacin.

There was no significant difference (p> 0.05) across treatment groups in all the haematological parameters except for the monocyte where T₁ was greater (p<0.05) than others. The PCV, Hgb, WBC, lymphocytes, heterophils, MCH and MCHC but not the RBC, basophils and MCV fell within normal ranges for broilers as stipulated by Mitruka and Rawnsley (1977). There were marginal increases in PCV, Hgb, RBC, MCV, MCHC and MCH after administration of PALM to *S. Enteritidis* infected birds. The WBC, heterophils and thrombocytes increased with the inoculation of *Salmonella* but decreased with the administration of *P. amarus* (T₃) and enrofloxacin (T₄). With respect to eosinophils, the treated groups were higher. There was rise in basophil count in T₂ probably due to salmonellosis which was abated by PALM and enrofloxacin. T₂ had numerical rise in monocytes count after which it decreased in T₃ and T₄.

Serum biochemistry values of broiler chickens challenged with oral dose of Salmonella enteritidis and treated with Phyllanthus amarus leaf extract: Table 2 shows the serum biochemistry values of broiler chickens challenged with oral dose of *Salmonella* Enteritidis and treated with *Phyllanthus amarus* leaf extract and enrofloxacin. The results revealed that all the parameters except ALP were not statistically different (p<0.05). With regard to total protein (TP) and albumin, T₁ was marginally higher than treated groups.

The reduction in other groups could have been occasioned by the presence of *S. Enteritidis* and test ingredients (*P. amarus* and enrofloxacin). The AST and ALT had no significant difference (p<0.05) among the treatment groups unlike ALP where there was significant difference (p>0.05) among the groups. There were however numerical rise in their concentrations in infected group (T₂) that later decreased with PALM and enrofloxacin treatments. The UA, BUN and creatinine were not influenced by treatments.

Table 1: The haematological values of broiler chickens inoculated with *Salmonella enteritidis* and treated with PALM and enrofloxacin

Parameter	T ₁	T ₂	T ₃	T ₄	±SEM
PCV (%)	30.50	27.50	28.00	30.50	1.08
Hgb (%)	9.85	8.70	9.25	10.20	0.35
RBC (x10 ⁶ /mm ³)	3.08	2.85	2.90	3.32	0.14
WBC (x10 ³ /mm ³)	12.55	16.75	15.40	12.55	0.92
Thrombocyte (%)	16.80	17.50	14.30	15.00	1.13
Lymphocyte (%)	58.00	58.00	64.00	59.50	1.96
Heterophyl (%)	35.00	36.00	29.00	34.50	2.01
Monocyte (%)	4.50 ^a	5.50 ^a	2.50 ^b	2.00 ^b	0.40
Eosinophil (%)	2.50	3.50	3.50	3.50	0.37
Basophil (%)	0.50	1.50	0.50	0.50	0.18
MCV (fl)	99.03	96.49	96.55	91.86	2.73
MCHC (g/dL)	32.30	31.64	33.04	33.44	0.39

a,b; means with different superscripts on the same row are statistically different from each other (p<0.05).

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Table 2: Serum biochemistry values of broiler chicken challenged with oral dose of *Salmonella enteritidis* and treated with *Phyllanthus amarus* and enrofloxacin

Parameter	T ₁	T ₂	T ₃	T ₄	±SEM
TP (g/dL)	7.25	6.80	6.20	6.70	0.41
Alb (g/dL)	2.45	1.60	1.65	2.15	0.32
Glob (g/dL)	4.80	5.20	4.55	4.55	0.19
AST (uL)	181.00	202.50	190.50	197.00	4.50
ALT (uL)	31.50	34.00	26.00	24.00	1.83
ALP (uL)	289.50 ^{bc}	317.50 ^a	272.00 ^c	306.50 ^b	6.77
UA (mg/dL)	5.43	5.78	5.92	5.71	0.34
BUN (mg/dL)	16.75	15.75	15.75	16.00	0.38
Creatinine (mg/dL)	0.65	0.55	0.50	0.55	0.04

a, b: means with different superscripts on the same row are statistically different from each other (p<0.05).

Key:- TP – Total protein, Alb – Albumin, Glog – Globulin, AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – Alkaline Phosphatase, BUN- Blood urea nitrogen, Creat - creatinine

These findings are in consonance with Ajit *et al.* (2012), Vivian *et al.* (2015) and Muthusamy *et al.* (2017) who opined that the increase in the vital hematological constituents like PCV, Hgb, RBC, and WBC in birds fed with the herbal ingredients (ginger, garlic and tulsi) is an indication of improved oxygen carrying capacity of the cells which translated to a better availability of nutrients for utilization to the birds consequently affecting their well being with an active immune system. This is likely attributable to the PALM supplement that has been proven to have antibiotic properties (Uzor *et al.*, 2016) inhibiting competition between host bird and pathogenic gut micro-flora, and by this, improved haematopoietic activity. This is corroborated by the study of Nwankpa *et al.* (2014) and Sakthi *et al.* (2017) who had significantly increased levels of RBC, Hb and PCV in rats given ethanol leaf extract of *P. amarus* and Unigwe *et al.* (2020) who got significant increase (p<0.05) in RBC count and marginal increases in PCV and Hgb of broiler chickens as PALM supplement increased. It has been noted that “some foliages contain a number of bioactive compounds such as vitamins, phenolic acids, flavonoids, isothiocyanates, tannins as well as saponins” (Vergara-Jimenez *et al.*, 2017) that are beneficial for the health of chickens (Rama Rao *et al.*, 2019; Sugiharto *et al.*, 2019). The findings of this study partly corroborate the claims of Omokore and Alagbe (2019) and Jimoh (2020) that *Phyllanthus amarus* does not influence the haematology of rabbits nor the general performance and health status of animals since most of the parameters were not statistically different and as well fell within physiologic normal ranges for broiler chickens as stipulated by Mitruka and Rawsley (1977). The marginal increases in haematological parameters could be associated with extra nutrients in PALM as well as its acquisition of antibiotic properties since Okiki *et al.* (2015) found abundance of protein and minerals whereas Babatunde *et al.* (2014) and Meena *et al.* (2018) had demonstrated the anti-bacterial potentials of *P. amarus* leaf. The reduction in haematological values in T₂ (*S. Enteritidis*

inoculated) in the present study is similar to the significantly decreased mean levels of RBC, PCV, Hb, MCV, MCH, MCHC, neutrophils and increased WBC and lymphocytes in *Salmonella typhi* infested wistar albino rats as obtained by Nwankpa *et al.* (2014).

Increased leukocyte production suggests that the body responds to infection (Chechik *et al.*, 1986). These features could be ascribed to the antibacterial activities of *P. amarus* and enrofloxacin that probably stopped bacterial induced inflammatory process. The possible consequence might be seen in the reduced ability of birds on *P. amarus* to effectively live up to the haemostasis function should there be vascular injury since thrombocytes release prothrombotic factors (Grant and Zucker, 1973; Kunicki and Newman, 1985). Also, contribution to immunological roles (Ferdous *et al.*, 2008) will be negatively affected. Eosinophilia is rarely found in chickens. However, if it occurs, it can be attributed to parasitism (Irizaary-Rovira, 2004). In chickens, the high number of basophils in the blood indicates that they are in abnormal conditions, such as stress due to sufficiently hot air or facing pathogen infection (Tamzil *et al.*, 2014). Meanwhile, the low number of basophils indicates that chickens are in healthier conditions (Maxwell *et al.*, 1992). Increased heterophils can be found in bacterial, fungal, and parasitic infections, inflammation, stress, toxicity, traumatic conditions, as well as leukemia” (Mitchell and Johns, 2008). The number of heterophils decreased due to the function of tannin in *P. amarus* having “toxic properties to bacterial cell membranes by inhibiting certain enzymes that will damage microbial or bacterial cells (Tomiya *et al.*, 2016). Increase in the number of monocytes, basophils, and eosinophils may be associated with various infectious or inflammatory conditions (Latimer and Bienzle, 2010). With regard to total protein (TP) and albumin, T₁ was marginally higher than the normal range as established by Harvey (2012) and Thrall *et al.* (2012) compared to other groups. The anti-nutritional compounds in *P. amarus* which include oxalate, phytate, hydrogen cyanide, nitrate and tannin (Gafar *et al.*, 2012) and concurrent

inoculation of *Salmonella* Enteritidis could have impacted negatively on protein absorption in them. Several physiological and pathological factors have been investigated to describe possible qualitative and quantitative alterations in the concentrations of blood proteins, reflecting the actual general health state and condition of the evaluated animals, including bird species (Tóthová *et al.*, 2017). Serum protein, albumin and globulin depend on availability of dietary protein (Obikaonu *et al.*, 2011) and too, the extract might have interfered with the equilibrium in the rate of synthesis or destruction of total protein from the system of the birds (Yusuf *et al.*, 2018). Globulin however, was higher than the normal range (Mitruka and Rawnsley, 1997) in all the groups. There was immunologic response to the inoculation of *S. Enteritidis* which subsided in T₃ and T₄ that received concomitant *P. amarus* and *S. Enteritidis* respectively. Globulin level has been used as indicator of immune responses and sources of antibody production (Bowes *et al.*, 1989). Serum albumin and globulin depend on availability of dietary protein (Obikaonu *et al.* 2011) and in this study the combined effects of *Salmonella*, *P. amarus* and enrofloxacin could have impacted negatively on the globulin vis-à-vis T₁ and T₂. More so, serum albumin is influenced by antigen exposure and is highly variable (Simaraks, 2015). The AST and ALT fell within the normal ranges for broilers (Mitruka and Rawnsley, 1997). Liver function tests (ALT, AST, ALP) provide information about the state of the liver by describing its functionality, cellular integrity and link with the biliary tract (Wang *et al.*, 2006; Agbaje *et al.*, 2009; Chanda *et al.*, 2015). The liver enzymes spiked in titre in T₂ probably as a result of SE inoculation. Infection with *S. Enteritidis* is mainly limited to the intestinal tract, but under certain circumstances may cross the mucosal barrier to disseminate and get established as some localized infectious focus. *S. Enteritidis* may involve the liver and evolve into an overt abscess (Mahajan *et al.*, 2014). Gast and Beard (1990) reported that after oral inoculation of adult Leghorn hens (20–88 weeks of age) with doses of 10⁹ cells of *S. Enteritidis*, they recovered *S. Enteritidis* from 53% of livers sampled during the first 5-weeks post-inoculation. Coble *et al.* (2012) equally reported a disruption of metabolic enzymes and pathways in the liver by *S. enteritidis* at 10-days post-infection. However, T₃ and T₄ which received PALM and enrofloxacin respectively had relative reduction in the enzymes. This is suggestive that *P. amarus* could be of help in reducing the liver enzymes should there be any reason for hike. This corroborates the results of Peters and Omeodu (2015) who observed reduction of ALT, AST and ALP when *P. amarus* extract treated groups of albino rats were compared with control group, suggesting recovery of

hepatocyte function. Similarly, Arunsi *et al.* (2020) reported significantly ($p < 0.05$) elevated ALT and AST when extracts of *Aspilia africana* leaves were used in rats. The uric acid, BUN and creatinine are used to measure the clearance ability of the kidney and in this present study they all fell within normal ranges (Fattah *et al.*, 2008). Renal function in chickens is indicated by serum uric acid concentration. Uric acid (UA) is the main end product of nitrogen metabolism in birds and is excreted via the faeces (Sturkie, 1986) and has been widely used in the detection of kidney damages and disease. In general, UA greater than 13 mg dL⁻¹ suggests impaired renal function in birds (Thrall *et al.*, 2012). Likewise, creatinine is one of the most sensitive biochemical markers employed in the diagnosis of renal damage because it is excreted through the kidney (Akande *et al.*, 2013). The results showed that *P. amarus* has no deleterious effect on kidney. The rise of uric acid in T₃ could be ascribed to higher protein due to *P. amarus* since Okiki *et al.* (2015) reported high crude protein content of 18.77±0.15% in *P. amarus*. High uric acid could be attributed to high quality of protein fed (Oduguwa and Ogunmodede, 1995) and higher amino acid breakdown as a consequence of higher protein supply.

Conclusions: PALM can be used to stabilize haematological and biochemical values in the event of negative alteration in quantities mainly due to microbial assault in broiler chickens. There was marginal improvement on haematological values of PALM treated group (T₃) as against the infected group (T₂). The rise in total WBC occasioned by SE was brought down by PALM suggesting anti-inflammatory activity of the extract. Spiked liver enzymes were reduced by the extract, suggesting possible reparative activity of PALM on the liver.

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