EFFECT OF CRUDE TANNIN EXTRACTS ON HAEMATOLOGY AND SERUM BIOCHEMICAL PROFILE OF BROILER CHICKENS

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ABSTRACT
The use of synthetic antibiotics as feed growth promoters remained banned by several countries because of their toxicological effects, microbe resistance and suspected carcinogenic potential. Therefore, the use of natural products as antibiotics replacement is a welcome opportunity in the livestock industry. This study was aimed at examining the effects of tannin extracts from Gliricidia sepium, a high tannin containing plant on the haematology and serum biochemistry of broiler chickens. Leaves of Gliricidia sepium, were harvested, weighed, air dried and then ground. Crude tannin extracts were applied to 0, 12, 24 and 36mL^{-1} drinking water. Eighty-four Abor Acres 1-day-old chicks were randomly assigned to four treatments, three replicates in Completely Randomized Design. Data were analysed using one-way analysis of variance (ANOVA). At week six, blood samples were drawn from birds via the jugular vein for serum biochemical and haematological analyses. With the exception of basophil values, which demonstrated a significant difference ($p<0.05$) with a higher value of (0.83%) at the 24 mL^{-1} inclusion level. The results indicated that all haematological parameters evaluated were not significantly ($p>0.05$) different among the treatments. However, the serum biochemical indices revealed a significant difference ($p<0.05$) in the levels of albumin, urea, and glucose in the blood while there were no significant differences ($p>0.05$) in other parameters. In conclusion, addition of gliricidia crude tannin extracts to drinking water act of 24 and 36 mL^{-1} generally enhanced the haematology and serum biochemistry of broiler chickens.
Keywords: Gliricidia, Crude tannin, Haematology, Serum biochemistry, Broiler chickens

INTRODUCTION

Antibiotic growth promoters (AGP) have been prohibited in the European Union and numerous other countries since 2006. As a result, the need to look for sustainable substitutes (Huyghebaert *et al*., 2011). In response to the ban, other products such as probiotics, prebiotics, minerals, enzymes, organic acids, spices, and herbs have emerged as potential substitutes. Among the alternative products, phytobiotics have gained attention due to their natural, non-toxic, and residue-free characteristics, Phytobiotics are plant-derived substances added to animal diets to enhance livestock productivity by improving feed quality, nutrient digestibility, absorption, and pathogen control in the gut (Abou-Bakr, 2011). According to Huyghebaert *et al.* (2011), plant polyphenols, especially tannins, hold promise as feed additives. The antimicrobial properties of tannins have been well-established, with studies on tannin toxicity towards yeasts, fungi and bacteria, extensively reviewed (Scalbert, 1991). Various mechanisms have been proposed to elucidate the antimicrobial effects of tannins, including, deprivation of essential substrates needed for microbial growth, direct interference with microbial metabolism by inhibiting oxidative phosphorylation, the inhibition of extracellular microbial enzymes and interactions with metal ions.

Gliricidia sepium is reported to be high in tannins (Rani *et al*., 2007) and it has been explored in poultry production as a result of its anti-viral, anti-inflammatory and anti-oxidant quality (Asaolu *et al.,* 2010). Blood is used to assess the clinical and nutritional health of experiment animals. The goal of this work was to elucidate the effect of crude tannin extracts on the haematology and blood biochemistry of broiler chickens.

MATERIALS AND METHODS

Experimental location

The experiment was carried out at the Poultry Unit, Teaching and Research Farm of the University of Ibadan, Ibadan, Oyo State, Nigeria.

EXPERIMENTAL MATERIAL

Collection of experimental materials

Gliricidia sepium leaves were harvested around Ibadan in Oyo state. Leaves harvested were weighed and air-dried and ground using a hammer mill and stored in moisture-free polyethylene bags until needed.

Screening Process

Gravimetric method involves using copper acetate as outlined by Josyln (1970), the total soluble tannin content in the samples was determined. Initially, 5g of dried samples was boiled with 50mL of distilled water in a 250 mL

conical flask for 20 min and then filtered. The filtrate was transferred to a 250 mL beaker, discarding the residue. Subsequently, 10 mL of 4% copper acetate solution was added to the hot filtrate and boiled for 10 minutes. After filtering the precipitate and discarding the filtrate, the residue was dried using Whatman No. 1 filter paper. The dried sample was removed from the filter paper into a pre-weighed crucible. The weight of the crucible with the sample was noted before incinerating it in a muffle furnace at 600°C for 7 hours. Once cooled in a desiccator, the weight was recorded. The total tannin content was calculated using a specific formula.

$$Tannin = \frac{\text{weight loss during ashing}}{\text{weight of the sample used}} \times 100$$

Extraction of tannins

According to Xuan *et al*. (2020), the decotion method was used to extract the tannin. Water was employed as the solvent. Using a mechanical shaker set up at a room temperature, the ground sample was submerged in water for 72 hours in a closed vessel while the mixture was heated continuously to 100^0C. After cooling the mixture to room temperature, the filtrate was taken out.

Experimental birds and experimental design

A total of 84 Arbor Acres day-old chicks were purchased from a reputable hatchery in Oyo, Nigeria. Upon reaching the farm, the broiler chicks were selected at random and assigned to four treatments, with three replicates each containing seven birds per replicate in a Completely Randomized Design.

Experimental diets

The proximate analysis of the diets was conducted following the AOAC (2005) standards. A starter diet was formulated with a 21% crude protein and 3000kcal/kg for the birds from day-old to 21 days and 19%CP and 3000kcal/kg from 22-42 days. Crude tannin was extracted in drinking water and grouped into four different treatments (T) based on inclusion level.

T1= CTE at 0ml/L in drinking water, T 2= CTE at 12mL/L in drinking water, T 3= CTE at 24mL/L in drinking water, T 4= CTE at 36ml/L in drinking water. Water and feed were given *ad libitum* throughout the study after daily routine such as general observation of birds, removals of dead birds, leftovers of the feed and water was removed the previous day.

Data collection

At six weeks from each replicate, blood samples were drawn from two birds through the jugular vein into container. Samples for haematological indices were collected in containers containing EDTA, while containers without anticoagulant were used for serum analysis following AOAC (2010) methods. Serum samples were separated by centrifugation for the analysis of biochemical indices.

Statistical Analysis

A Completely Randomized Design (CRD) was used for the study. SAS software, version 9.2 for Windows (SAS 2020), was used for one -way analysis of variance (ANOVA) on data obtained. Duncan's Multiple Range Test was used to compare means.

RESULTS AND DISCUSSION

The effect of crude tannin on haematological parameters of broilers chickens is shown in Table 1. The results showed nonsignificant ($p>0.05$) difference in Packed cell volume, haemoglobin, red blood cell, White blood cell, Platelets, monocytes, eosinophils, lymphocytes among control and other treatments. However, a significant ($p<0.05$) effect was observed on Basophils. The value (0.00%) was recorded at T2 with crude tannin extract (CTE) at 12ml/L in drinking water which was significantly lower to all other group. The value of RBC in T4(36mL CTE in drinking water) increased compared to other treatment with crude tannin extract.

The recorded value for Basophil across the treatments still falls within the normal range, indicating no adverse effects on the haematological indices of the birds. The results of this study contradict the report by Lee *et al*. (2010) who suggested that the inclusion of tannic acid (HT) at dietary levels of (0.0125%) and (0.1%) had an adverse effect on plasma iron and haematological indices in pigs. The value of WBC in T4(36mL/L CTE in drinking water) increased compared to other treatment with crude tannin extract and control, but the values obtained were still within the normal range for broiler chickens. The value of RBC in T4(36ml/L CTE in drinking water) increased compared to other treatment with crude tannin extract. The result obtained for RBC in this study is in agreement with the recent research done by Gessica *et al*. (2019) observed that increased RBC counts in broiler chicks fed a tannin-containing extract. Though no PCV value was higher than 35%, and the results fell within the normal ranges for broilers (22-45%), generally speaking, a PCV value of more than 56% indicates dehydration in most birds (Al-

Nedawi *et al*., 2018). The serum biochemical analysis of broiler chickens given crude tannin extract was within the normal range.

Table 1. Effect of crude tannin extracts in drinking water on haematological parameters of broiler chickens

Indices	T1(0ml/L)	T2(12ml/L)	T3(24ml/L)	T4(36ml/L)	Normal Range
Packed cell volume (%)	32.00±1.90	30.17±2.71	31.17±3.76	31.67±1.63	22-35
Haemoglobin (g/dL)	10.17±0.63	9.60±0.79	9.97±1.28	10.22±0.61	7.0-13.0
Red blood cells ($x10^6$uL)	3.17±0.24	3.01±0.24	3.15±0.28	3.17±0.21	2.0-4.0
Lyphocytes (%)	64.83±3.60	61.50±4.32	63.33±5.32	63.50±3.94	40-100
Nutrophils (%)	28.17±4.71	29.83±3.43	30.17±5.38	29.83±3.31	25-40
Monocytes (%)	3.00±0.63	2.33±1.37	2.50±1.22	3.00±1.10	0.0-3.0
Eosinophil (%)	3.83±2.14	4.33±0.82	3.17±0.75	3.33±2.07	0.0-4.0
Basophils (%)	0.17±0.41[a]	0.012±0.35[a]	0.83±0.41[b]	0.33±0.52[a]	0.0-2.0
White blood cells ($x10^3$uL)	15.79±12.34	15.15±24.28	15.59±26.02	16.28±18.71	12-30
Platelets (uL)	12.21±91.52	12.05±75.29	10.26±44.24	12.83±13.24	10-15

[abc,] Mean values with same superscript in the same column are significantly different (P<0.05*).*

Table 2. Effect of crude tannin extracted in drinking water on serum biochemical indices of broiler chickens

Parameters	T1(0ml/L)	T2(12ml/L)	T3(24ml/L)	T4(36ml/L)	Normal range
Glucose(mg/dL)	211.64±12.3[b]	192.50±19.2[a]	192.10±14.63[a]	211.05±12.48[b]	
Cholesterol(mg/dL)	108.96±14.86	101.47±27.32	86.38±17.47	99.01±14.68	87-192
Albumin(g/dL)	1.18±0.29[a]	1.48±0.13[b]	1.29±0.15[ab]	1.22±0.17[a]	1.17-2.74
Total Protein(g/dL)	2.43±0.40	2.54±0.07	2.71±0.33	2.57±0.16	2.0-5.5
AST (I.U/L)	218.20±16.10[a]	235.33±3.79[b]	216.58±10.67[a]	215.80±9.63[a]	70-240
ALT (I.U/L)	18.06±5.14	19.27±1.30	20.94±3.75	18.52±2.34	15-25
Urea (mg/dL)	4.03±0.56[a]	4.65±0.52[ab]	4.98±0.81[b]	5.20±0.26[b]	1.9-12.5

[abc,] Mean values with same superscript in the same column are significantly different (P<0.05). AST= Aspartate transaminase, ALT= Alanine transaminase

The results from table 2 revealed that Glucose, Urea, Albumin and Aspartate amino transferase (AST) were significantly different (p<0.05). The glucose level was significantly higher in T1 (control) with value (211.64mg/dL) compared to other treatments. The level of Albumin in T2(12mL) with value (1.48g/dL) was higher compared to others and lower in T1(control) with value (1.18g/dL). The AST value in T2(12mL) was also observed to be significantly higher compared to the value in other treatment. Urea was also observed to be significantly lower at T1(control) with value (4.03mg/dL) compared with values in other treatment. The total protein was higher in treatment containing 24mL CTE which indicate good health. Sturkie (2000) indicated that the levels of cholesterol in birds are influenced by their physical and nutritional well-being. Cholesterol is synthesized from consumed fats and produced within the cells; reduced cholesterol levels can lower the risk of cardiovascular disease while high level is a sign of greater risk of cardiovascular disease. Nonetheless, according to Gessica *et al*. (2019), the values obtained in this study fall within the normal range (75.00-196.00mg/dL) for a healthy chicken. Uric acid levels typically vary from 1.9 – 12.5 mg/dl. The urea values found in this investigation were greater in T4 (36ml/L CTE in drinking water) than in other treatments.

CONCLUSION AND RECOMMENDATIONS

Feeding broiler chickens with crude tannin extract up to 36ml/L in drinking water could enhance the health status of broiler chickens without any harmful effects but with improvement in the health status of the birds. Further research should be done to determine the toxicity level of tannin.

REFERENCES

Abou-Bakr, S. 2011. Effect of some plant extracts on fungal and aflatoxin production. *International Journal of Academic Research,* 3: 116- 120.

AL-Nedawi, A.M. (2018). Reference Haematology for commercial Ross 308broilers, Online *Journal of Veterinary Research*, 22(7):566-570.

Association of Analytical Chemist (AOAC) 2010. Association of Analytical Chemist. Official Methods of Analysis 17th Edition AOAC Ic, Arlington, Virginia, USA.

Gessica, P, Matheus D.B., Matheus, F, Mauricio, B., Renata, A., Casagrande, L.G.G., Bruno, F.F., Andreia V., Lenita M. S., Macrel, M. B., Thierry, G.D., Fabio, S. and Aleksandro, S.D. 2019. Effects of tannin containing diets on performance, gut disease control and health in broiler chicks. Animal Production Science, https:// doi.org/10.1071/AN18393.

Huyghebaert, G., Ducatelle, R. and Van Immerseel, F. 2011. An update on alternatives to antimicrobial growth promoters for broilers. *Veterinary Journal*. 187: 182–188

Hydrolyzabletannins(chebulagicacidandpunicalagin) target viral glycoproteinglycosaminoglycan interactions to inhibit herpes simplex virus 1 entry and cell-to-cell spread. J. Virol. 85, 4386–4398. doi: 10.1128/JVI. 01492-10.

Lee, S., Shinde, P., Choi, J., Kwon, I., Lee, J., Pak, S., Cho, W. and Chae, B. (2010). Effects of tannic acid supplementation on growth performance, blood haematology, iron status and faecal microflora in weanling pigs. *Livestock Science,* 131:281-286.

Scalbert, A. (1991). Antimicrobial properties of tannins. Phytochemistry, 30, 3875– 3883.

Sturkie, P.D. (2000). Avian Physiology 5th ed Academic press. SAN Diego, California, U.S.A.

Xuan Cuong, D., Xuan Hoan, N., Huu Dong, D., Thi Minh Thuy, L., Van Thanh, N., Thai Ha, H., Xuan Chinh, D**.** (2020). Tannins: Extraction from Plants. IntechOpen. doi: 10.5772/intechopen.86040.