EFFECT OF CRUDE TAINNIN EXTRACTS FROM *GLIRIDIA SEPIUM* ON INTESTINAL MICROFLORA AND CHYME PH OF BROILER CHICKEN

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ABSTRACT
Tannins have been reported to have antibiotic effect on consuming animals. In this study the effect of application of crude tannin extracts in drinking water of chickens in place of synthetic antibiotics on intestinal microflora and chyme pH of broiler chicken. Gliricidia sepium leaves were harvested, weighed, air-dried and milled using hammer mill. The tannins were extracted in aqueous medium as crude tannin extract (CTE) and then ad libitum 0, 12, 24, and 36mL-1 in water. Eighty-four 1- day old Abor acres chicks were randomly allotted to four treatments, three replicates in Completely Randomized Design. The birds were fed common diets and their respective water ad libitum. At week 6 the birds were slaughtered by severance of the jugular veins and the chyme pH, and the microbial populations within the duodenum, jejunum and ileum were examined. All data were subjected to one-way analysis of variance (ANOVA). The digesta pH within the ilea of broiler fed diet with CTE at 24 mL-1 in drinking water was significantly lower compare to all other groups (P>0.05). The Total bacteria count within the jejunum of birds fed 36 mL-1 was significantly lower (p>0.05) in contrast to other treatments. The coliform population in broiler fed diet with CTE at T2(12mL-1) within the jejunum was significantly lower than those fed T1(0 mL-1), T3(24 mL-1) and T4(36 mL-1) in drinking water (p>0.05). The population of Lactobacillus in jejunum of broiler fed diet with CTE at 0mL-1in drinking water was significantly higher compare to all other groups. There were no significant (p>0.05) differences in total fungi count within all the intestinal segment with the exception of broiler fed with CFE at T3 (24 mL-1) in drinking water in duodena. Based on the results from this study it could be concluded that the supplementation of gliricidia crude tannin extract at 36mL-1 in drinking water reduced bacteria population in small intestine.

Keywords: Crude tannin, Chyme, Coliform, Lactobacillus, Fungi, Gliricidia sepium leaves.

INTRODUCTION

The harmful effects of synthetic antibiotics use led the ban of antibiotics in EU and have prompted researchers to think about alternatives to antibiotics (Diarra and Malouin, 2014). The aim of these alternatives is to maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health.

Phytogenic feed additives derived from plants, herbs and spices have been reported to improve animal performance. Tannins are the main polyphenolic secondary metabolites distributed widely in the range of 5 to 10% of dry vascular plant materials found mainly in bark, stems, seeds, roots, buds, and leaves ((Barbehenn and Constabel, 2011).

Tannins are a complex group of polyphenolic compounds reported to possess the capacity to form reversible and irreversible complexes with proteins, polysaccharides, alkaloids, nucleic acids and minerals. Different forms of tannins have diverse biological activities: Anti-tumour, anti-mutagenic, anti-diabetic, anti-proliferative, anti-bacterial and anti-mycotic (Masek *et al.,* 2014). The level of tannins in *G. sepium* were found to be more than in *acacia nilotica* and *leuceana leucocephala* leaves (Abu *et al*., 2024, unpublished report). However at low dosage, tannins have shown bacteriostatic and bactericidal properties *in vitro* against pathogenic bacteria, including *Escherichia coli*, Salmonella spp, Proteus spp. and Clostridium spp. (Lee *et al.,* 2010). In some reports high concentrations of tannins have been shown to have anti-nutritional effects in monogastric animals because tannins can decrease feed intake, nutrient digestibility, and growth performance of chickens (Garcia *et al.,* 2004). Jamroz *et al*. (2009) assessed the effects of dietary addition of sweet chestnut tannins at low concentration (0.025, 0.05 and 0.1%) on the performance, intestinal microbial populations and

histological characteristics of intestine wall in chickens and reported that tannin supplementation had no effects on feed conversion and carcass quality, but tannin at 0.1% reduced final body weight. *E. coli* and coliform bacteria in the small intestine of 28-d-old chickens were also reduced at the tannin levels of 0.05% to 0.1%. However, till date the effects of tannin on broiler chickens remain inconclusive.

Gliricidia sepium leaves has been reported have anti-viral, anti-inflammatory and anti-oxidant properties (Asaolu *et al.,* 2010). *Gliricidia sepium* leaves have a total condensed tannin content range from 25-186 mg/g of dry weight (Schofield *et al.,* 2001). This present study was aimed at studying the effect crude tannins from *Gliricidia sepium* on intestinal microflora and chyme pH of the broiler chicken

MATERIALS AND METHODS

Experimental site

This study was conducted at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The farm is situated in southwestern Nigeria at 70 201N, 3 0 501 E at an altitude of 200-300 m above sea level.

Experimental Materials

Collection of Experimental Materials

Gliricidia sepium leaves were harvested on the campus of the University of Ibadan. The harvested leaves were weighed and air-dried to reduce the moisture content and also to prevent loss of some crucial nutrients. The air-dried leaves were milled using hammer mill and stored separately in polyethylene bags kept under moisture free condition prior to use.

Extraction and determination of crude tannins

Tannin was extracted from the ground leaves by using a water bath filled with tap water at 80°C. The pH of the extract was adjusted by using acetic acid, this was then be followed by the sedimentation process for 24 h. The supernatant of the extract was collected and the level of tannic acid was determined by Pholin-Ciocalteu assay, using tannic acid as a standard solution.

Experimental Diets

Starter diet was formulated to contain 21% crude protein CP and 3000kcal/kg for the birds from day-old to 21 days and finisher diet19% CP and 3000kcal/kg 22-42 days. The proximate analyses of the diets were determined according to the standards of AOAC (2005). Crude tannin was extracted in drinking water and grouped into four different treatments (T) based on inclusion level.

T1= *CTE at 0mL^{-1} in drinking water,* T2= *CTE at 12mL^{-1} drinking water,* T3= *CTE at 24mL^{-1} drinking water,* T 4= *CTE at 36mL^{-1} drinking water*

Experimental Birds and arrangement

A sum of 84 Abor acres 1-day-old broiler chicks were procured from a reputable hatchery in Ibadan, Oyo State, Nigeria. On arrival, the chicks were randomly allotted to four treatments, three replicates with 7 birds per replicate in Completely Randomized Design.

Operation and Health Management

Feed and water were given *ad libitum* throughout the study. The birds were fed their respective starter diets throughout the experiment. Medication and vaccination were administered as at when due.

Gut Microbiome Analysis

At 6 weeks of age, 3 broilers from each replicate were slaughtered and their intestinal tracts were removed. The duodenum, jejunum and ileum were divided into three parts for the investigation of the digesta pH, microflora and (*Lactobacillus spp. and E. coli)*.

GUT pH determination

Ten (10g) of the digesta were collected from the duodenum, jejunum and ileum, transferred to plastic tubes and then diluted with phosphate buffer saline (PBS) to make 1:10 dilution. The pH of each sample was investigated by using a pH meter.

Microflora composition

For bacterial count, the samples were weighed and diluted in peptone water to an initial 10G1 dilution. Microbial populations were quantified via serial dilution (10G1 to 10G7) of samples in PBS before inoculation onto petri dishes of sterile agar.

Selective Media: *Lactobacillus spp* and *coliform* were quantified on de Man Rogosa and Sharpe (MRS agar) and Violet Red Bile Agar (VRBA), respectively. The plates were incubated at 37ºC for

Lactobacillus, anaerobically (73% N, 20% CO_2, 7% H_2) and aerobically for coliform. The plates were counted between 24 and 48 h after inoculation. The Colonial counts were defined as distinct colonies measuring at least 1 mm in diameter.

Statistical Analysis

The study designed was completely randomized design (CRD) and all data collected were subjected to one-way analysis of variance (ANOVA) according to the SAS software, version 9.2 of the SAS System for Windows (SAS 2020). Duncan's Multiple Range Test was used to separate mean.

RESULTS

Table 1 showed that there were no significant($p>0.05$) differences in the digesta pH within duodenum and jejunum for all groups. The digesta pH within the ilea of broiler fed diet with crude tannin extract (CTE) at 24mL-^{1} in drinking water was significantly lower compare to all other groups.

TABLE 1: Effects of Crude Tannin extracted in drinking water on Chyme pH

Chyme pH	T1(0 mL^{-1})	T2(12mL^{-1})	T3(24 mL^{-1})	T4(36 mL^{-1})
Duodenum	5.77±2.03	6.83±0.07	5.71±1.99	6.92±0.10
Jejunum	5.70±1.98	5.72±2.09	5.72±1.91	6.89±0.10
Ileum	6.96±0.15^{b}	7.05±0.05^{b}	3.46±3.47^{a}	7.12±0.06^{b}

$^{ab.,}$ *Mean values with same superscript in the same column are not significantly different (P>0.05).*

The result from Table 2 revealed that the TBC within the jejunum and ileum were significantly different($p<0.05$). The TBC within the jejunum of T4(36 mL^{-1}) broiler was significantly lower in contrast to other treatments. The ileum of T2(12mL^{-1}) had the least value and the highest value was recorded for T1(0 mL^{-1}). However no significant differences were observed in the TBC within duodenum. There were no significant ($p>0.05$) differences in TFC within all the intestinal segment with the exception of broiler fed with CFE at T3(24 mL^{-1}) in drinking water in duodena.

TABLE 2: Effects of Crude Tannin extracted in drinking water on Total Bacteria and Fungi Count

Parameters	T1(0 mL^{-1})	T2(12mL^{-1})	T3(24 mL^{-1})	T4(36 mL^{-1})
TBCx10-4cfu/g				
Duodenum	46.83±23.09	41.50±22.61	7.83±4.25	30.00±27.78
Jejunum	43.33±5.69^{b}	31.50±17.80^{ab}	18.50±20.42^{ab}	12.67±2.47^{a}
Ileum	81.17±38.26^{b}	25.83±13.29^{a}	30.17±18.23^{a}	39.33±12.22^{ab}
TFCx10-4cfu/g				
Duodenum	0.67±1.15^{a}	0.00±0.00^{a}	7.67±2.02^{b}	0.00±0.00^{a}
Jejunum	0.00±0.00	0.50±0.50	6.33±10.97	0.00±0.00
Ileum	0.67±1.15	0.00±0.00	5.33±8.39	2.83±3.69

$^{ab.,}$ *Mean values with same superscript in the same column are not significantly different (P>0.05*). *TBC= Total bacteria count, TFC= Total fungi count, cfu; colony forming unit*

The result showed that there were significant differences in the TFFCO within the duodenum with the least value (1.83) recorded for broiler fed treatments T1 and T2. The TCC in broiler fed diet with CTE at T2(12mL-^{1}) within the jejunum was significantly lower than those fed T1(0 mL^{-1}), T3(24 mL^{-1}) and T4(36 mL^{-1}). There were no significant differences among the duodenum and ileum for Lactobacillus count. The population of Lactobacillus in jejunum of broiler fed diet with CTE at 0mL-^{1}in drinking water was significantly higher compare to all other groups.

DISCUSSION

The reduction in bacteria population within the jejunum of birds fed 36 mL^{-1} indicating that supplementation of crude tannin extract at 36mL-^{1} decreased the number of bacteria in jejunal of broiler chicken. However results from this study was in disagreement with the report from Worapol and Thongchai (2017) who reported that populations of lactobacillus spp., and *E.coil* within the duodenum jejunum and ileum of broiler chicken fed control group, positive control and an antibiotic-

free diet with tannic acid at 10, 30 and 50mL^{-1}in drinking water were not different. Lactobacillus spp. can produce bacteriostatic bacteriocin-like compounds as well as acids which decrease the gut pH

TABLE 3: Effects of Crude Tannin extracted in drinking water on population of Total faecal contamination count, Coliform bacteria, Lactobacillus *spp.*

PARAMETERS	Treatment	Duodenum	Jejunum	Ileum
	TI	4.50±2.18	3.33±1.76^{ab}	5.00±5.63
TCC	T2	2.83±2.36	1.17±0.76^{a}	5.67±1.15
	T3	3.33±0.58	7.00±2.00^{b}	6.17±4.73
	T4	2.17±1.26	3.00±4.36^{ab}	1.17±1.26
LBTCT	TI	4.50±3.64	8.33±0.76^{b}	7.33±4.65
	T2	3.33±0.58	1.17±2.02^{a}	3.83±4.07
	T3	6.17±3.82	3.50±2.60^{a}	2.67±1.26
	T4	2.50±2.78	0.83±1.44^{a}	3.67±0.76

$^{ab.,}$ Mean values with same superscript in the same column are not significantly different (P>0.05). TFFCO=Total faecal contamination count, TCC= Total coliforms count, LBTCT Lactobacillus count, TI=0 mL^{-1} T2=12mL^{-1}, T3=24mL^{-1}, T4=36mL

(Chateau *et al.,* 2010) The digesta pH of the broiler fed diet with CTE at 24mL-^{1} in drinking water was lower than other groups. This result was similar with the report from Worapol and Thongchai (2017) who reported that the digesta pH in the ilea of the broiler that received tannin acid extracted from cassava leaves at 30mL-^{1} was lower than positive and control group. Although he stated that the phenomenon could not be explained because parameters regarding the small intestine such as lactobacillus spp., population were not different. The result from this current could be attributed to the different in lactobacillus spp. Population in the ileum of the broiler chickens.

CONCLUSION

Supplementation of gliricidia crude tannin extracts at 36mL^{-1} in drinking water reduced bacteria population in small intestine of broiler chickens.

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