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Nutrient Composition, Metabolites and Microbial Counts in Litter Material of Broiler Chicken Fed Condensed Tannins Containing Guava Leaf Meal Supplemented Diets: Reduce Environmental Pollution and Alternate Feedstuffs for Ruminants

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ABSTRACT

Present study was conducted in 240 day-old broiler chicks to assess the effect of condensed tannins (CT) containing guava leaf meal (GLM) supplemented diet on nutrient composition, litter metabolites viz. pH, moisture and ammonia concentration) and microbial counts after completion of feeding trial of 42 days. Chicks were randomly allocated into 4 dietary treatments, with 4 replicates of 15 chicks in each pen in a completely randomized block design (CRD). Chicks were fed basal diets supplemented with GLM @ 1.5, 2.5 and 3.5 percent, in T_1, T_2 and T_3 groups, respectively, while, T_0 group fed only basal diet. Litter materials of chicks showed good nutrient profiles and properly sterilized litter material act as alternative feed ingredient for ruminants feed. Supplementation of GLM in the diets of chicks significantly ($P<0.05$) reduced moisture, pH, NH_3 concentration, harmful *Eschericia coli* and *Clostridia spp*. counts in the litter materials and reduced environmental pollution. The litter material of GLM supplemented (T_1, T_2 and T_3) chicken showed significantly higher ($P<0.05$) CP contents as compared to un-supplemented control. It can be concluded that properly sterilized litter material of GLM supplemented chicks act as cheaper alternative feedstuffs for ruminant's ration and reduce environmental pollution by lowering NH_3 concentration, harmful *E. coli* and *Clostridia* counts in broiler litter material.

Keywords: Condensed tannins, Environmental pollution, Guava leaf meal, Litter material, Microbes, Nutrient composition

Million tons of poultry litter materials are produced annually worldwide. Broiler litter is a combination of accumulated excreta, feathers and bedding material which is a valuable source of nutrients such as nitrogen, phosphorus, sulphur, potassium and energy etc. The litter material is a valuable resource and can be used in many ways. It has varied nutrient composition from other agro-industrial by-products. Although, it is a good source of crude protein and minerals, while, energy values showed wide variation.

However, the poultry litter as a source of dangerous and unwanted pathogenic bacteria which could be resistant to antimicrobials and thus requires treatment before being fed. Although, its utilization as alternate feedstuffs, it needs to be processed properly and technology driven nutritional intervention to reduce its pathogenic microbial load (Elliott and Collins, 1982) and environmental pollution. Quality of litter is of great concern because it affects performance,

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health, carcass quality, and welfare of broilers and the farmers. Identifying suitable and affordable interventions is of particular importance in developing countries, as broiler production makes a significant contribution to the livelihoods of small-scale farmers. Now days properly sterilized litter material can also be one of the non protein nitrogenous (NPN)compounds as alternate feedstuffs for ruminant ration in many developing countries, as it is relatively cheaper source of nitrogen for ruminants (Tsadik *et al*., 2008).

Though, there is a growing concern about the environmental pollution caused by such an activity, especially air pollution and spread of disease causing pathogenic microorganism, as a result of huge amount of poultry excreta create nuisance and pollute the environment. If the proper guidelines/ mitigation strategies are not followed, the poultry litter generate undesirable odours, gases, suspension of particulate matter and dust, etc. (Meda *et al*., 2011; Copeland, 2014). The most significant gaseous pollutants in the poultry sheds are ammonia (NH_3), carbon dioxide (CO_2), carbon monoxide (CO), methane (CH_4) and hydrogen sulphide (H_2S, Barrasa *et al*., 2012).

Besides, exposure, duration and concentration of pollutants inside poultry sheds may be associated with development of respiratory diseases in birds and humans (Naas, 2004). Particulate matter emitted from poultry sheds may contain pathogenic microorganisms, such as avian-influenza virus, *Escherichia coli* and *Salmonela spp.* etc. (Cambra-Lopez *et al*., 2010). Out of these gases, NH_3 is the main pollutant affecting the health of birds and workers (Menegali *et al*., 2012). Complications due to NH_3 exposure in poultry rearing have long been recognized. At higher concentration, it causes irritation of eyes and respiratory system and has a toxic effect over the physiological metabolism, leading to reduce feed intake, weight gain, and inefficient feed conversion and decline profit margin (Kilic and Yaslioglu, 2014). This negatively affects health status of human beings, poultry as well as of the surrounding ecosystem.

Dietary manipulation, composting and proper heat treatment are the practical methods to process the litter; although total nitrogen (N) losses occurs mainly due to NH_3 volatilization, that can reduce N value of litter as a fertilizer or alternative feedstuffs (Tiquia and Tamb, 2000). Here the dietary manipulation in the ration of experimental broiler chicken is the incorporation of condensed tannins (CT) containing guava leaf meal (GLM) with the hypothesis that the CT containing GLM showing potential role on pathogenic microorganism as well as on NH_3 production.

Furthermore, improper management system and failures in computing all the balance of the released gases and pathogenic microorganisms can contribute to the underestimation of the polluting potential of poultry farms. Therefore, it is necessary to identify the means and type of environmental pollution associated with poultry production as well as how we can safely utilized litter material as alternative feedstuffs for feeding of ruminants and how we can set eco-friendly sustainable mitigation strategies to reduce the impacts caused by their emissions. Keeping all these points in view, the present study was planned to evaluate the impact of CT containing GLM supplementation in the diet of broiler chickens on nutrient composition, metabolites, NH_3 levels and microbial load in broiler litter materials after completion experimental feeding trial of 42 days. Therefore, the present study warrant's investigation.

MATERIALS AND METHODS

The feeding schedule and experimental design of broiler chicken fed with and without Condensed tannins (CT) containing guava leaf meal (GLM) are presented in the table 1. Sixteen separate deep litter pen were prepared before the arrival of chicks. The rice husk was used as bedding materials for experimental chicks. Two hundred and forty chicks were randomly allocated into 4 dietary treatments, with 4 replicates of 15 chicks in each pen in a completely randomized block design (CRD). Chicks were fed basal diets formulated by incorporating feed ingredients procured from local market viz. crushed maize, soybean meal, rice bran, fish meal, meat cum bone meal, common salt, L-lysine Hcl, DL-methionine, di-calcium phosphate, lime stone powder, trace minerals and vitamin premix supplemented with guava leaf meal (GLM) at the rate of 1.5, 2.5 and 3.5 percent, respectively in T_1, T_2 and T_3 groups, while, chicks of T_0 group fed only basal diet to fulfil the nutrient need as per ICAR (2013). All the chicks were provided adlib feed and water and followed standard management practices for rearing of chicks.

Table 1: Experimental design and feeding schedule of experimental broiler chickens

240 day-old unsexed broiler chicks (Completely randomized block design for 42 days)															
T0				**T1**				**T2**				**T3**			
60 Chicks				**60 Chicks**				**60 Chicks**				**60 Chicks**			
R1	**R2**	**R3**	**R4**	**R1**	**R2**	**R3**	**R4**	**R1**	**R2**	**R3**	**R4**	**R1**	**R2**	**R3**	**R4**
15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Basal diet				**Basal diet**				**Basal diet**				**Basal diet**			
GLM @0.0%				GLM @1.5%				GLM @2.5%				GLM @3.5%			

A 3-phase (pre-starter, starter and finisher) feeding was followed for experimental broiler chicks as per ICAR (2013).

Table 2: Nutrient composition (on DM basis) of litter materials

Groups	**Nutrient composition (%)**						
	CP	**EE**	**CF**	**TA**	**AIA**	**Ca**	**P**
T_0	10.86^{c}	1.93^{a}	23.24^{a}	23.67^{a}	14.63^{a}	2.34	1.86
T_1	9.21^{b}	2.24^{a}	23.99^{a}	23.81^{a}	15.02^{a}	2.56	1.44
T_2	8.17^{a}	2.54^{ab}	25.67^{ab}	25.72^{ab}	16.88^{b}	2.77	1.39
T_3	8.06^{a}	2.69^{b}	28.08^{b}	28.93^{b}	19.22^{c}	2.83	1.01

^{abc}Means with different superscript within a column differ significantly (P<0.05).

Litter samples were collected at the end of feeding trial of 42 days from 5 locations within each pen (4 equidistant from each corner and one central), thoroughly mixed and subsequently analyzed for pH, moisture content, ammonia concentration, microbial counts and nutrient composition. The pH was recorded using digital pH meter after 30 g of macerated litter were added to 250 ml of deionized water, agitated for 5 min, and suspended for 30 min. The NH_3 (ppm) concentration in broiler litter material was determined by modified Chaney and Marbach (1962) method using two reagents viz. nitroprusside with phenol and hypochlorite with alkali.

Microbial counts in broiler excreta/ litter material were assessed by classical culture techniques using selective medium for specific group of microbes. Proximate analysis of experimental feeds and litter materials were performed according to AOAC (2012). Litter moisture was measured after drying for 24 h at 105°C. Calcium and phosphorus contents were determined as per the methods described by Talpatra *et al*. (1941) and AOAC (2000), respectively. Results obtained were processed and analysed using SPSS 16.0 software. Significance was declared at P<0.05 levels by using Duncan's multiple range test. Statistical procedures were done as per Snedecor and Cochran (2004).

RESULTS AND DISCUSSION

The chemical composition of the litter materials with and without GLM supplementation is shown in Table 2. Litter material of chicks fed GLM supplemented groups (T_1, T_2 and T_3) showed lower (P<0.05) crude protein (CP) contents as compared to litter material of un-supplemented group (T_0). Present results are in harmony with the findings of Min *et al*. (2015) who observed that increase in uric acid is consistent with NH_3 reduction in poultry litter treated with pine bark tanninthat's why increased CP contents in litter materials. Higher CP contents and lower NH_3 concentrations in the litter materials of CT containing GLM supplemented groups might be related with the NH_3 reduction in litter materials of supplemented groups. However, other proximate principles viz. ether extract, crude fibre, total ash and acid insoluble ash were found to be highest in the litter materials of T_3 group followed by T_2, T_1 and the lowest in the litter materials of T_0 group, while, the calcium and phosphorus contents of litter material did not differ significantly irrespective of groups.

Table 3: Effect of CT containing GLM on litter metabolites and bacterial counts of broiler chickens after completion of feeding trial of 42 days

Bacterial species	Groups				P value
	T_0	T_1	T_2	T_3	
Litter metabolites					
Moisture (%)	$18.28^{c} \pm 0.18$	$17.22^{c} \pm 0.08$	$15.67^{b} \pm 0.36$	$13.88^{a} \pm 0.51$	0.000
pH	$7.69^{c} \pm 0.13$	$7.52^{bc} \pm 0.07$	$7.28^{ab} \pm 0.09$	$7.13^{a} \pm 0.06$	0.012
NH_3 (ppm)	$71^{d} \pm 9.77$	$66^{c} \pm 2.85$	$61^{b} \pm 6.08$	$49^{a} \pm 2.52$	0.016
Bacterial counts (log10 CFU/g of litter material)					
Clostridium spp.	$8.84^{b} \pm 0.16$	$8.81^{b} \pm 0.23$	$8.27^{a} \pm 0.13$	$8.12^{a} \pm 0.08$	0.026
Eschericia coli	$9.96^{b} \pm 0.23$	$9.83^{b} \pm 0.09$	$9.23^{a} \pm 0.11$	$9.06^{a} \pm 0.06$	0.004

[abcd]Means with different superscript with in a row differ significantly (P<0.05).

The litter metabolites (moisture, pH and NH_3 concentration) and microbial loads (*E. coli* and *Clostridia*) of broiler chicken with and without GLM supplementation are presented in the table 3. Litter materials of chicks fed GLM supplemented diets (@ 1.5, 2.5 and 3.5) had decreased (P<0.05) pH, NH_3 concentration, *E. coli* and *Clostridia spp*. counts than that of un-supplemented control. As the level of GLM in the diet increased pH, NH_3 concentration, *E. coli* and *Clostridia spp*. counts decreased significantly (P<0.05) in the litter materials. However, the litter materials of chicks fed GLM supplemented diet had significantly (P<0.05) lower moisture contents. The higher litter pH, moisture and temperature are the important factors that affect NH_3 concentration (Coufal *et al*., 2006).

Lower moisture contents, pH and NH_3 concentrations in the litter materials of supplemented groups as compared to un-supplemented control might be due to anti-microbial activity of CT in GLM, which lowered litter pH and ultimately reduce NH_3 concentration. An association between litter moisture and NH_3 emission was also observed by Liu *et al*. (2007) in their experimental study. The presence of CT in the excreta of GLM supplemented chickens might have contributed to the reduction of substrate for microbial growth, and thus the formation of NH_3 (Pathak *et al*., 2017). Previous workers reported that low to moderate level of CT from tree leaf meal mixture (LMM) supplementation improved gastrointestinal N balance, microbial protein (MP) synthesis but had negative impact on gastrointestinal parasites (*Haemonchus contortus*) of lambs, sheep and goats (Pathak *et al*. 2015, 2017; Khan *et al*., 2019 and Singh *et al*., 2016), whereas Min *et al*. (2014) had observed changes in bacterial and methanogenic populations in the gastrointestinal tract (GIT) of goats supplemented with CT. Manipulations of NH_3 conversion from uric acid, reduces NH_3 volatilisation and N loses from poultry litter.

Furthermore, conversion of uric acid to NH_3 is attributed to microorganisms that use uric acid as N source (Kim *et al*., 2009). CT also reduces NH_3 release in rumen through reduction of protein degraded by microorganisms due to binding of CT with proteins making a complex of CT-protein that is resistant to microbial enzymes in rumen (Naumann *et al*., 2017; Pathak *et al*., 2015, 2017). Similarly, Min *et al*. (2012) reported decline protein degradation with subsequent reduction in NH_3 production in the rumen of goats. NH_3 is related to environmental pollution and interrelated to the health of human, poultry and environment. It is an added advantage if litter material has a low pH, because the conversion of excretory uric acid into NH_3 is decreased at acidic pH (Moore *et al.*, 1995), while, as the pH rises above 7, the ionized NH_3 shifts to un-ionized form and thus more NH_3 is available for volatilization (Elliott and Collins, 1982). Therefore, properly processed litter materials free from pathogens were used as feedstuffs for feeding of ruminants in many developing countries (Mavimbela *et al*., 1997; Tsadik *et al*., 2008; Akinfala and Komolafe, 2011) as it was relatively cheap NPN source of N for ruminants (Van Ryssen, 2001). The CT containing GLM supplementation in broiler diets may be an attractive natural modification to preserve CP contents of alternative feedstuff by reducing NH_3 production side by side reducing *E. coli* and *Clostridia* counts by breaking the chain of pathogenic microorganism spread in the ecosystem.

CONCLUSION

It can be concluded that dietary supplementation of condensed tannins containing guava leaf meal in broiler chickens reduced NH_3 concentration and pathogenic *E. coli* and *Clostridia* counts in broiler litter material. Properly processed and sterilized litter material act as cheaper alternative feedstuffs, considered as a safe, environment-friendly and sustainable approach to save nutrients for better ruminant production practices and reduce environmental pollution. Further research is needed to know the exact mechanism by which CT containing GLM target on specific group of microorganism's reduction, and their specific metabolites generated in the gastrointestinal tract of broiler chicken which shows suppression of NH_3 production and mitigate environmental pollution is warranted.

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