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Original Article

Influence of Pine Bark Tannin on Bacterial Pathogens Growth and Nitrogen Compounds on Changes in Composted Poultry Litter

■Author(s)

Arzola-Alavarez C[I] https://orcid.org/0000-0003-0474-0834
Castillo-Castillo Y[I] https://orcid.org/0000-0002-1902-8869
Anderson RC[II] https://orcid.org/0000-0003-2683-4700
Hume ME[II] https://orcid.org/0000-0001-9093-6045
Ruiz-Barrera O[I] https://orcid.org/0000-0002-7279-7325
Min BR[III] https://orcid.org/0000-0001-5827-3480
Arzola-Rubio A[I] https://orcid.org/0000-0003-1606-9654
Beier RC[II] https://orcid.org/0000-0002-3445-1008
Salinas-Chavira J[IV] https://orcid.org/0000-0002-3149-3413

[I] Universidad Autónoma de Chihuahua, Chihuahua, Chihuahua, Mexico.
[II] USDA/ARS, College Station, Texas, USA.
[III] USDA/ARS/CPRL, Bushland, TX, USA.
[IV] Universidad Autónoma de Tamaulipas, Cd. Victoria, Tamaulipas, Mexico.

■Mail Address

Corresponding author e-mail address
Jaime Salinas-Chavira
Universidad Autonoma de Tamaulipas
Facultad de Medicina Veterinaria y Zootecnia - Nutricion Animal - km. 5 carretera cd. victoria a cd. mante Victoria Tamaulipas 87000 Mexico.
Phone: (0202) 01119992240
Email: jsalinasc@hotmail.com

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ABSTRACT

To study the antimicrobial and uric acid-preserving activity of pine bark tannin on poultry litter composting, antibiotic-free wood chip-based poultry litter was distributed (11 g) to 50-mL conical centrifuge tubes and immediately amended with 1.3 mL0.4 M sodium phosphate buffer (control) or with 1.3 mL condensed tannins from pine bark (*Pinus palustris*; 9 % tannin wt/vol in water). All tubes (*n* = 3 tubes/treatment) were inoculated with a novobiocin and naladixic acid-resistant *Salmonella typhimurium* (STNN) to achieve 3.0 $\log_{10}$ CFU/g and incubated at 37°C for 3 days to simulate an initial compost period. Wildtype *E. coli* and the challenge STNN strain as well as concentrations of ammonia, uric acid and urea were measured on days 0 and 3. Pine bark tannin treatment decreased ($p<0.01$) STNN populations in the litter by 0.6 log units compared to the controls. Wildtype *E. coli* populations were unaffected by tannin treatment ($p>0.05$). Ammonia accumulation decreased ($p<0.01$) 23% in tannin-treated litter compared to the control (2.8 ± 0.1 μmol/g). Conversely, the residual uric acid concentration was 1.6-fold higher ($p=0.02$) in litter treated with the pine bark tannin than in the control litter. Urea concentrations were unaffected by tannin treatment ($p>0.05$). Results suggest that pine bark tannin treatment may preserve uric acid and reduce ammonia volatilization in composted litter while aiding Salmonella control.

INTRODUCTION

Poultry litter can contain dangerous and unwanted pathogenic bacteria which could be resistant to antimicrobials and thus requires treatment before being fed. Composting is a practical method to process the litter; although total nitrogen (N) can represent losses of about 59% of the initial concentration, and this occurs mainly due to NH_3 volatilization, that can reduce the value of litter as a fertilizer and feed additive (Tiquia & Tamb, 2000). Additions of biochar (BC) to poultry litter composted for fertilizer lowered N losses by 52% (Steiner *et al.*, 2009), but it did not clear that the N fixed with BC can be used effectively by ruminants. Besides composting, chemical compounds such as sodium chlorate have been used to control pathogens in the gastrointestinal tract of ruminants (Anderson *et al.*, 2002; Edrington *et al.*, 2003; Callaway *et al.*, 2003; Callaway *et al.*, 2004: Anderson *et al.*, 2005; Taylor *et al.*, 2012; Arzola *et al.*, 2014; Copado *et al.*, 2014). Short-chain nitro compounds also have shown bactericidal effects on *Salmonella* spp., *Escherichia* spp. and other gastric and intestinal bacteria (Arzola-Alvarez *et al.*, 2016; Arzola *et al.*, 2017; Correa *et al.*, 2017; Ruiz-Barrera *et al.*, 2017).

Tannins are a distinct family of chemical compounds, having application in the food industry as well as in animal nutrition and wine production. As one of the most abundant and ubiquitous groups of plant metabolites, tannins are an integral part in animal diets. Recently, Min *et al.* (2015) reported that a moderate level of pine bark tannin supplementation improved gastrointestinal nitrogen balance, but had negative impact on fiber, lignin, and protein digestibility of goats, whereas the same author, (2014) had earlier observed changes in bacterial and methanogenic populations in the GI tract of goats supplemented with tannins. Beauchemin *et al.* (2007) found no effect of feeding quebracho tannin extract on reducing enteric methane emissions from growing cattle, although there was evidence of a protein binding effect which potentially could improve nitrogen retention in the host. Dawson *et al.* (1999) reported that feeding quebracho tannin influenced ruminal and post-ruminal metabolism resulting in reduced nutrient utilization and impaired animal performance. Scalbert (1991) postulated that tannins had direct and indirect antimicrobial activity. At the presente moment, there are no conclusive results on the mechanism that underlies the N transformation in composting poultry litter treated with pine bark tannins. Ammonia is generated by uric-acid and urea-metabolizing microorganisms during poultry manure composting (Kim *et al.*, 2009). The main concerns of ammonia are related to environment pollution and related to the health of animals in the barns. The present study was conducted to examine the antimicrobial activity, uric acid-preserving and ammonia production of poultry litter compost treated with pine bark tannin.

MATERIAL AND METHODS

Preparation of poultry litter compost

Approximately one-year old poultry litter having reared 2 to 3 flocks without antibiotic or coccidiostat exposure was collected from the Texas A&M University poultry farm and screened through a 17 mm diameter sieve. Condensed tannins from pine bark (*Pinus palustris*) were extracted using a Sephadex LH-20.

Litter was distributed (11 g) to 50 mL conical centrifuge tubes (n = 3 tubes/treatment), amended with 1.3 mL of pine bark tannin (9 % tannin wt/vol) or 0.4 M sodium phosphate buffer, pH 6.5, alone (control), and then inoculated with a novobiocin and naladixic acid-resistant *Salmonella typhimurium* (STNN) to achieve 3.0 $\log_{10}$ colony forming units (CFU)/g. Tubes were closed with caps, sealed with parafilm and then incubated anaerobically in a Gas Pack jar flushed with 100% CO_2 at 37°C for 3 days.

Analytical methods

Tubes sampled at 0 and day 3 of incubation were serially diluted (10-fold) and plated to 3M *E. coli*/coliform petri-film for enumeration of wildtype *E. coli* and to novobiocin- and naladixic acid- supplemented (20 and 25 µg/mL, respectively) Brilliant Green Agar for quantitative recovery of the challenge STNN strain. *E. coli*/coliforms were counted according to 3M *E. coli*/Coliform Petrifilm as per manufacturer's instructions (3M, Minneapolis, MN, USA). The initial 1:10 dilutions of each sample were also analyzed colorimetrically for the determination of uric acid using an Uric Assay Assay Kit (Sigma-Aldrich, St. Louis, MO, USA), urea using the QuantichromTM Urea Assay Kit (BioAssay Sysyems, Hayward, CA, USA). Ammonia was analyzed colorimetrically as described by Chaney &Marbach, 1962). Total N was determined via combustion by the Soil, Water and Forage Testing Laboratory at the Texas AgriLife Extension Services' Department of Soil and Crop Sciences.

Statistical Analysis

Log base 10CFU of wildtype *E. coli* and STNN as well as uric acid, urea and ammonia concentrations were subjected to analysis of variance in a completely randomized design. Four treatments were considered: T1 no pine bark tannin and sampled at 0 days (control), T2 no pine bark tannin and sampled at 3 days, T3 pine bark tannin and sampled at 0 days, T4pine bark tannin and sampled at 3 days of composted poultry litter. Means of treatments were compared using the Least Significant Difference (LSD) test. Significant effect was considered at $p<0.05$.

RESULTS

The effect of pine bark tannin on *Salmonella* and *E coli* counts as uric acid, urea and ammonia concentrations in composted poultry litter are shown in Table 1. *Salmonella typhimurium* populations increased ($p<$ 0.01) as the days of composting increased; however, Pine bark tannin decreased ($p<0.01$) STNN populations in the litter after 3 d of composting by 0.6 log units compared to controls. Wild type *E. coli* populations were unaffected ($p>0.05$) by tannin treatment but increased ($p<0.01$) increasing days of composting.

Uric acid was higher and ammonia was lower ($p<0.01$) in pine bark tannin-treated poultry litter after 3 days composting. On 0 d of incubation, these variables were not influenced ($p>0.05$) by pine bark tannin treatment. Urea concentrations were increased

Table 1 – Effect of pine bark tannin on bacterial pathogens growth and nitrogen compounds in composed poultry litter (mean ± SD)

	Untreated		Pine bark tannin		
	0 Day	3 Day	0 Day	3 Day	*p* value
Uric Acid, µmol/g	20.90 ± 3.32a	15.46 ± 1.26b	20.90 ± 3.32a	25.17 ± 2.77a	0.02
Urea, µmol/g	3.49 ± 0.55b	10.33 ± 2.39a	3.49 ± 0.55b	10.01 ± 1.01a	< 0.01
Ammonia, µmol/g	1.55 ± 0.19c	2.82 ± 0.11a	1.55 ± 0.19c	2.15 ± 0.14b	< 0.01
Salmonella typhimurium, $\log_{10}$ CFU/g	3.89 ± 0.04c	6.01 ± 0.15a	3.89±0.04c	5.40 ± 0.32b	< 0.01
Escherichia coli, $\log_{10}$ CFU/g	5.29 ± 0.39b	6.48 ± 0.04a	5.29±0.39b	6.43 ± 0.17a	< 0.01

[abc] In each row, values with different letter are statistically different (p< 0.05)

(p=0.02) due to days of composting but were unaffected (p>0.10) by pine bark tannin treatment.

The effects of pine bark tannin on final pH and total nitrogen after 3 days composting poultry litter are showed In Table 2. Pine bark tannin had no influence (p=0.23) on final pH and tended to greater (p=0.09) total nitrogen in composted poultry litter.

Table 2 – Effect of pine bark tannin on final pH and total nitrogen after 3 days composting poultry litter (mean ± SD)

Treatment	pH	Total nitrogen (%)
Untreated	6.6 ± 0.32	3.06 ± 0.07
Pine bark tannin	6.3 ± 0.15	3.22 ± 0.10
p value	0.23	0.09

DISCUSSION

Salmonella typhimurium reduced populations in poultry litter treated with Pine bark tannin after 3 d of composted. Scalbert (1991) postulated that the antimicrobial activity of tannin could be attributed to inhibition of extracellular microbial enzymes with removal of substrates required for microbial growth or by direct effect on microbial metabolism inhibiting oxidative phosphorylation. Zdunczyk *et al.* (2018) observed that turkeys fed diets with faba bean seeds with high or low tannin content had a decrease in the counts of *Salmonella* bacteria in the gastrointestinal tract. Reyes *et al.* (2017) reported antibacterial effects against *Salmonella* infection in mice treated with tannin-derived components: gallic acid and tannic acid. Rubinelli *et al.* (2017) reported that sodium bisulfate alone or in a mixture with tannin reduced *S. typhimurium* rapidly of in vitro cultures of cecal and fecal samples of broiler chickens.

In the present study, *E. coli* populations were not inhibited by tannin treatment in composted poultry litter. However, Ribeiro *et al.* (2018) found that extract of different plants had inhibitory effects on the growth of *E coli*. They observed that removing tannins from the extracts, the antimicrobial effect disappeared, indicating the importance of tannins on *E. coli* growth-inhibition. These differences could be due to the conditions in the culture of microorganisms. Ogawa & Yazaki (2018) documented that *E. coli* was inhibited by tannin extracts only in aerobic conditions because tannins autoxidize releasing hydrogen peroxide inhibiting *E. coli*. This effect may not occur in anaerobic medium.

The increase in uric acid is consistent with ammonia reduction in the litter treated with pine bark tannin after 3 days composting. Uric acid is degraded to ammonia by microbial enzymes in the poultry manure. Manipulations of this process reduces ammonia volatilisation and nitrogen loses from poultry manure. In part, the conversion of uric acid to ammonia is attributed to microorganisms that use uric acid as a nitrogen source. Kim *et al.* (2006) found that poultry litter treated with nitrocompounds inhibited uric acid-utilising microorganisms manure and Ruiz-Barrera *et al.* (2017) observed decreased rates of ammonia accumulation of more than 70% compared to untreated poultry manure. Further research on poultry litter treated with pine bark tannins on uric-acid utilising microorganisms is warrantied.

Tannins also reduce ammonia released in rumen through the reduction of dietary protein degraded by microorganisms in rumen due to the binding of tannins with proteins forming a complex of tannin-protein that is resistant to microbial enzymes in rumen (Naumann *et al.,* 2017). Consistently pine bark tannins have reduced crude protein degradation with subsequent reduction in ammonia production in the rumen of goats (Min *et al.,* 2012).

There is not information about chemical complexes formed among pine bark tannin with proteins or other nitrogen compounds of poultry manure.

Other effect of tannins is the methane reduction during rumen fermentation. Salami *et al.* (2018) observed that tannins inhibited methanogens or protozoa abundance in rumen fermentations. We have not found information about the influence of pine bark tannins on microbial fermentation related to methane production of composted poultry manure.

Urea concentrations were not influenced by pine bark tannin treatment; however, total nitrogen tended to be greater in poultry litter treated with pine bark tannin and may be related with the ammonia reduction and preservation of uric acid in the treated litter.

CONCLUSIONS

The reduction in ammonia production by pine bark tannins in the current study may be considered as a safe, environmentally compatible and sustainable technology to recycle nutrients contained in poultry litter for good animal production practices. Pine bark tannin treatment may be an attractive natural amendment to poultry litter to preserve the crude protein value of this feedstuff during early periods of composting while concurrently aiding in the reduction of pathogenic microbes. Further research into the effects of pine bark tannins on specific microorganisms and metabolites generated during composting poultry litter is warranted.

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DECLARATION OF INTEREST STATEMENT

No potential conflict of interest was reported by the authors

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