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Research article

Effects of Korean Red Ginseng marc with aluminum sulfate against pathogen populations in poultry litters

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A B S T R A C T

Background: The aim of this study was to evaluate the effects of Korean Red Ginseng marc with aluminum sulfate as litter amendments on ammonia, soluble reactive phosphorus, and pathogen populations in poultry litters.
Methods: Increasing levels of Korean Red Ginseng marc with aluminum sulfate were applied onto the surface of rice hull as a top-dress application; untreated rice hulls served as controls.
Results: Treatment with Korean Red Ginseng marc with aluminum sulfate or aluminum sulfate alone resulted in lower litter pH ($p < 0.05$), as compared with that of the controls. There were some differences ($p < 0.05$) between treatments with Korean Red Ginseng marc with aluminum sulfate or aluminum sulfate alone and controls at 2–4 wk (not at 1 wk). Ammonia levels reduced on an average by 29%, 30%, and 32% for 10 g, 20 g Korean Red Ginseng marc with aluminum sulfate, and aluminum sulfate alone, respectively, as compared with controls at 4 wk. During the experiment, Korean Red Ginseng marc with aluminum sulfate or aluminum sulfate treatment had an effect ($p < 0.05$) on soluble reactive phosphorus content, as compared with the controls (not at 4 wk). A decrease in *Salmonella enterica* and *Escherichia coli* was observed ($p < 0.05$) in litter amended with both Korean Red Ginseng marc with aluminum sulfate and aluminum sulfate alone, as compared with the control, except at 1–3 wk for *Salmonella enterica* and 1 wk and 4 wk for Escherichia *coli*, respectively.
Conclusion: The results showed that using Korean Red Ginseng marc with aluminum sulfate (blends), which act as acidifying agents *by reducing the pH of the litter*, was equally effective as aluminum sulfate in reducing the environmental impact.

1. Introduction

Poultry litter is recognized as an excellent nutrient source and fertilizer in growing plants because of its high nitrogen and phosphorus (P) content. Compared with different types of animal manure, poultry litter that is a mixture of bedding material and manure, has high organic matter and macronutrient and micronutrient contents, leading to the formation and release of ammonia (NH_3) as volatile compounds [1,2]. In addition, poultry litter provides an ideal environment for microbial proliferation, which is effectively controlled by litter management practices. The major environmental problems of the intensive rearing conditions related to poultry production and the land application of poultry litter have become important concerns in recent years. There is direct evidence that air quality in poultry houses and agricultural fields can cause environmental change, which in some cases may adversely affect agriculture viability in the short term as well as the long term. Consequently, the main environmental impacts caused by improperly managed poultry litter can be broadly categorized as follows: (1) high levels of NH_3 emissions from poultry houses that

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affects poultry health and production or causes soil acidification; (2) environmental effects of over-application of P in aquatic systems that can lead to algal blooms and eutrophication; and (3) increased numbers of pathogenic organisms such as viruses, *Salmonella* and *Escherichia coli* [2–4].

In order to solve the above-mentioned problems in poultry litter management, alternative methods are needed to facilitate a reduction in the environmental impact of poultry houses. Currently, acidifying amendments, which are easily available for use in poultry houses, are one of the most commonly applied techniques to decrease NH_3, P, and potential pathogens in poultry litters. For example, aluminum sulfate is commonly applied to poultry houses to alleviate the negative environmental impact. Several benefits of aluminum sulfate application have been reported, including the following: improved animal production, reduction of NH_3 levels in the poultry house, pathogen populations in litter, soluble reactive P, and energy cost during the winter season [5]. Moore et al. [6] also reported a significant reduction in NH_3 fluxes (99%) from houses treated with aluminum sulfate, as compared with controls during the first 4 wk, which would result in a healthier environment for chickens. In another study of Moore and Edwards [7], aluminum in aluminum sulfate that binds with the P in litter showed an approximately 75% decrease in P runoff in pastures [7]. Line and Bailey [8] observed that increased level of litter treatment resulted in a slightly delay of *Campylobacter* colonization onset in broiler chicks.

Another alternative is the use of Korean Red Ginseng marc, a fibrous and insoluble byproduct of the Korean Red Ginseng extraction process. In general, Korean Red Ginseng is produced by repeatedly steaming the ginseng at 98–100°C for 2–3 h and drying it, which can increase the storage period and improve medicinal efficacy [9,10]. In South Korea, Korean Red Ginseng byproducts (Korean Red Ginseng marc) are rendered into poultry feed for poultry production purposes [11]. However, Korean Red Ginseng byproducts become environmental contaminants when disposed as waste in soil. To date, there are no studies or reports on the use of Korean Red Ginseng marc as a litter treatment in the poultry industry.

In this study, we evaluated the effects of Korean Red Ginseng marc with aluminum sulfate as litter amendments on NH_3, soluble reactive phosphorus (SRP), and pathogen populations in poultry litters. The results of the study could be useful in determining the optimal levels to reduce the environmental impact of the poultry industry.

2. Materials and methods

2.1. Animals

All procedures of the present study were carried out in accordance with the guidelines of the animal policy at Yoosim Farms (Youngju, South Korea). The experiment was conducted on 160 male and 80 female chicks (1-d-old Arbor acres) obtained from a commercial hatchery for 28 d and randomly placed in 16 pens with a density of 0.07 m^2/bird. Each treatment had a randomized complete block design with four replicate pens of 15 birds (10 males and 5 females per pen). Chicks were raised on a depth of 5 cm with rice hulls as bedding materials. They were allowed *ad libitum* access to commercial diets and water through one tube feeder and an automatic bell drinker in each pen. The feeding program consisted of a commercial starter diet for the first 3 wk (0–21 d) and a finisher diet for the 4^{th} week (22–28 d). Temperature, lighting, and ventilation could be controlled to suit the chicks.

2.2. Treatments

The materials used in this experiment were Korean Red Ginseng marc (Yoosim, Youngju, South Korea) and aluminum sulfate, a form of dry acid salt that neutralizes alkalinity (Samchun Pure Chemical Corporation, Pyeongtaek, South Korea). These materials were applied onto the surface of the rice hull as a top-dress application. There were four treatment groups: CON, no treatment; T1, 10 g Korean Red Ginseng marc + 90 g aluminum sulfate per kg rice hulls; T2, 20 g Korean Red Ginseng marc + 80 g aluminum sulfate kg rice hulls; T3, 100 g aluminum sulfate per kg rice hulls.

2.3. Litter sampling and NH_3 gas measurements

Litter samples from each pen were collected weekly from four random locations for 4 wk and thoroughly mixed by hand. A subsample of 100 g was added to a plastic bag and maintained frozen until analysis. NH_3 emissions from rice hulls were taken weekly at four random locations in each pen and analyzed using the multigas analyzer (Yes Plus LGA, Critical Environment Technologies Canada Inc., Delta, Canada).

2.4. Chemical analysis

The pH and SRP content of the litter were determined with a 1:10 litter/water extract ratio, as described previously [12]. In brief, 10 g of poultry litter from each sample was weighed in a 200 mL polycarbonate centrifuge tube and diluted with 100 mL deionized water. The entire mixture was shaken using a mechanical shaker for 2 h and centrifuged at 6,000 rpm for 15 min. After that, pH measurements were made immediately in an unfiltered condition using a pH meter. SRP samples were filtered in the laboratory through a membrane filter of 0.45 μm to remove suspended solids. All filtered samples were acidified using hydrochloric acid of pH 2.0 and then frozen for analysis [12]. SRP analysis was estimated using the ascorbic acid technique with an auto-analyzer according to American Public Health Association method 424-G [13].

2.5. Microbial analysis

To determine *Salmonella enterica* and *E. coli*, samples (10 g) were prepared by adding 100 mL of phosphate-buffered saline buffer (pH 7.0) and mixed using a stomacher. Then, 0.1 mL of the litter suspension (10^0) was serially diluted from 10^1 to 10^7 dilutions. Serial dilutions were spread-plated on a sterile Petri plate with Difco TM SS agar (Becton, Dickinson and Company, Sparks, MD, USA) and Difco TM Violet Red Bile agar (Becton, Dickinson and Company, Sparks, MD, USA) for counting *Salmonella enterica* and *E. coli*, respectively. Plates were incubated at 37°C for 24 h, after which the colonies as average colony forming units (cfu)/g litter were counted at 1 wk through to 5 wk.

2.6. Statistical analysis

All data analyses were performed with analysis of variance using the general linear model of SAS (SAS Institute, 1990), with the pen as the experimental unit. Differences between means were compared using Tukey's honest significant difference test. Significance was set at $p < 0.05$.

3. Results and discussion

Effects of the addition of Korean Red Ginseng marc with aluminum sulfate to poultry litter on pH as a function of time were shown in Table 1. The litter pH showed statistically significant

Table 1
Effects of the addition of Korean Red Ginseng marc with aluminum sulfate to poultry litter with pH as a function of time

Time (wk)	Treatment[4]				SEM[5]	p
	CON	T1	T2	T3		
1	6.78[1)]	6.04[2)]	6.14[2)]	6.15[2)]	0.07	0.0027
2	7.76[1)]	7.06[2)]	6.90[2)]	6.40[3)]	0.15	0.0002
3	8.53[1)]	7.24[1),2)]	7.19[2)]	7.08[2)]	0.16	0.0008
4	8.72[1)]	7.50[3)]	7.45[1)]	7.32[2)]	0.17	<0.0001

1),2),3) Means on the same row followed by difference superscripts differ significantly ($p < 0.05$)
4) CON: no treatment; T1: 10 g Korean Red Ginseng marc + 90 g aluminum sulfate per kg rice hulls; T2: 20 g Korean Red Ginseng marc + 80 g aluminum sulfate per kg rice hulls; T3: 100 g aluminum sulfate per kg rice hulls
5) Values are means ± SEM
SEM, standard error of the mean

Table 2
Effects of the addition of Korean Red Ginseng marc with aluminum sulfate to poultry litter on ammonia emissions as a function of time

Time (wk)	Treatment (ppm)[4]				SEM[5]	p
	CON	T1	T2	T3		
1	0.00	0.00	0.00	0.00	0.00	—
2	1.63[1)]	0.14[2)]	0.07[2)]	0.03[2)]	0.09	0.0147
3	12.20[1)]	4.85[2)]	4.69[2)]	2.40[3)]	0.31	<0.0001
4	23.06[1)]	16.46[2)]	16.10[2)]	15.70[2)]	0.56	0.0431

1),2),3) Means on the same row followed by difference superscripts differ significantly ($p < 0.05$)
4) CON: no treatment; T1: 10 g Korean Red Ginseng marc + 90 g aluminum sulfate kg rice hulls; T2: 20 g Korean Red Ginseng marc + 80 g aluminum sulfate per kg rice hulls; T3: 100 g aluminum sulfate per kg rice hulls
5) Values are means ± SEM

differences among treatments ($p < 0.05$) through all weeks. Litter pH of the control ranged from 6.78 (at 1 wk) to 8.72 (at 4 wk). In treatments with Korean Red Ginseng marc with aluminum sulfate (T1 and T2) or aluminum sulfate alone (T3) at 1 wk, the litter pH values for T1, T2, and T3 were 6.04, 6.14, and 6.15. However, after 2 wk, they ranged from 7.06 to 7.50 in T1, 6.90 to 7.45 in T2, and 6.40 to 7.32 in T3. These pH values were lower than those of the controls during the study. In the current study, addition of Korean Red Ginseng marc with aluminum sulfate (T1 and T2) was effective in reducing litter pH, as compared with aluminum sulfate (T3), which was within the range reported in other studies [14].

NH_3 emissions observed from Korean Red Ginseng marc with aluminum sulfate added to poultry litter, as a function of time, were presented in Table 2. No significant difference in NH_3 emissions could be detected among treatments during the 1st wk after the beginning of the experiment. However, there were some differences ($p < 0.05$) between treatments with Korean Red Ginseng marc with aluminum sulfate or aluminum sulfate alone and controls at 2–4 wk. Overall NH_3 emissions in all groups were undetectable (<2 ppm) through 2 wk, but at 3 wk, NH_3 levels in the controls were 12.20 ppm, as compared with 4.85 ppm, 4.69 ppm, and 2.40 ppm in T1, T2, and T3, respectively. In comparison with aluminum sulfate alone, the rate of decrease in NH_3 emissions for litter treated with blends (Korean Red Ginseng marc and aluminum sulfate) was statistically similar or slightly different. As shown in Table 2, NH_3 levels had an average reduction of 29%, 30%, and 32% for T1 (16.46 ppm), T2 (16.10 ppm), and T3 (15.70 ppm), respectively, as compared with controls (23.06 ppm) at 4 wk. These results were in agreement with the research of Moore et al. [5] and DeLaune et al. [15], who reported that adding aluminum sulfate to poultry manure significantly decreased NH_3 volatilization. In a laboratory study to simulate open-lot feedyards, Shi et al. [16] showed that pH adjustment for controlling NH_3 emissions with aluminum sulfate amendments resulted in decreased pH and reduced NH_3 volatilization (98%). They found a strong relationship between pH and NH_3 emissions, with rapidly increasing NH_3 emissions between pH 6 and 8.

Generally, the poultry build up litter with an alkaline pH of about 8.0–9.0, and NH_3 is created through the breakdown of uric acid in poultry litter. Thus, NH_3 depends on temperature, moisture content, and pH in poultry litter and its production can occur if litter management is not effective [17]. In other words, it should be noted that controlling litter pH is the most important step for reducing NH_3 emissions from poultry house. Decreases in pH and in NH_3 emissions have also been explained by the acidification of the litter with aluminum sulfate, which in turn converted NH_3 to NH_4^+, such as ammonium sulfate [$(NH_4)_2SO_4$] [5,12]. Therefore, we concluded that blends (Korean Red Ginseng marc with aluminum sulfate) have the ability to reduce pH and NH_3 due to their ability to act as acidifiers [2,4] and not through any biological activity.

SRP data from Korean Red Ginseng marc with aluminum sulfate treated poultry litter were summarized in Table 3. During the experiment, Korean Red Ginseng marc with aluminum sulfate or aluminum sulfate treatment had an effect ($p < 0.05$) on SRP content, as compared with the controls (although not at 4 wk). At 1 wk, SRP contents in control were 13.20 mg/kg, as compared with 5.01 mg/kg, 4.2 mg/kg, and 2.3 mg/kg for T1, T2, and T3, respectively. From 2 wk to 4 wk, SRP content for controls was 28.00 mg/kg, 13.40 mg/kg, and 14.00 mg/kg, while other treatments ranged from 0.7 mg/kg to 10.6 mg/kg. The results showed that the ability of Korean Red Ginseng marc with aluminum sulfate (T1 and T2) to reduce SRP content in comparison with aluminum sulfate, are likely due to the lower litter pH [18]. Similar to the result of the current study, the P content in litter receiving aluminum sulfate was, on average, associated with a significant reduction in the water-soluble P content [2]. According to several researchers [19–21], aluminum sulfate not only decreases water-soluble P in poultry litter by forming insoluble $AlPO_4$ hydroxides and $Al(OH)_3$-phosphate surface adsorption complexes, but also continues to be effective at reducing P levels in runoff from fields and soils where the litter is applied. However, the mechanism by which Korean Red Ginseng marc with aluminum sulfate lowers litter SRP is currently unclear.

Effects on pathogen populations of the addition of Korean Red Ginseng marc with aluminum sulfate in litters as a function of time were shown in Table 4. A decrease in *S. enterica* was observed ($p < 0.05$) at 4 wk and 5 wk in litter mixed with both Korean Red Ginseng marc with aluminum sulfate (T1 and T2; ranged from 4.13–3.73 log 10 cfu/g) and aluminum sulfate alone (T3; 3.17 log 10 cfu/g at 4 wk and 3.37 log 10 cfu/g at 5 wk), as compared with the control (3.86 log 10 cfu/g at 4 wk and 4.36 log 10 cfu/g at 5 wk).

Table 3
Effects of the addition of Korean Red Ginseng marc with aluminum sulfate to poultry litter on soluble reactive phosphorus as a function of time

Time (wk)	Treatment (mg/kg)[3]				SEM[4]	p
	CON	T1	T2	T3		
1	13.20[1)]	5.01[2)]	4.20[2)]	2.30[2)]	0.98	0.0051
2	28.00[1)]	3.70[2)]	0.70[2)]	1.20[2)]	1.99	0.0220
3	13.40[1)]	5.90[2)]	3.30[2)]	4.50[2)]	1.22	0.0158
4	14.00	10.60	9.10	9.50	1.89	0.3827

1),2) Means on the same row followed by difference superscripts differ significantly ($p < 0.05$)
3) CON: no treatment; T1: 10 g Korean Red Ginseng marc + 90 g aluminum sulfate per kg rice hulls; T2: 20 g Korean Red Ginseng marc + 80 g aluminum sulfate per kg rice hulls; T3: 100 g aluminum sulfate per kg rice hulls
4) Values are means ± SEM

Table 4
Effects of the addition of Korean Red Ginseng marc with aluminum sulfate to poultry litter on pathogenic populations in poultry litters as a function of time

Time (wk)	Treatment[4]				SEM[5]	p
	CON	T1	T2	T3		
***Salmonella enterica* (log 10 cfu/g)**						
1	ND	ND	ND	ND	—	—
2	3.97	ND	ND	ND	0.33	—
3	4.15[3)]	5.76[1)]	5.01[2)]	4.81[2),3)]	0.22	0.0011
4	3.86[1)]	3.75[1)]	3.58[1),2)]	3.17[2)]	0.18	0.0056
5	4.36[1)]	4.13[1),2)]	3.73[2),3)]	3.37[3)]	0.25	0.0019
***Escherichia coli* (log 10 cfu/g)**						
1	5.37	5.08	5.13	4.93	0.43	0.6722
2	6.01[1)]	5.21[1),2)]	5.20[1),2)]	3.90[2)]	0.45	0.0068
3	6.35[1)]	6.28[1)]	5.93[1),2)]	5.45[2)]	0.26	0.0202
4	5.68	5.47	4.93	5.54	0.35	0.1682
5	5.56[1)]	5.46[1)]	4.39[2)]	4.97[1),2)]	0.34	0.0045

[1),2),3)] Means on the same row followed by difference superscripts differ significantly ($p < 0.05$)
[4)] CON: no treatment; T1: 10 g Korean Red Ginseng marc + 90 g aluminum sulfate per kg rice hulls; T2: 20 g Korean Red Ginseng marc + 80 g aluminum sulfate per kg rice hulls; T3: 100 g aluminum sulfate per kg rice hulls
[5)] Values are means ± SEM
ND, not detected

However, *S. enterica* at 1–2 wk was not detected in any treatment, except at 2 wk for the control. At 3 wk, *S. enterica* in the control groups (4.15 log 10 cfu/g) was lower ($p < 0.05$) than in treatments with Korean Red Ginseng marc with aluminum sulfate (5.76 log 10 cfu/g for T1 and 5.01 log 10 cfu/g for T2) and aluminum sulfate alone (4.81 log 10 cfu/g). *E. coli* levels decreased slightly ($p < 0.05$) with increasing levels of Korean Red Ginseng marc with aluminum sulfate (T1 and T2) and aluminum sulfate alone (T3) throughout the experimental period, but there were no significant differences in *E. coli* levels at 1 wk and 4 wk. *E. coli* in poultry litter varied from 3.90 to 6.28 log 10 cfu/g with Korean Red Ginseng marc with aluminum sulfate and aluminum sulfate alone during the 5-wk period, whereas *E. coli* for the control group was 5.37 log 10 cfu/g, 6.01 log 10 cfu/g, 6.35 log 10 cfu/g, 5.68 log 10 cfu/g, and 5.56 log 10 cfu/g at 1 wk through to 5 wk. In terms of reducing pathogens, it is interesting to note that Korean Red Ginseng marc with aluminum sulfate (T1 and T2), which acts as acidifying agents, was equally effective as aluminum sulfate alone in poultry litter. This implied that acidifying the litter with these blends creates an unfavorable environment for pathogens in the litter. Our findings supported the results of Payne et al. [22] and William et al. [23], which showed that manipulating the pH of the litter to ≤ 4 creates an unfavorable environment for most bacterial growth and reduction in pathogen populations was greatest with litter amendments. For example, pathogens such as *E. coli*, *Globicatella*, *Listeria*, *Mycobacterium*, *Salmonella*, and *Streptococcus* can easily grow in new or used chicken litter because of the more favorable pH. Thus, adding acidifiers to the litter results in pH reduction that suppresses pathogenic growth and activity in the litter [24], producing a favorable environment for healthy microorganism to flourish [25].

In conclusion, the overall results of this study showed that increasing levels of Korean Red Ginseng marc with aluminum sulfate in blends might provide an effective litter management practice for decreasing NH_3 emissions, SRP contents, and pathogen populations of poultry litter, the three major issues of concern. Furthermore, the lower litter pH could potentially be associated with the control of these negative environmental factors. However, future studies are still needed to evaluate the mechanism of Korean Red Ginseng marc with aluminum sulfate with respect to the reduction in environment impact or the optimal levels as litter amendments.

Conflicts of interest

The authors have no conflicts of interest.

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