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Chitosan Reduces Water Solubility of Phosphorus in Poultry Litter

An Undergraduate Honors College Thesis

in the

Department of Biological and Agricultural Engineering
College of Engineering
University of Arkansas
Fayetteville, AR

by

Iain M. Bailey

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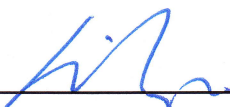
This thesis is approved.

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Dr. Brian Haggard

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INTRODUCTION

Chitin is the second most abundant natural polysaccharide on earth (Chen et al., 2003). Found as the main structural component in crustacean shells, it serves as the chemical precursor to chitosan (Chen et al., 2003). Chitosan is formed from chitin through the process of chemical deacetylation, which removes some or all of the acetyl groups from each of the carbohydrate monomers and exposes the amino groups (Onsosyen and Skaugrud, 1990). Depending upon the pH of the medium or reacting aqueous solution, the amino groups can become protonated and cause the molecule to become cationic (Onsosyen and Skaugrud, 1990). Chitosan can be characterized by the purity of the sample, the average molecular weight (MW) of the polysaccharide chain, and the deacetylation degree (DD) that the chitin underwent upon transformation to chitosan. Chitosan can also be characterized by source: commercial chitosan is usually derived from crab shells, whereas other varieties can be derived from shrimp and crawfish discards.

This combination of cationic and structural flexibility makes chitosan highly reactive with a large spectrum of different chemicals in aqueous solutions (Onsosyen and Skaugrud, 1990; Cook et al., 2011). Especially when coupled with an inorganic salt to prevent the molecule from elongating in solution, chitosan shows a good affinity for chelating both anions and cations. Its ability to flocculate solids has been studied most commonly in commercial wastewater treatment applications and manure separation (No and Meyers, 1989; Onsosyen and Skaugrud, 1990; Gamage and Shahidi, 2007). More recently, chitosan has been used to flocculate algae in streams and even immobilize algae to promote nutrient removal (No and Meyers, 1989; Divakaran and Sivasankara Pillai, 2002). These characteristics of chitosan suggest that it could

have some useful applications in poultry production, potentially reducing the water solubility of certain elements in poultry litter.

Poultry litter has been land applied as fertilizer at prescribed rates for decades. Historically, poultry litter was applied at rates based upon forage nitrogen (N) needs, but more recently, phosphorus (P) content in the litter and soil have guided application rates (Sharpley, 1999). These changes in management were prompted by concerns over accelerated eutrophication, where P has been noted or even assumed to be the factor limiting algal growth. The loss of P in runoff from land applied poultry litter is regulated by the amount of water extractable P (WEP), where WEP application rates are positively related to runoff concentrations and loads (Kleinman et al., 2002; DeLaune et al., 2004; Haggard et al., 2005a). The water solubility of P in poultry litter can be reduced by chemical amendments (Moore and Miller, 1994), and some chemicals also reduce ammonia (NH₃) volatilization during poultry production (Moore et al., 2000). Thus, it is conceivable that chitosan addition to poultry litter could reduce the water solubility of P and possibly other trace elements.

The goal of this study was to evaluate the effects of chitin and chitosan on reducing NH₃ volatilization and water solubility of P in poultry litter. We hypothesize that chitosan will significantly decrease the amount of WEP relative to poultry litter and even that treated with chitin. However, we do not anticipate reduced NH₃ volatilization from chitosan-amended poultry litter despite Cook et al. (2011) observing increased total and organic N content of chitosan-treated poultry litter relative to control after a 56-d incubation. The effects of chitin and chitosan additions to poultry litter were compared to aluminum sulfate (alum, Al₂(SO₄)₃), which has been shown to reduce P solubility and NH₃ loss during incubations.

MATERIALS AND METHODS

The study was performed through a series of three experiments which examined the effects of chitin and chitosan, as well as alum, on NH_3 release and water solubility of P and other trace elements. A control and five separate amendments were used in Experiment 1, including alum, three grades of chitosan, and coarse-ground chitin (Table 1). A single source of unaltered poultry litter was divided into 10 g samples, mixed with treatments (1% w/w as is) and incubated at room temperature for three weeks in closed containers; four replicates were used for each treatment. After incubation, litter samples were analyzed for WEP content and trace element content at the University of Arkansas Agricultural Diagnostic Service Lab. Water extractable elements were determined following standard litter protocols, i.e. 1:100 ratio of dry weight poultry litter to water (Kleinman et al., 2007). The filtrate from the extraction procedure was analyzed for P, potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), and aluminum (Al) using an ICP-OES. The intent of this study was to evaluate the effects of chitin and chitosan grades on WEP relative to alum-treated litter and a control (untreated litter) at a low treatment dose (1% w/w); the trace element data will not be discussed.

Table 1. Summary of treatments used in experimental series.

Number	Treatment	Chemical Name	Description
1	<i>Control</i>	no chemical amendment	untreated
2	<i>Alum</i>	aluminum sulfate	ground
3	<i>Coarse Chitin</i>	chitin from shrimp shells	poly(N-acetyl-1,4,beta-D-glucopyranosamine)
--	<i>Chitosan</i>	--	--
4	<i>Grade C</i>	chitosan	medium molecular weight; poly(D-glucosamine) deacetylated
5	<i>Grade B</i>	chitosan from shrimp shells	poly(1,4-beta-D-glucosamine) $\geq 75\%$ deacetylated
6	<i>Grade A</i>	chitosan from shrimp shells	practical grade

Experiment 2 was similar to the first, except all amendments were applied at 1%, 5%, and 10% (w/w). The amendments were added to the litter at rates typically recommended of alum dosage for the control of NH₃ volatilization (1% w/w) or that for control of WEP (5-10% w/w) (Moore, 2011; Penn and Zhang, 2011). The same litter source was divided into 5 g samples for analysis, including four replicates for each treatment; amendments and litter were well mixed and then incubated at room temperature for three weeks. Samples were again taken to the Agricultural Diagnostic Service Lab and analyzed for water extractable elements using the protocol described above.

Experiment 3 shifted the focus from water extractable elements to effects on NH₃ volatilization, but amendments were only applied at 5% and 10% (w/w) rates. A new litter source was used and analyzed for pH, conductivity, water content, WEP, total N (TN), total P (TP), and other elemental concentrations at the Agricultural Diagnostic Service Lab. The litter was divided into 20 g samples, which were well mixed with each amendment and then

transferred to separate Erlenmeyer flasks. A 15 mL vial with 10 mL of deionized water and 4 drops of concentrated HCl was placed uncovered and upright in each flask. The flasks containing the samples and vials were covered and were incubated at room temperature for eight weeks; each treatment included 4 replicates. Vials were collected and replaced after weeks 1, 2, and 8; vial collection and replacement extended from 2 to 8 weeks because of initial results. The acidic water in the vials was analyzed for total $\text{NH}_3\text{-N}$ (as ammonium, $\text{NH}_4\text{-N}$) at the Arkansas Water Resources Center Water Quality Lab using a Lachat 8500 following EPA Method 351.2. Following the incubation, the litter was also analyzed for water extractable elements and TN content. Chitin was not analyzed per the results of Experiments 1 and 2.

Statistical Analysis of Data

Multi-sample analyses of the data for each experiment were performed using computer-based software (Statistix 9.0; Analytical Software, Tallahassee, FL). A one-way analysis of variance (ANOVA) analysis was performed on each group of samples, using an alpha of 0.05 to determine statistically significant differences between treatments. Treatment means were separated via least significant difference (LSD) for each of the three experiments. Significant differences between treatment means were denoted by lettered groups (e.g. A, B, AB, BC, etc.), where any common letters between treatment means represented no significant difference between those treatment means.

RESULTS

Experiment 1

As expected, the control samples had the greatest WEP content (2135 mg kg⁻¹ dry weight basis) after incubation, and poultry litter treated with 1% alum showed the least WEP content (1768 mg kg⁻¹; Figure 1). Chitin (3) and chitosan (5, poly-(1,4-β-D-glucopyranosamine)) at 1% treatment showed no significant reduction in WEP content versus untreated litter and were significantly greater than the poultry litter samples treated by 1% alum. However, there was no significant difference in WEP across the three chitosan treatments at 1% in this first experiment and WEP contents in two of the chitosan treatments (4, poly-(D-glucosamine) deacetylated, 1826 mg kg⁻¹; 6, practical grade, 1853 mg kg⁻¹) were not significantly different than WEP from 1% alum-treated poultry litter.

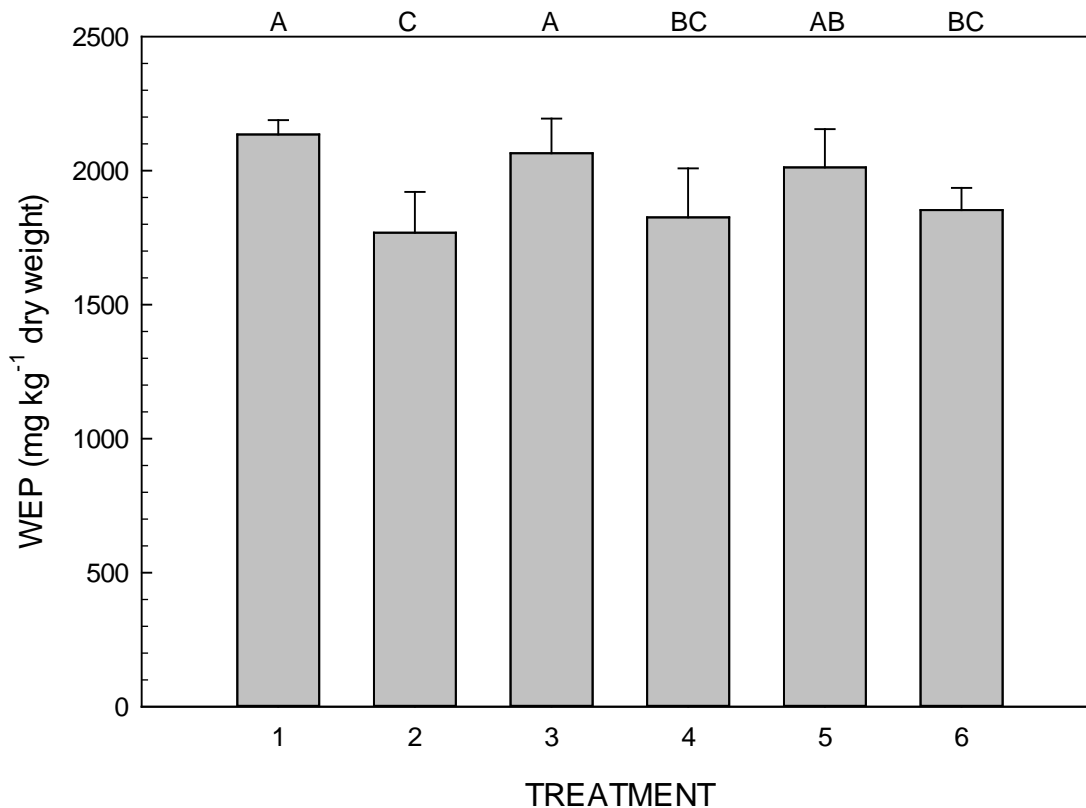


Figure 1. Experiment 1 on the effects of the treatments on water-extractable phosphorus (WEP) in poultry litter, where each chemical was applied at 1% by mass based on the mass of poultry litter as is. [The treatments are: **1** – Control, **2** – aluminum sulfate (alum) 1%, **3** – chitin from shrimp shells 1% (poly(N-acetyl-1,4,beta-D-glucopyranosamine)), **4** – chitosan 1% (poly(D-glucosamine) deacetylated), **5** – chitosan 1% (poly(1,4-beta-D-glucosamine) \geq 75% deacetylated), **6** – chitosan from shrimp shells, practical grade; letters above the bar graph show significant differences (ANOVA, LSD, $P < 0.05$).]

Experiment 2

At the 1% w/w treatment rate, the results were not as predictable as those from Experiment 1 (Figure 2). The WEP content of chitin-treated poultry litter (2689 mg kg⁻¹ dry weight basis) was not significantly different than the control (2382 mg kg⁻¹), and the WEP content in the untreated poultry litter was not significantly different from the three chitosan treatments at 1% (4, 2289 mg kg⁻¹; 5, 2131 mg kg⁻¹; 6, 2106 mg kg⁻¹). However, the three

chitosan treatments had WEP contents which were not significantly different than the 1% alum treated poultry litter (1971 mg kg⁻¹).

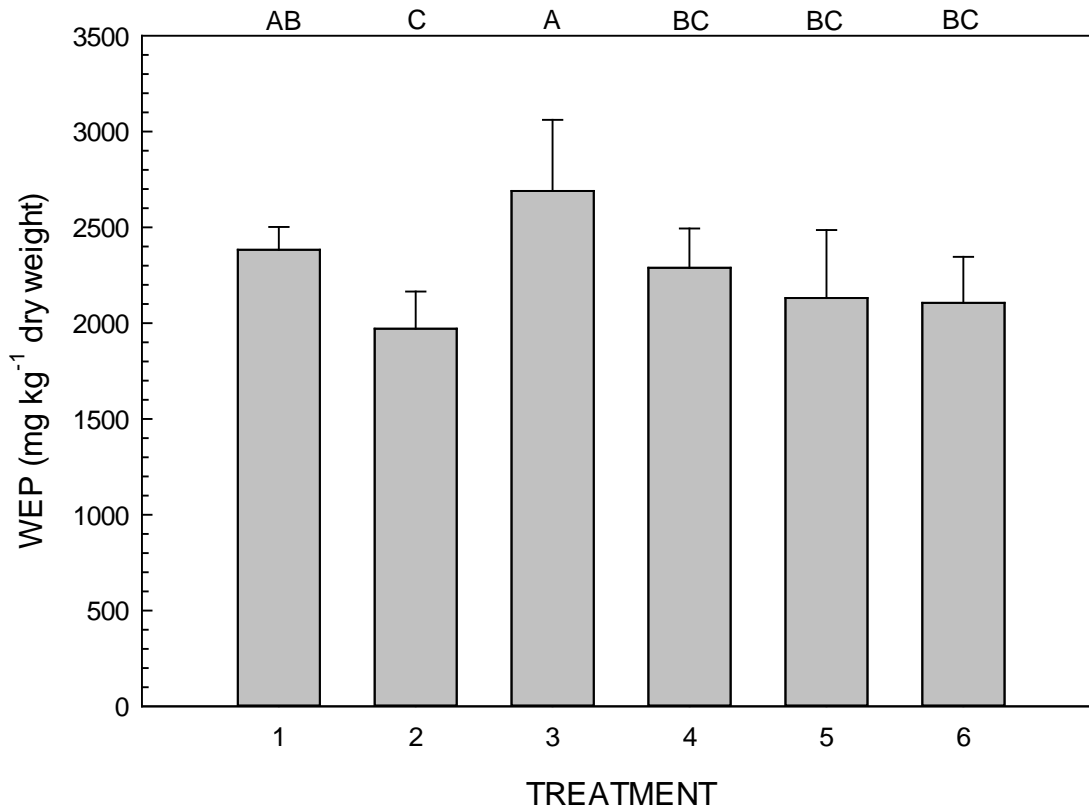


Figure 2. Experiment 2 on the effects of the treatments on water-extractable phosphorus (WEP) in poultry litter, where each chemical was applied at 1% by mass based on the mass of poultry litter as is. [The treatments are: **1** – Control, **2** – aluminum sulfate (alum) 1%, **3** – chitin from shrimp shells 1% (poly(N-acetyl-1,4,beta-D-glucopyranosamine)), **4** – chitosan 1% (poly(D-glucosamine) deacetylated), **5** – chitosan 1% (poly(1,4-beta-D-glucosamine) ≥75% deacetylated), **6** – chitosan from shrimp shells, practical grade 1%; letters above the bar graph show significant differences (ANOVA, LSD, P<0.05).]

Treatments at 5% w/w showed more pronounced WEP trends than those at 1% w/w (Figure 3). There was no significant difference in WEP content (2875 mg kg⁻¹) of chitin-treated poultry litter versus the control samples (2703 mg kg⁻¹). The three varieties of chitosan showed significantly less WEP than control, however, and there was no significant difference in WEP content between the chitosan-treated poultry litters (4, 1629 mg kg⁻¹; 5, 1697 mg kg⁻¹; 6, 1861 mg kg⁻¹) and alum-treated samples (1451 mg kg⁻¹).

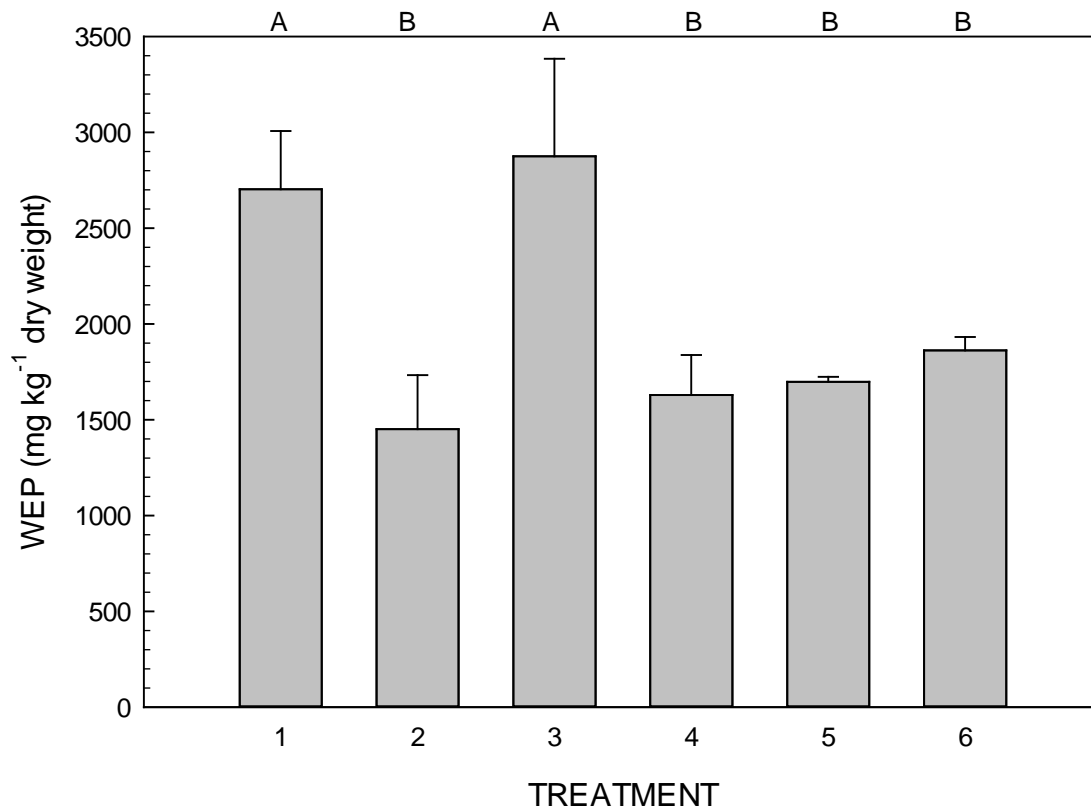


Figure 3. Experiment 2 on the effects of the treatments on water-extractable phosphorus (WEP) in poultry litter, where each chemical was applied at 5% by mass based on the mass of poultry litter as is. [The treatments are: *1* – Control, *2* – aluminum sulfate (alum) 5%, *3* – chitin from shrimp shells 5% (poly(N-acetyl-1,4,beta-D-glucopyranosamine)), *4* – chitosan 5% (poly(D-glucosamine) deacetylated), *5* – chitosan 5% (poly(1,4-beta-D-glucosamine) \geq 75% deacetylated), *6* – chitosan from shrimp shells, practical grade 5%; letters above the bar graph show significant differences (ANOVA, LSD, $P < 0.05$).]

10% w/w treatment results differed from those of 5% and 1% (Figure 4). At this treatment rate, WEP levels (2469 mg kg⁻¹) in chitin-treated litter showed no significant difference from control (2528 mg kg⁻¹) and were significantly greater than WEP levels in alum-treated samples (678 mg kg⁻¹). Poultry litter treated with all three chitosan varieties had WEP contents (4, 1511 mg kg⁻¹; 5, 1366 mg kg⁻¹; 6, 1544 mg kg⁻¹) significantly greater than alum-treated poultry litter but were significantly less than control.

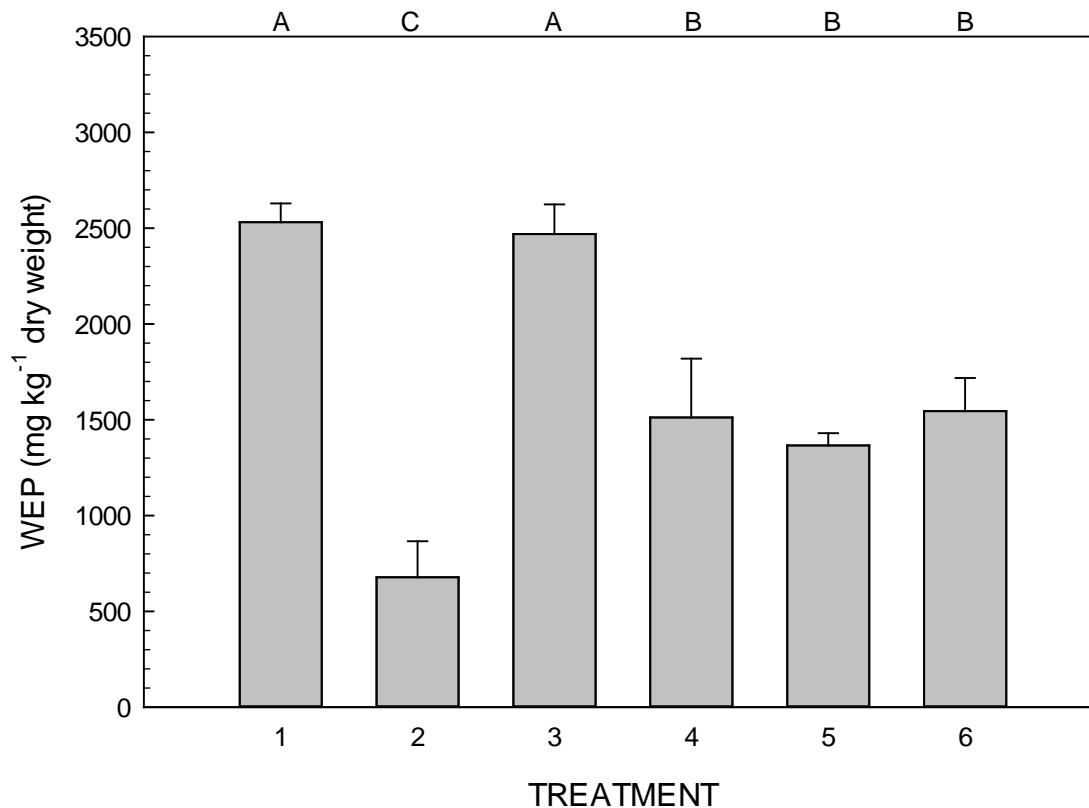


Figure 4. Experiment 2 on the effects of the treatments on water-extractable phosphorus (WEP) in poultry litter, where each chemical was applied at 10% by mass based on the mass of poultry litter as is. [The treatments are: **1** – Control, **2** – aluminum sulfate (alum) 10%, **3** – chitin from shrimp shells 10% (poly(N-acetyl-1,4,beta-D-glucopyranosamine)), **4** – chitosan 10% (poly(D-glucosamine) deacetylated), **5** – chitosan 10% (poly(1,4-beta-D-glucosamine) \geq 75% deacetylated), **6** – chitosan from shrimp shells, practical grade 10%; letters above the bar graph show significant differences (ANOVA, LSD, $P < 0.05$).]

Experiment 3

After week 1, results showed that NH_3 concentrations from the vials in the alum-treated flasks were significantly less than those in the control vials (Table 2). All varieties of chitosan tested showed no significant difference from control except chitosan 6, 10%, which actually had a greater NH_3 concentration than control, unexpectedly. This difference was likely due to one outlier out of the four replicates. All varieties of chitosan had significantly greater vial NH_3 concentrations compared to alum (5% and 10%). Week 2 results showed that vial NH_3

concentrations were not significantly different between any chitosan treatment and control litter. However, chitosan 6, 5% and chitosan 5, 10% both were not significantly different than alum, 5%. But, alum treatment of poultry litter at these rates resulted in the least vial NH₃ concentrations (in general).

Table 2. Mean ammonia (NH₃-N) concentrations in acid trap vials during the 8 week incubation of poultry litter at room temperature.

Treatment	Description	Week 1 (mg L ⁻¹ NH ₃ -N)	Week 2 (mg L ⁻¹ NH ₃ -N)	Week 8 (mg L ⁻¹ NH ₃ -N)
1	Control	23.4 ^{B*}	34.7 ^{A,B}	480.0 ^A
2	Alum, 5%	5.3 ^C	11.3 ^{C,D}	171.3 ^{A,B}
3	Alum, 10%	1.6 ^C	3.9 ^D	65.7 ^B
4	Chitosan 6, 5%	20.5 ^B	24.3 ^{B,C}	249.6 ^{A,B}
5	Chitosan 6, 10%	37.1 ^A	41.9 ^A	370.2 ^{A,B}
6	Chitosan 5, 5%	23.0 ^B	29.6 ^{A,B}	338.9 ^{A,B}
7	Chitosan 5, 10%	20.3 ^B	21.2 ^{B,C}	226.8 ^{A,B}
8	Chitosan 4, 5%	24.8 ^B	30.8 ^{A,B}	453.9 ^A
9	Chitosan 4, 10%	29.3 ^{A,B}	30.4 ^{A,B}	441.1 ^A

*Superscript letters within a column denote significant difference based on means separation using least significant difference (ANOVA, LSD, P<0.05)

The results of weeks 1 and 2 were similar and suggested that chitosan had no effect on NH₃ volatilization from litter, so it was decided the next vial sampling would occur after week 8, a six week incubation. Week 8 results showed no significance between control, alum at 5%, and all varieties of chitosan. Alum at 10% showed a significantly less NH₃ concentration than control and both chitosan 4 treatments.

DISCUSSION

Effect on Water Extractable Phosphorus

The results of the first experiment suggested that at least two varieties of chitosan tested (4 and 6) have a significant effect on WEP content versus control. Even at 1%, less than the 5% extension recommended application rate of alum (Moore, 2011; Penn and Zhang, 2011), these two varieties showed an average of 14.5% and 13.2% decreases in WEP content compared to untreated litter, respectively. These values are not significantly different from the 17.2% decrease versus control observed in alum-treated samples. The promising results from this experiment suggested that chitosan was worth testing at the extension recommendations.

In the subsequent experiment, chitosan-treated samples were not significantly different in WEP content than control at 1% w/w treatment. However, these values were also not significantly different from the WEP content decrease observed by alum-treated samples (17.3%). Although chitosan 6, practical grade, showed the closest performance to alum at this rate, the three grades of chitosan did not perform significantly differently compared to each other. Treatment rates at extension recommendations affected the results dramatically for all three varieties of chitosan and control. Chitosan 4, 5, and 6 showed 39.7%, 37.2%, and 31.2% decreases in WEP content, respectively, compared to untreated litter. Again, all three varieties at 5% w/w performed comparably to each other and alum (46.3% decrease compared to control). At 10%, each chitosan variety was not as effective as alum, but all showed decreased WEP compared to untreated poultry litter.

These results suggested that processed chitin as chitosan, in all three varieties, performs comparably to alum in the chelation of P in poultry litter, especially at 5% w/w treatment. At

10% w/w, however, alum's performance far surpasses that of the varieties of chitosan tested. Thus according to this experiment, 5% w/w is the most favorable treatment rate if chitosan were to succeed alum as an amendment to poultry litter to decrease WEP. Future research should determine if chitosan is a cost-effective management option in poultry production and evaluate how P chelated by chitosan transforms in soils. It is conceivable that chitosan-treated poultry litter could have reduced runoff P when land applied because WEP has been found to control P release during rainfall-runoff studies (Haggard et al., 2005b)

Effect on Ammonia Volatilization

The data from the third experiment showed, because of the significantly reduced NH_3 concentrations in the vials with alum-treated samples, that the experiment performed as expected. It also suggested that chitin and chitosan did not significantly reduce volatilized NH_3 from poultry litter in these lab experiments. These results contradict those of Cook et al. (2011), which suggested that chitosan reduces N loss, whether through NH_3 volatilization or other processes. N loss by Cook et al. (2011) was estimated indirectly by assessing the TN content of the litter samples post-treatment. However chitosan, depending on its deacetylation degree, can range between 5 to 8% TN content (Ravi Kumar, 2000). At the prescribed 10% w/w treatment rate in the Cook et al. (2011) experiment, this could result in up to a 40% increase in the TN content of treated poultry litter assuming untreated litter at 2 to 4% TN content (Wang et al., 2006). Thus, the increase in TN may have been caused solely by the native N in the chitosan amine groups, not chitosan's ability to chelate N and prevent NH_3 volatilization.

The mechanism of most amendments employed to reduce NH_3 release from litter is to acidify the litter matrix, reducing the pH. For example, the addition of alum will reduce the

poultry litter pH, reducing NH_3 volatilization by converting it to non-volatile, water-soluble ammonium (NH_4^+). Chitosan would not reduce pH, so limited effect on NH_3 loss is expected. However, chitosan can easily be dissolved in concentrated acetic acid and may be applied to litter in liquid form. The acid may decrease NH_3 volatilization similarly to other amendments and may also further protonate amine groups of chitosan, which could improve WEP and trace element chelation.

Conclusion

Chitosan, in several variations, does show increased chelation of WEP compared to untreated litter. Chitosan efficacy is a function of the amount of treatment added to litter and its efficacy compared to alum also varies with treatment level. In most instances, however, chitosan's performance was not significantly different from that of alum as a litter amendment. Depending upon future studies into cost-effectiveness and different methods of chitosan application to litter, chitosan may prove to be a viable alternative to alum for litter treatment with respect to WEP chelation. Chitosan's ineffectiveness at decreasing NH_3 volatilization from litter, however, may pose a disadvantage to its use against alum. Future studies may also reveal agricultural benefits of the presumably increased TN content of chitosan-treated litter because of the native N contained in chitosan amine groups.

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