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ABSTRACT

Phosphorus (P) is an important factor in the eutrophication of freshwater, and watershed sources include effluent discharges and the landscape. Poultry litter applied to the landscape can be a potential source of P, which is dependent on rainfall, runoff and dissolution. Chitosan, the deacetylated form of the biopolymer chitin, has been shown to have an effect on reducing water extractable phosphorus (WEP) in poultry litter when applied as a powder. The intent of this study was to measure the effect that poultry litter treatment (PLT), acetic acid and incubation time have on chitosan's ability to reduce WEP in poultry litter. The results were that (1) the presence of PLT in the litter inhibits chitosan's ability to reduce WEP; (2) chitosan dissolved in acetic acid (0.005, 0.01, 0.02, and 0.05 g mL^{-1}) does not decrease WEP at any point during a 7 week incubation period; and (3) chitosan in a powder form reaches its full effectiveness after three weeks of incubation. Chitosan could be an effective coamendment to poultry litter with other treatments in order to reduce WEP.

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MEET THE STUDENT-AUTHOR

Zachary Simpson

I am from Vilonia, Arkansas and graduated from Vilonia High School in 2010. I had an interest in environmental health and sustainability which led to joining the biological engineering program at the University of Arkansas. In May 2014, I graduated with a B.S. in Biological Engineering with a minor in Sustainability. During my time at the university, I was active in the Biological Engineering Student Club and am a member of the American Society of Agricultural and Biological Engineers. I plan to pursue my interests in water quality and watershed management by attending graduate school in Biological Engineering at the University of Arkansas in the fall of 2014.

I would like to thank Dr. Brian Haggard, the project mentor, for making this possible and for his teaching. I would also like to thank Brina Smith, an associate of the Arkansas Water Resources Center, who guided me through all of the laboratory work for this project and provided a great amount of support.

INTRODUCTION

Phosphorus (P) has been a concern for water quality because it is considered to be one of the primary factors limiting algal growth and influencing eutrophication (Parry, 1998; Correll, 1998). The enrichment of freshwaters causes increased primary production (i.e. algal growth), leading to changes in aquatic communities (Smith, 1998; Swingle, 1966), diurnal changes in dissolved oxygen (Alabaster, 1959; Alabaster, 1961; Floyd, 1992), anoxic bottom waters during lake and reservoir stratification (Diaz and Rosenberg, 2008; Floyd, 1992), and even taste and odor issues in drinking water supplies (Walker, 1983). Phosphorus and other nutrients enter freshwaters through defined discharges and diffuse sources from the landscape.

The diffuse sources are transported during rainfall-runoff events from the landscape, including agricultural fields and urban development. The agricultural sources include P stored in soils and what is applied to the landscape in fertilizers and animal manures. In northwest Arkansas, poultry production and application of poultry litter (manure plus bedding) represent an important diffuse source of P in watersheds. Several studies have shown that the water extractable phosphorus (WEP) content of poultry litter is positively correlated to P concentrations in runoff during rainfall simulation studies (Haggard et al., 2005; Kleinman and Sharpley, 2003; Kleinman et al., 2007; Vadas et al., 2004). This relation has prompted research on ways to minimize the WEP content of poultry litter; for example, aluminum sulfate (alum) has been shown to reduce WEP in poultry litter (Dao, 1999) and therefore reduce P concentrations in runoff from field plots (Moore et al., 2000; Shreve et al., 1995; Smith et al., 2001).

A biologically derived coamendment, in the form of chitosan, has also been researched for its ability to reduce WEP in animal manures (Bailey, 2012) among its other uses (Garcia et al., 2009; Kumar and Majeti, 2000; Rabea et al., 2003; Rinaudo, 2006). The preliminary lab studies have shown that WEP in poultry litters was reduced when chitosan was applied at 1-10% rates (as is basis), and chitosan was as effective as alum at the 1-5% application rates (Bailey et al., 2014). To further understand the ability of chitosan to reduce WEP content in poultry litter, the goal of this study is to evaluate factors that alter WEP reduction in poultry litter treated with chitosan. We hypothesized that chitosan delivered in acetic acid solution, which is commonly used to dissolve chitosan in other applications, will produce a significantly greater reduction of WEP content in poultry litter than dry application of chitosan powders. We also hypothesized that there is greater reduction of WEP content in poultry litter as incubation time progresses, especially with the dry application of chitosan powders.

MATERIALS AND METHODS

Poultry litter was collected from the stacking barn and compost at University of Arkansas poultry facilities, which grows birds under contract for Simmons Foods (Siloam Springs, Ark.). These poultry facilities used Poultry Litter Treatment (PLT, sodium bisulfate, $NaHSO_4$) during bird production to reduce ammonia (NH_3) volatilization, and PLT also influences litter chemistry (Pope and Cherry, 2000; Sweeney et al., 1996). In the first experiment using PLT treated litter, a control and four different application rates (percent on dry weight basis) were used for each delivery method, which is delivery as a powder or dissolved in dilute (2%) acetic acid solution. The PLT treated litter was homogenized and divided into 20-g samples (dry weight equivalent), mixed with the treatment, and incubated at room temperature for two weeks. The treatments consisted of a control (untreated), a control treated with only dilute acetic acid, four application rates of ChitoClear® chitosan (90% or more pure) in powder form (i.e., 0.5%, 1.5%, 3%, and 5% dry weight equivalent, g chitosan g^{-1} poultry litter), provided by Dr. Zaharoff, and then chitosan delivered as dissolved in acetic acid (0.05%, 0.1%, 0.2%, and 0.5% dry weight equivalent, g chitosan g^{-1} poultry litter); for each treatment, 4 replicates were used. After incubation, the poultry litter samples were extracted for water extractable phosphorus (WEP) using a 1:100 dry litter-to-water ratio (Kleinman et al., 2007) and then the filtrate was analyzed using the inductively coupled argon plasma optical emission spectrometry (ICP-OES) at the University of Arkansas Soil Diagnostic Lab, Fayetteville, Ark. The WEP_{ICP} content was compared across treatments using analysis of variance (ANOVA) with mean separation (least significant difference, LSD) at $\alpha = 0.05$. The filtrate was also analyzed using the ascorbic acid method for soluble reactive P (SRP) to measure WEP_{SRP}.

In the second experiment, a new source of poultry litter that was not treated with PLT was collected from the University of Arkansas experimental poultry facilities at the Arkansas Agricultural Research and Extension Center in Fayetteville. This litter was handled as previously described in experiment one, and then both litters (PLT and non-PLT amended) were used in the next experiment. Four different types of chitosan (all at 90% or greater purity) were used in this experiment (Table 1), including the one used in first experiment and the same three used in a previous study (Bailey, 2012; Bailey et al., 2014). A control and four treatments (each chitosan form applied at 10% on dry weight basis) were used for each litter source, where the chitosan was applied in powder form not dissolved in dilute acetic acid. Five replicates were used for each control and treatment, where 6-g dry weight equivalent poultry litter was incubated. The treatments were applied; the litter was mixed, incubated for 7 weeks, and then WEP was measured on subsamples after 1, 4, and 7 weeks. After the selected incubation time, up to 2 g (dry weight) of the samples were extracted to measure WEP (Kleinman et al., 2007) as modified in the first experiment. The WEP solutions were filtered using a Whatman-40 filter (GE Healthcare Life Sciences, Pittsburgh, Pa.) via gravity filtration (primary filtration) and the filtrate as analyzed for SRP using the modified ascorbic acid reduction method, which is analogous to WEP_{SRP}. Analysis of variance with mean separation (LSD) at $\alpha = 0.05$ was used to compare treatments. Calculation of WEP_{SRP} removed across all chitosan treatments compared to the control was also done for each incubation period.

In the third experiment, only the non-PLT litter source was used based on the results from experiments one and two. Approximately, 8 g of poultry litter (dry weight equivalent) were separated into containers. This experiment featured the following treatments: a control, a control with just acetic acid (approximately 0.8 mL), 10% (dry weight basis) chitosan in powder form, and varying application rates of chitosan delivered in a dilute acetic acid solution (i.e., 0.05%, 0.10%, 0.20%, and 0.50% chitosan on a dry weight basis, g chitosan g^{-1} poultry litter). The chitosan used was the medium molecular weight chitosan, and incubation times were set ranging from 1 to 7 weeks for all treatments. The treatments were sampled at the selected incubation times, and then extracted following the same process as in experiment two and analyzed for WEP_{SRP}. The same statistical analysis as for the previous two experiments was repeated in the third experiment to compare treatment means.

Table 1. A list of chitosan types used in experiment 2.

Number	Type of Chitosan
1	ChitoClear®, provided by Dr. Zaharoff.
2	≥75% deacetylated chitosan†
3	Practical grade chitosan‡
4	Medium molecular weight chitosan§

† Sigma-Aldrich, C3646-25G.
‡ Sigma-Aldrich, 417963-25G.
§ Sigma-Aldrich, 448877-50G.

RESULTS AND DISCUSSION

Experiment 1. The results from the first experiment were unexpected since the WEP_{ICP} content of the poultry litter samples treated with chitosan in powder form and the samples treated with chitosan dissolved in acetic acid were not significantly different from the control (3942 mg kg^{-1}, Table 2). The PLT litter treated with 0.20 (dry weight basis) chitosan dissolved in acetic acid had WEP_{ICP} content (3986 mg kg^{-1}) numerically greater than the control. The PLT litter treated with 0.50% (dry weight basis) chitosan dissolved in acetic acid had WEP_{ICP} content (4143 mg kg^{-1}) numerically greater than the control and was significantly different from WEP_{ICP} content of some of the other chitosan treatments. These results were contrary to the observations made in previous studies (Bailey, 2012; Bailey et al., 2014), which showed that chitosan applied to poultry litter in powder form significantly reduced WEP_{ICP} content.

The first experiment was conducted to follow Bailey (2012), where WEP was measured using ICP-OES at the University of Arkansas System Division of Agriculture Soil Diagnostic Lab (i.e., following Kleinman et al., 2007). However, the filtrate was also analyzed for SRP using a colorimetric method, which is designated as WEP_{SRP}. These two methods differ, where WEP_{ICP} represents the total P measured in the filtrate whereas WEP_{SRP} represents the reactive P measured in the filtrate. However, analysis of the same samples using both analytical methods showed a significant, positive correlation between WEP_{ICP} and WEP_{SRP} (Fig. 1). Since both analyses were comparable and SRP analysis was more practical in the laboratory, SRP using spectrometry analysis was used for the rest of the experiments.

Experiment 2. Since the first experiment showed such unexpected results, several factors were called into question: the source of the poultry litter, the source of chitosan used, and also the length of the incubation. Experiment 1 used poultry litter that had been treated with PLT, or sodium bisulfate ($NaHSO_4$), and is used in commercial poultry production to reduce ammonia volatilization. The bisulfate, HSO_4^-, reduces litter pH which reduces ammonia volatilization and therefore improves bird health (Sweeney et al., 1996). This chemical amendment was suspected to have an effect on chitosan's ability to reduce WEP in the litter. In order to examine its effect, a new source of poultry litter that had not been treated with PLT was obtained for the second experiment.

To test whether the source of chitosan played a role in the first experiment's results, three new sources of chitosan, all used by Bailey (2012), and the source of chitosan in the first experiment were included in the second experiment. The second experiment tested the new sources of chitosan and the original source on both sources of poultry litter (PLT and non-PLT treated) at a rate of 10% (dry weight basis), which was within the range of treatment shown to be effective at reducing WEP_{ICP} (see also Bailey et al., 2014).

For the poultry litter that had been treated with PLT, the results after a 4 week incubation showed that WEP_{SRP} of PLT litter treated by all sources of chitosan were not significantly different than WEP_{SRP} of the control (4172 mg kg^{-1}, Table 3). The samples treated with chitosan had numerically greater amounts of WEP_{SRP} than that of the

Table 2. Water extractable phosphorus (WEP_{ICP}) in poultry litter amended with Poultry Litter Treatment (PLT) after mixing with chitosan delivered as powder or dissolved in acetic acid (n = 4) and incubated at room temperature for two weeks (Experiment 1).

	WEP_{ICP} (mg kg^{-1} dry litter)		
Treatment	Mean	Standard Deviation	Homogeneous Groups[†]
Control	3942	247	AB
AA Control[‡]	3769	77	B
0.5% Powder[§]	3774	32	B
1.5% Powder	3867	95	B
3.0% Powder	3869	244	B
5.0% Powder	3904	167	AB
0.05% Dissolved[¶]	3761	210	B
0.10% Dissolved	3859	165	B
0.20% Dissolved	3986	90	AB
0.50% Dissolved	4143	245	A

[†] Homogenous groups based on means separation using least significant difference, ($\alpha = 0.05$).

[‡] AA designates acetic acid, where this treatment received the same volume of AA without chitosan.

[§] Chitosan applied as a dry powder.

[¶] Chitosan applied dissolved in acetic acid.

control samples. These results showed that none of the sources of chitosan that had been shown to reduce WEP by Bailey (2012) were able to have a similar effect on the litter treated with PLT. This suggests that chitosan is not effective at reducing WEP, when poultry litter is treated with PLT. The addition of PLT to litter adds an excess of sulfate (SO_4^{2-}) ions, which might inhibit chitosan's ability to remove P from solution and reduce WEP due to the competition with phosphate as the anion with which to form electrostatic complexes (Rinaudo, 2006). The results for the poultry litter not treated with PLT were much different. The WEP_{SRP} content of the control non-PLT litter (4448 mg kg^{-1}) was significantly greater than the WEP_{SRP} content of the four chitosan treatments, and the chitosan treatments were not statistically different. These results match with the results seen by Bailey (2012), which showed that WEP_{ICP} was significantly reduced by chitosan application. These results showed that the chitosan source used in the first experimentwas not the factor that resulted in the lack of WEP_{SRP} reduction.

Experiment 2 also showed that incubation time has an effect on chitosan's ability to reduce to WEP_{SRP}. Subsamples from the non-PLT litter source were extracted after 1, 4, and 7 weeks of incubation. The amount of WEP_{SRP}

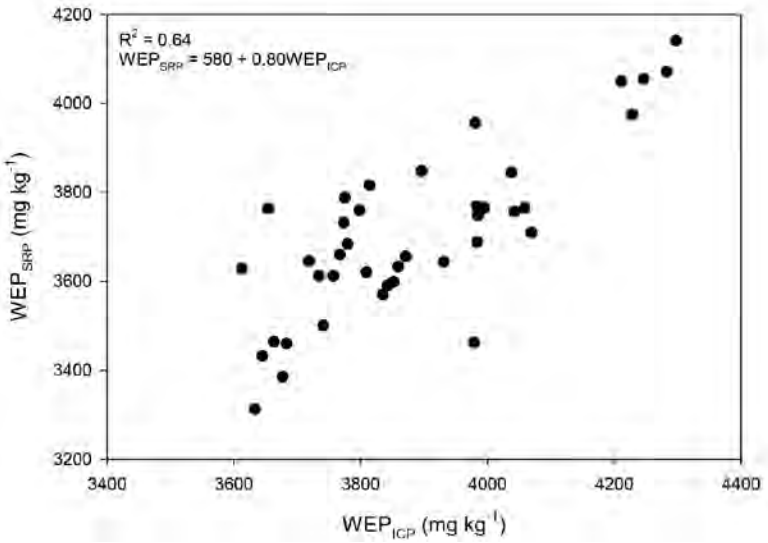

Fig. 1. Comparison of water extractable phosphorus (WEP) content by spectrometry (WEP_{SRP}) and by ICP-OES (WEP_{ICP}) for samples from experiment 1.

Table 3. Water Extractable Phosphorus (WEP_{SRP}) from two sources of poultry litter treatment with various sources of chitosan at a 10% dry weight basis application rate (Experiment 2) following a four week incubation.

			WEP_{SRP} (mg kg^{-1} dry litter)		
Application Rate	Litter Source	Chitosan Source[†]	Mean	Standard Deviation	Homogeneous Groups[‡]
	PLT[§]		4172	393	A
10%	PLT	1	4527	385	A
10%	PLT	2	4466	378	A
10%	PLT	3	4559	170	A
10%	PLT	4	4566	408	A
	Non-PLT[¶]		4448	70	A
10%	Non-PLT	1	3833	68	B
10%	Non-PLT	2	3830	67	B
10%	Non-PLT	3	3841	81	B
10%	Non-PLT	4	3918	42	B

[†] Refer to Table 1 for description of chitosan source.

[‡] Homogenous groups, based on means separation with least significant difference (α = 0.05) within a litter source.

[§] Poultry litter that has been treated with Poultry Litter Treatment (PLT).

[¶] Poultry litter that has not been treated with PLT.

removed across all chitosan treatments compared to the control is illustrated in Fig. 2. While chitosan had some effectiveness after 1 week of incubation, its performance appeared to peak after 4 weeks of incubation and remained about the same for the rest of its incubation. The experiments performed by Bailey (2012) used incubation times that exceeded 4 weeks, based on the time incubated in the lab and then analyzed at the Soil Diagnostic Lab for WEP_{ICP}. So, it can be concluded from experiment 2 that chitosan reduced WEP in poultry litters not treated with PLT and that it needs to be mixed with litter for 4 weeks to maximize the reduction.

Experiment 3. Having determined that the treatment of PLT to poultry litter has an effect on chitosan's ability to reduce WEP_{SRP} in the second experiment, the third experiment was a modified version of the first experiment that excluded the presence of PLT. The source of the litter used was the non-PLT litter from the second experiment. This allowed us to investigate the effect that dissolving chitosan into acetic acid has on its ability to reduce WEP. Since the second experiment showed that the sources of chitosan used did not produce significantly different results, which source of chitosan to use was not heavily considered.

After one week of incubation, the results showed that the chitosan powder (4354 mg kg^{-1}, Table 4) was the only treatment to reduce WEP_{SRP} in comparison to the control (4586 mg kg^{-1}); WEP_{SRP} content in the litter treated with chitosan powder was significantly different from the control, but it was applied at a rate an order of magnitude greater than the chitosan dissolved in acetic acid. Since this experiment (and the first experiment) intended to

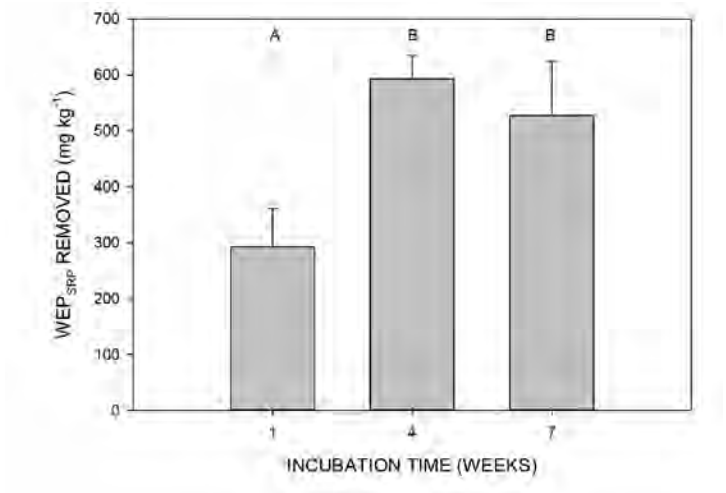

Fig. 2. Comparison of removal ability of WEP_{SRP} for all chitosan treatments compared to the control after various incubation times for experiment 2.

Table 4. Water Extractable Phosphorus (WEP_{SRP}) from poultry litter (without PLT) treated with chitosan as a powder or dissolved in acetic acid (Experiment 3) and incubated at room temperature for 1 week.

	WEP_{SRP} (mg kg^{-1} dry litter)		
Treatment[†]	Mean	Standard Deviation	Homogeneous Groups[‡]
Control	4596	215	B
AA Control[§]	4730	91	AB
10% powder	4354	213	C
0.05% Dissolved[¶]	4895	146	A
0.10% Dissolved	4796	191	AB
0.20% Dissolved	4848	87	A
0.50% Dissolved	4840	159	A

[†] Chitosan used is 4 in Table 1.

[‡] Homogenous groups based on means separation using least significant difference (α = 0.05).

[§] AA designates acetic acid, where this treatment received the same volume of AA without chitosan.

[¶] Chitosan applied dissolved in acetic acid.

use a constant and reasonable amount of acetic acid (1 mL of acetic acid per 10 g of litter) for the chitosan dissolved in acetic acid treatments, the highest concentration that was possible was the 0.50% (dry weight equivalent) treatment. At this concentration, the solution becomes very viscous and especially difficult to dissolve more chitosan into the dilute acetic acid. To apply more chitosan to the litter like the 10% dry weight equivalent powder treatment, the amount of acetic acid added would increase as well to levels that would likely not reflect a reasonable real-world application.

The four chitosan dissolved in acetic acid treatments (0.05%, 4895 mg kg^{-1}; 0.10%, 4796 mg kg^{-1}; 0.20%, 4848 mg kg^{-1}; 0.50%, 4840 mg kg^{-1}) all had WEP_{SRP} contents numerically greater than the control, and only the WEP_{SRP} content of the 0.10% treatment was significantly not different from the control. Interestingly, the control with just acetic acid applied (4730 mg kg^{-1}) was also numerically greater than the control, but not significantly different. The expectation was that dissolving chitosan into dilute acetic acid would increase the effectiveness, or the reduction in WEP. However, the use of acetic acid in poultry litter treatment likely increases WEP. It is also impractical to apply chitosan dissolved in acetic acid at rates equivalent to dry application, because of the volume of acetic acid required.

Three weeks of incubation had results with the same trend as discussed above (Table 5). The 10% powder treatment (4372 mg kg^{-1}) had the least WEP_{SRP} content and was significantly different from all of the treatments. The next lowest WEP_{SRP} content was found in the control (4757 mg kg^{-1}). Of the treatments that involved acetic acid, only the 0.50% chitosan dissolved in acetic acid (4993 mg kg^{-1}) was significantly not different than the control. The 0.50% treatment was also the only one that was significantly different from the control with acetic acid (5334 mg kg^{-1}). The other three chitosan dissolved in acetic acid treatments (0.05%, 5171 mg kg^{-1}; 0.10%, 5306 mg kg^{-1}; 0.20%, 5202 mg kg^{-1}) were not significantly different from the acetic acid control nor the 0.50% treatment. It is possible that the dilute acetic acid might hydrolyze bound P in the litter, resulting in the increase in WEP_{SRP}.

Seven weeks of incubation gave results that differ slightly than the previous incubations (Table 6). The control and the 10% powder treatment (4972 and 4764 mg kg^{-1}, respectively) were not statistically different from each other. This contradicts what was shown in the previous two sets of extractions, where chitosan powder appeared to reduce WEP_{SRP} in comparison to the control. The acetic acid control (5353 mg kg^{-1}) and the other chitosan dissolved in acetic acid treatments (0.05%, 5324 mg kg^{-1}; 0.10%, 5502 mg kg^{-1}; 0.20%, 5342 mg kg^{-1}; 0.50%, 5404 mg kg^{-1}) were not statistically different from each other but were statistically greater in WEP_{SRP} than the control and chitosan powder treatment.

Since the original results after seven weeks of incubation were unexpected with respect to the control and chitosan powder treatment, the data was closely investigated. The control and the powder treatment had one WEP value that was a possible outlier, where it was much lower in the control and then much greater in the powder treatment. Removing the possible outlier from among the powder treatments is supported by the observation that the treatment had visibly less chitosan powder. The alternative results show that, as was predicted, the chitosan powder treatment (4656 mg kg^{-1}) was significantly less in WEP_{SRP} than all other treatments including the control (5076 mg kg^{-1}). This was consistent with the previous extractions in the third experiment, and it also supported that observed in the previous studies on chitosan (Bailey et al., 2014).

Table 5. Water Extractable Phosphorus (WEP_{SRP}) from poultry litter (without PLT) treated with chitosan as a powder or dissolved in acetic acid (Experiment 3) and incubated at room temperature for 3 weeks.

	WEP_{SRP} (mg kg^{-1} dry litter)		
Treatment[†]	Mean	Standard Deviation	Homogeneous Groups[‡]
Control	4757	66	C
AA Control[§]	5334	280	A
10% powder	4372	277	D
0.05% Dissolved[¶]	5171	113	AB
0.10% Dissolved	5306	191	A
0.20% Dissolved	5202	200	AB
0.50% Dissolved	4993	281	BC

[†] Chitosan used is 4 in Table 1.

[‡] Homogenous groups based on means separation using least significant difference (α = 0.05).

[§] AA designates acetic acid, where this treatment received the same volume of AA without chitosan.

[¶] Chitosan applied dissolved in acetic acid.

These results are evidence against the hypothesis that chitosan in acetic acid at a practical application rate would have a greater effect on the reduction of WEP_{SRP} in poultry litter. The presence of acetic acid appears to possibly increase WEP_{SRP}. The highest chitosan dissolved in acetic acid treatment (0.50%) almost appeared to have the desired effect at 3 weeks of incubation, however, the control remained statistically less than the treatment at 7 weeks of incubation. The results of the chitosan powder treatment resemble that of the second experiment; chitosan powder has a peak effectiveness on reducing WEP_{SRP} after 3 weeks. Thus, it does not seem beneficial to dissolve chitosan into acetic acid at these low treatment levels when applying to poultry litter. However, acetic acid would likely reduce litter pH and therefore inhibit ammonia volatilization but it would possibly increase WEP and the potential loss of P during rainfall runoff events.

CONCLUSION

Chitosan's ability to reduce WEP was inhibited by the presence of PLT in the poultry litter. The source of poultry litter must be untreated with PLT in order for chitosan to have its desired effect, i.e. reduce WEP. Application of chitosan dissolved in acetic acid (0.05%, 0.10%, 0.20% and 0.50% dry weight basis, g chitosan g^{-1} poultry litter) was ineffective and the presence of acetic acid alone potentially increases WEP. The time of incubation did have an effect on the reduction of WEP, suggesting chitosan's effectiveness peaks after 3 weeks of incubation. Future studies may find alternative methods of applying chitosan to poultry litter to improve effectiveness, such as using a different acid solution in place of acetic acid. Furthermore, the next step needs to be applying poultry litter treated with chitosan to field plots where rainfall simulation studies can be used to evaluate P transport in runoff waters.

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Table 6. Water Extractable Phosphorus (WEP_{SRP}) from poultry litter (without PLT) treated with chitosan as a powder or dissolved in acetic acid (Experiment 3) and incubated at room temperature for 7 weeks.

	WEP_{SRP} (mg kg^{-1} dry litter)		
Treatment[†]	Mean	Standard Deviation	Homogeneous Groups[‡]
Control	4972	268	B
AA Control[§]	5353	230	A
10% powder	4764	285	B
0.05% Dissolved[¶]	5324	388	A
0.10% Dissolved	5502	166	A
0.20% Dissolved	5342	65	A
0.50% Dissolved	5404	182	A

[†] Chitosan used is 4 in Table 1.

[‡] Homogenous groups based on means separation using least significant difference (α = 0.05).

[§] AA designates acetic acid, where this treatment received the same volume of AA without chitosan.

[¶] Chitosan applied dissolved in acetic acid.

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