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Evaluation of nitrogen retention and microbial populations in poultry litter treated with chemical, biological or adsorbent amendments

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ABSTRACT

Poultry litter is a valuable nutrient source for crop production. Successful management to reduce ammonia and its harmful side-effects on poultry and the environment can be aided by the use of litter amendments. In this study, three acidifiers, two biological treatments, one chemical urease inhibitor and two adsorber amendments were added to poultry litter. Chemical, physical and microbiological properties of the litters were assessed at the beginning and the end of the experiment. Application of litter amendments consistently reduced organic N loss (0–15%) as compared to unamended litter (20%). Acidifiers reduced nitrogen loss through both chemical and microbiological processes. Adsorbent amendments (water treatment residuals and chitosan) reduced nitrogen loss and concentrations of ammonia-producing bacteria and fungi. The use of efficient, cost-effective litter amendments to maximum agronomic, environmental and financial benefits is essential for the future of sustainable poultry production.

1. Introduction

Poultry litter (a mixture of manure and bedding materials) is a valuable nutrient source for plant growth that contains high levels of phosphorous (P), potassium (K), nitrogen (N) and other trace minerals (Kelleher et al., 2002). Sustainable use of this commodity has been impeded by the industrialization of livestock production and the consequential replacement and/or supplementation of manure application with chemical fertilizers. However, current environmental (emissions regulations) and financial (high price of chemical fertilizers; low profit margins) concerns make implementation of improved animal manure and litter management methodologies a priority. A more holistic approach to poultry production would emphasize litter management to improve animal health, retain fertilizer value and to optimize recycling of minerals back to the soil (returning around 70% of the feed minerals) (Martinez et al., 2009; McCrory and Hobbs, 2001).

A major issue with poultry litter is the loss of N as ammonia (NH_3) due to microbial mineralization of urea and uric acid, which represent up to 80% of the total N in litter (Kelleher et al., 2002; Ritz et al., 2004). Ammonia levels in poultry houses have increased due to tighter house designs and infrequent litter removal. Elevated NH_3 can cause health issues with the birds (i.e., damage to the respiratory tract, increased susceptibility to disease) and has been shown to reduce bird weight and feed efficiency (Moore et al., 1999, 2000; Ritz et al., 2004). Additionally, NH_3 volatilization releases the malodorous and environmentally damaging gas into the atmosphere and reduces the value of poultry litter as a fertilizer due to N loss (McCrory and Hobbs, 2001).

An array of poultry litter treatments has been used to reduce NH_3 emissions including physical, chemical and/or biological methods. Physical treatments include biofilters, scrubbers, moisture and ventilation control. The most common practice for removing NH_3 from broiler facilities is by ventilation that exhausts to the atmosphere (Ritz et al., 2004). This practice has negative consequences including increasing the contribution of poultry facilities to atmospheric pollution and environmental deposition which results in acidification of soils and water sources (McCrory and Hobbs, 2001; Shah et al., 2007). Current methods to treat the air leaving the house are also expensive and do not address the effects of gas build-up within the house.

Acidifier amendments have been studied extensively for NH_3 reduction and have provided the most promising and consistent results. The most common litter amendments are dry acids including Al+Clear® ($Al_2(SO_4)_3 \cdot 14H_2O$), Poultry Litter Treatment®

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(93% $NaHSO_4$) and Poultry Guard® (clay soaked in 36% H_2SO_4). These compounds reduce NH_3 volatilization by lowering the pH of the litter and converting volatile NH_3 into non-volatile ammonium (NH_4^+). Acidifiers have also been shown to improve bird health and productivity (McWard and Taylor, 2000; Moore et al., 2000; Terzich et al., 1998), reduce pathogen persistence (Line and Bailey, 2006; Pope and Cherry, 2000; Rothrock et al., 2008b) and reduce metals and chemical run-off (Moore et al., 1998; Warren et al., 2006).

Recent research has shown that in addition to chemical inhibition of NH_3 by lowering pH, acidification also reduces populations of bacteria with genes like urease and uricase (Cook et al., 2008; Rothrock et al., 2010). Given the importance of microbial enzymes to NH_3 production, microbial inhibition by acidifiers likely contributes significantly to the success of these amendments. A result of acidification, however, is a 3–4 order of magnitude increase in the concentration of fungal urease and uricase producers (Cook et al., 2008; Rothrock et al., 2010). Rothrock et al. (2010) demonstrated that this shift from bacterial to fungal urease producers correlated with organic nitrogen mineralization. Therefore, fungi may be responsible for the delayed but predictable nitrogen mineralization that occurs in poultry houses several weeks after application of acidifier amendments. Delaying or reducing the onset of fungal growth may extend the effective duration of acidifiers thereby further reducing organic nitrogen mineralization and increasing litter nitrogen content.

Biological treatments include blends of enzymes, enzyme inhibitors, nutrients (i.e., fructooligosaccharides) and/or microorganisms. Augmenting water, feed or litter with biologicals enhances or alters the native microbial population with the goal of increasing NH_3 degradation rates, preventing the formation of toxic compounds, improving feed efficiency and/or competitively excluding undesirable microorganisms (Cole et al., 2006; Patterson and Burkholder, 2003; Shah et al., 2007). Researchers have had success when using biological treatment to inhibit pathogens (Cole et al., 2006) and reduce the effects of fungal toxins (Awad et al., 2006). Those that have evaluated biologicals for reduction of ammonia volatilization from poultry litter have met with mixed success (DeLaune et al., 2004; Shah et al., 2007).

Adsorbents including water treatment residuals (WTR) and chitosan may serve as cost-effective litter treatments. WTR are Fe, Al, or Ca salts added to raw water to remove colloids, sediment and contaminants (Makris et al., 2010). This material is generated in large quantities by most municipalities and can be readily obtained at a minimal cost (Hovsepyan and Bonzongo, 2009). WTR have been studied extensively in recent years and have been shown to have the capacity to bind heavy metals (including As, Pb, Hg, Cd, and Zn) (Brown et al., 2005; Hovsepyan and Bonzongo, 2009; Nagar et al., 2009). Aluminum hydroxide containing WTR were previously shown to effectively bind phosphorous in poultry litter and were suggested as a low cost alternative to alum amendment (Makris et al., 2005).

Chitosan is a derivative of chitin one of the most abundant sources of carbon on earth. Chitosan is produced by alkaline *N*-deacetylation of chitin and garners its ability as an adsorbent due its poly-cationic nature (Babel and Kurniawan, 2003; Vold et al., 2003). The properties of chitosan can be manipulated by chemical modification to increase acid stability, mechanical strength and adsorption capacity (Chatterjee et al., 2009). It has also been shown to have anti-microbial properties, supposedly due to adsorptive properties and enzyme inhibition (Badaway and Rabea, 2009; Lin et al., 2009). Chitosan is also of interest to industry due to its ability to scavenge heavy metals, including Hg^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+}.

The goal of this study was evaluate poultry litter amendments for their ability to reduce total and organic N loss through chemical and microbiological processes.

Better understanding litter amendments that are currently used and evaluation of novel cost-effective, environmentally conscience amendments will provide new information important to sustainable, value-added poultry production.

2. Materials and methods

2.1. Litter collection, litter amendments and sampling

Poultry litter (sawdust bedding) was collected from random locations inside a commercial broiler (*Gallus gallus domesticus*) house in KY prior to placement of the fifth flock. The physiochemical characteristics of the litter are shown in Table 1. After collection, the litter was thoroughly mixed prior to adding 350 g into triplicate plastic incubation containers (21.8 × 16.3 × 13.2 cm) for each treatment.

Acidifiers, biological treatments, chemical inhibitors and adsorber amendments were added on day 0 prior to incubation (Table 2). The three acidifier treatments were added to litter at 10% w/w (35 g). Acidifiers included: Al+Clear® (AC; General Chemical, Parsippany NJ), Poultry Litter Treatment® (PLT; Jones-Hamilton Co., Walbridge, OH), and Poultry Guard® (PG; Wynco Distributors, LLC, Fruitland, MD). To control the fungal bloom in acidified litter, liquid Clinafarm®EC (CL, Janssen Animal Health, Union, NJ) was added into triplicate incubation containers in addition to 10% AC; CL treatment was re-applied on day 15 and on day 30, according to manufacturers specifications. The biological treatment (BT; Vytol BioSystems, Inc., West Point, NE) containing a proprietary blend of bacteria, enzymes and nutrients (10 mg dry powder) was mixed into the litter matrix. The commercial urease inhibitor, Agrotain Plus® (AP; Corydon, KY) was added to the litter as directed by the manufacturer (3.5 mL of a 1:20 solution) and was re-applied on day 15 and day 30. Two novel treatments, wastewater treatment residual (WTR; Bowling Green, KY) and Chitosan (CH; Sigma-Aldrich, St. Louis, MO) were added 10% w/w to the litter matrix.

In all treatments, the moisture content was raised to 538.5 g H_2O kg^{-1} of dry mass (35% w/w) using deionized water (initial litter moisture content was 271.5 g H_2O kg^{-1} of dry mass (23% w/w)) (Cook et al., 2008). All containers were then vigorously shaken to ensure a homogenous mixture of poultry litter and amendments. A 25 g sub-sample was removed from each container on day 0 and the containers were stored in the dark at room temperature. Whenever liquid amendments were re-applied, an equal volume of deionized water was added to the remaining treatments. At the end of the study (56 d), containers were vigorously shaken and 10 subsamples were pooled to obtain a homogenous, representative 25 g sample from each container. A 23 g sub-sample was stored at 4 °C pending physiochemical analysis, while the remaining 2 g subsamples was frozen (−20 °C) pending molecular microbial analysis.

2.2. Physiochemical analysis of the poultry litter

Moisture was determined by drying the litter at 65 °C overnight and comparing the weight before and after drying. Litter pH was determined using a combination electrode (Fisher Scientific, Hampton, NH) at a 5:1 deionized water to litter ratio. Total N and C were determined by combustion (Watson and Wolfe, 2003) of the litter using a Vario Max CN analyzer (Elementar Americas, Inc. Mt. Laurel, NJ). The NH_4^+–N content of litter was determined after a 1:60 litter to KCl (2 M) extraction (Peters and Wolfe, 2003) followed by flow injection analysis (FIA) using the Quickchem FIA+, method # 12-107-06-2-A (Lachat Instruments, Milwaukee, WI). The NO_3–N content was also assessed after KCl extraction using Quickchem FIA+, method # 12-107-04-1-B (Lachat Instruments, Milwaukee, WI). Organic N was estimated by subtracting the

Table 1
Physiochemical properties of amended poultry litters.

Amendment	Total Mass Amendment Loss (%)[a]	pH		Total C	Organic N (g kg^{-1})	Total N (g kg^{-1})		Organic N (g kg^{-1})		NH_4–N (g kg^{-1})		NO_3–N (g kg^{-1})	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Unamended (N)	13.54 ± 6.13	8.20 ± 0.03	8.57 ± 0.07	415.5 ± 11.9	392.1 ± 11.6	43.3 ± 3.5	42.8 ± 4.7	38.9 ± 3.4	35.8 ± 4.6	4.2 ± 0.3	6.8 ± 0.3	0.22 ± 0.11	0.26 ± 0.20
Al+Clear® (AC)	26.94 ± 2.98	4.31 ± 0.18	6.52 ± 0.25	371.0 ± 3.6	365.1 ± 12.9	40.3 ± 0.8	59.9 ± 1.6	35.3 ± 0.9	51.0 ± 0.8	4.4 ± 0.1	8.6 ± 0.7	0.19 ± 0.17	0.26 ± 0.12
PLT® (PLT)	10.85 ± 4.27	3.33 ± 0.18	5.24 ± 0.11	355 ± 11.5	345.0 ± 5.6	37.7 ± 1.7	42.7 ± 0.2	32.9 ± 1.9	37.3 ± 0.4	4.6 ± 0.5	5.2 ± 0.2	0.19 ± 0.06	0.25 ± 0.044
Poultry Guard® (PG)	18.09 ± 0.63	3.95 ± 0.28	6.34 ± 0.10	388.9 ± 18.6	300.2 ± 1.1	42.8 ± 2.6	51.4 ± 0.8	38.0 ± 2.7	42.6 ± 0.3	4.4 ± 0.1	8.6 ± 0.6	0.30 ± 0.09	0.26 ± 0.27
Al+Clear & Clinafarm® EC (CL)	24.35 ± 2.29	4.42 ± 0.22	5.94 ± 0.35	384.1 ± 10.3	363.2 ± 8.2	40.1 ± 1.0	57.4 ± 2.4	35.1 ± 0.4	50.4 ± 1.1	4.7 ± 0.7	6.8 ± 1.7	0.27 ± 0.03	0.29 ± 0.23
Agrotain Plus® (AP)	10.56 ± 1.43	8.26 ± 0.03	8.64 ± 0.04	414.9 ± 5.6	417.3 ± 16.5	42.3 ± 1.4	44.8 ± 2.2	37.7 ± 1.7	37.2 ± 2.1	4.3 ± 0.4	7.2 ± 0.1	0.34 ± 0.03	0.40 ± 0.05
Biological Treatment (BT)	8.65 ± 0.70	8.17 ± 0.03	8.92 ± 0.01	413.0 ± 8.9	400.1 ± 6.1	43.0 ± 2.1	43.2 ± 1.9	39.3 ± 2.1	36.2 ± 2.2	3.4 ± 0.2	6.6 ± 0.4	0.26 ± 0.01	0.33 ± 0.62
Chitosan (CH)	9.33 ± 0.91	7.84 ± 0.01	8.05 ± 0.13	422.8 ± 7.9	366.9 ± 36.8	37.6 ± 1.7	41.9 ± 4.7	31.4 ± 0.1	35.6 ± 0.3	3.8 ± 0.5	3.4 ± 0.7	2.42 ± 2.75	2.96 ± 0.84
Water Treatment Residuals (WTR)	7.99 ± 0.66	7.97 ± 0.04	8.20 ± 0.06	395.0 ± 5.8	346.8 ± 25.0	35.9 ± 0.4	37.4 ± 1.0	30.1 ± 0.0	29.2 ± 0.1	3.4 ± 0.1	4.6 ± 0.8	2.35 ± 0.04	3.53 ± 0.50

Values represent the average ± standard deviation of triplicate samples.
[a] Total mass lost as a percent of the original mass.

Table 2
Amendments evaluated for physical and biological influence on poultry litter.

Amendment	Type	Composition	Action
Unamended (N)	None	N/A	N/A
Al+Clear® (AC)	Acidifier	Aluminum sulfate $[Al_2(SO_4)_3 \bullet 14H_20]$	Reduce pH below 7
PLT® (PLT)	Acidifier	Sodium Bisulfate ($NaHSO_4$)	Reduce pH below 7
Poultry Guard® (PG)	Acidifier	Acidified Clay (36% H_2SO_4)	Reduce pH below 7
Al+Clear & Clinafarm® EC (CL)	Acidifier & Anti-fungal	Aluminum sulfate & Clinafarm® (Enilconazole)	Enilconazole prevents the formation of ergosterol in fungal cell walls
Agrotain Plus® (AP)	Chemical	N-(n-butyl) thiophosphate triamide (NBPT) & dicyandiamide	Urease inhibitor; Retards Nitrification
Biological Treatment (BT)	Biological/ Enzymatic	Bacteria, enzymes and nutrients	Bioaugmentation-Proprietary mixture of microbial strains & enzymes
Chitosan (CH)	Adsorbent	Poly-cationic Biopolymer	Complexes with metal cations
Water Treatment Residuals (WTR)	Adsorbent	Al-oxyhydroxide	Coagulant and floculant to remove impurities

NH_4^+–N and NO_3–N values from the total N value. Organic N mineralization and total N loss were determined by mass balance. All carbon and N values were adjusted for moisture content and are reported on a dry weight basis.

2.3. Molecular analysis of the poultry litter

DNA was extracted from poultry litter samples (0.3 g) using the Q-Biogene FastDNA® Spin Kit for soil (Q-Biogene, Irvine, CA) according to manufacturer's specifications. Quantitative, real-time PCR (qPCR) was used to determine the concentration of targeted groups in DNA from the poultry litter extracts including: total bacteria (16S rRNA gene analysis), total fungi (18S rRNA gene analysis), bacterial urease producers common to poultry litter (bacterial urease), *Aspergillus*-like urease producers (fungal urease), *Bacillus*-like uricase producers (bacterial uricase), and *Aspergillus*-like uricase producers (fungal uricase) using primers, probes (if necessary), and assay conditions as previously described (Rothrock et al., 2010). All qPCR assays were run on the DNA Engine Opticon 2 (MJ Research, Inc., Waltham, MA) in a total volume of 25 μl. PCR assays with probe were carried out using 1X Qiagen HotStarTaq® Master Mix (Qiagen, Valencia, CA), 3.0 mM $MgCl_2$, 600 nM each primer, 200 nM of probe and sample DNA or standard (from 10^2 to 10^8 copies). QuantiTect™ SYBR® Green PCR kit was used for SYBR Green assays with 300 nM each primer, sample DNA or standard (10^1–10^8 copies).

2.4. Statistical analyses

Data were statistically analyzed by means and standard errors (proc MEANS), linear regression (proc REG), and analysis of variance (proc ANOVA), and least significant difference at a 0.05 probability level ($LSD_{0.05}$) for multiple comparisons among means with SAS Version 9.2 (SAS Institute, Inc. 2008. SAS. Release 9.2., Cary, NC).

3. Results and discussion

3.1. Properties of amended and control litters

The goal of this study was to evaluate poultry litter treatments for their effect on N mineralization and bacterial populations. Eight poultry litter amendments (chemical, biological or physical

treatments) were evaluated for their ability to reduce total and organic N loss (Table 2). Total C, total N, total bacteria and fungi, and microbiological groups specifically associated with ammonia production (urease and uricase producers) were measured at the beginning and the end of the experiment. Organic N represented approximately 80% of the total N present in the litter. The initial litter physiochemical properties, 35–42% total C and 3.5–4.3% total N, were in the expected range (Table 1). Initial nutrient concentrations varied significantly among treatments (Table 1), therefore, to evaluate the effect of litter amendments, data were analyzed based on percent change between initial and final concentrations of total and organic nitrogen (Fig. 1), bacterial (Fig. 2) or fungal (Fig. 3) populations. All litter amendments significantly reduced the loss of total and organic N as compared to the unamended litter (Fig. 1A, B).

3.2. Acidifier amendments

Acidifier amendments inhibit NH_3 volatilization by lowering the pH of the litter, thereby converting volatile NH_3 into non-volatile NH_4^+. The pH of all acidified litter treatments started between 3 and 4 and increased to 5 or 6 by the end of the study (Table 1). In this study, acidifiers were found to be among the most effective amendments for reducing N loss (Fig. 1A, B). Poultry Guard (acidified clay) was the least effective of the acidifiers, although still significantly better than unamended litter. In a previous study, Rothrock et al. (2010) found that results for PG were similar to those for AC. Therefore, the differences seen in this study may be due to the more limited sampling (beginning and end points) and the shorter duration of the study. In agreement with previous studies, there were significant ($P < 0.05$) decreases in the concentration of total bacteria and bacterial urease and uricase producers (except bacterial uricase in PLT) in all acidified litters (Fig. 2A, B and C) and concomitant increases (up to 5 orders of magnitude) in the concentration of total fungi, and fungal urease and uricase producing populations (Fig. 3A, B and C). Interestingly, the mass loss from the AC and CL treatments was significantly higher than in all other treatments; this trend was also observed in a previous study (Cook et al., 2008). The combination of greater mass loss and nitrogen retention increases the economic benefits of this amendment (i.e., lower transportation costs and greater nutrient value).

In previous studies, *Aspergillus* spp. were found to be the dominant fungi in acidified litter and the dominant fungal urease and uricase producing population (Rothrock et al., 2010, 2008a,b). Reduction of this population may extend the utility of acidifiers. Therefore, one of the AC treatments was also treated with Clinafarm®EC, an enilconazole micro-emulsion that prevents formation of ergosterol in fungal cell walls. The product was designed for use in hatcheries for prevention of aspergillosis. In this study, fungal concentrations were significantly reduced as compared to the AC treatment with no CL (Fig. 3A). However, N losses from this treatment were similar to the AC treatment with no CL (Fig. 1A, B). This is likely due to the reduced (2 orders of magnitude decrease) but still high ($2.5 \pm 0.35 \times 10^7$ cells g^{-1}) concentrations of fungi in that treatment. Therefore, the bloom was curtailed, but not eliminated with CL treatment. CL effectiveness might be improved or extended by re-applying more often, however, this approach may make the

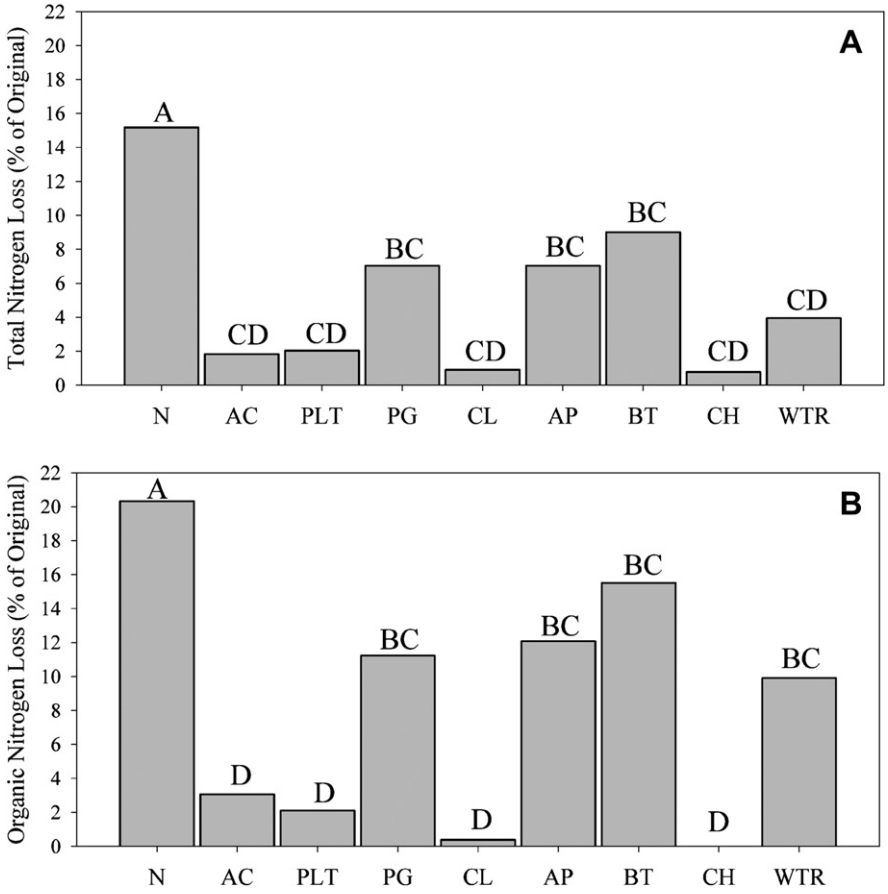

Fig. 1. Percent of total (A) or organic (B) nitrogen lost per gram of litter based on N mass balance with the following amendments: N, unamended; AC, Al+Clear®; PLT, Poultry Litter Treatment®; PG, Poultry Guard®; CL, Al+Clear® plus Clinafarm®EC; AP, Agrotain Plus®; BT, biological treatment; CH, Chitosan; or WRT, Water treatment residuals. Values are the average of triplicate samples. Treatments with the same letters are not significantly different ($P = 0.05$).

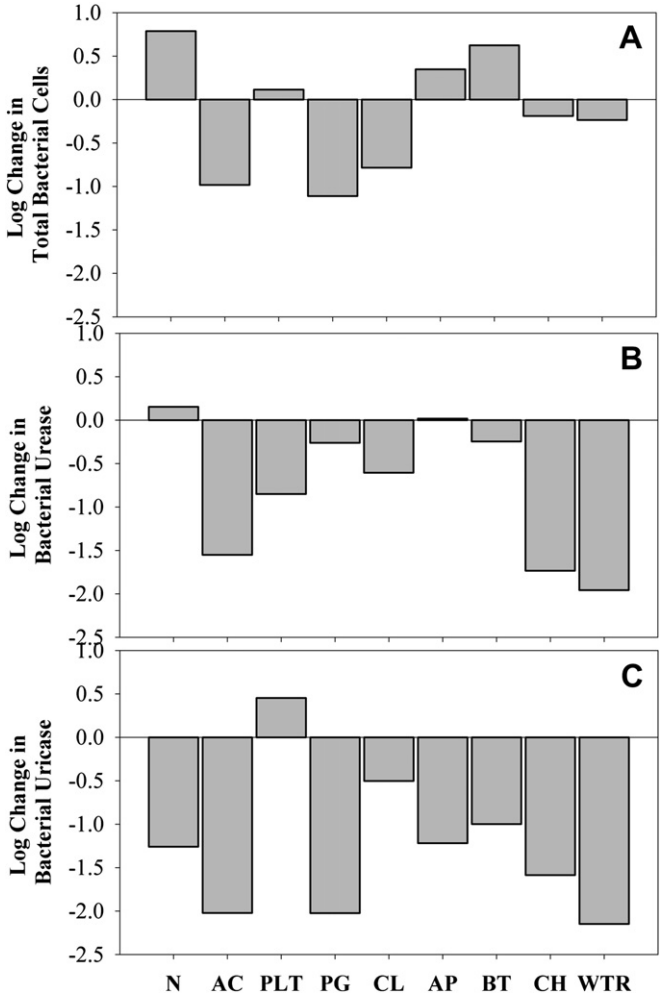

Fig. 2. Log change in total (A), urease positive (B) or uricase positive (C) bacterial cells in poultry litter with the following amendments: N, unamended; AC, Al+Clear®; PLT, Poultry Litter Treatment®; PG, Poultry Guard®; CL, Al+Clear® plus Clinafarm®EC; AP, Agrotain Plus®; BT, biological treatment; CH, Chitosan; or WRT, Water treatment residuals. Values are the average of triplicate samples.

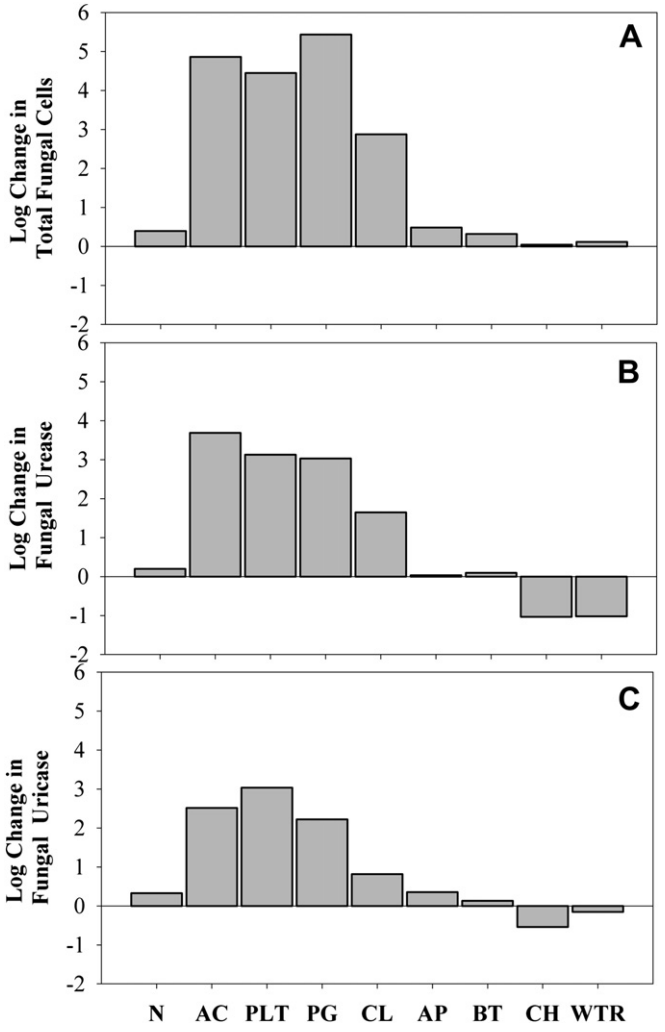

Fig. 3. Log change in total (A), urease positive (B) or uricase positive (C) fungal cells in poultry litter with the following amendments: N, unamended; AC, Al+Clear®; PLT, Poultry Litter Treatment®; PG, Poultry Guard®; CL, Al+Clear® plus Clinafarm®EC; AP, Agrotain Plus®; BT, biological treatment; CH, Chitosan; or WRT, Water treatment residuals. Values are the average of triplicate samples.

use of this treatment cost-prohibitive. In this study, CL was re-applied bi-monthly. In preliminary studies, CL was re-applied to the litter on a weekly basis, however no significant improvement in N retention or fungal reduction was observed as compared to the bi-monthly application (data not shown).

3.3. Biological treatments

The biological treatment (BT) used in this study, a powder containing a blend of bacteria, enzymes and nutrients, was specifically designed to control NH_3 production from poultry litter. In this study, BT significantly decreased total and organic N loss as compared to the unamended litter (Fig. 1A, B), although the microbial cell concentrations were very similar to controls (Figs. 2 and 3). Given the results of this study, more research on this material is warranted to evaluate the effect of environmental differences in moisture, temperature and pH on its effectiveness and reproducibility. More detailed information about mode of action of these amendments would also aid in the design of studies to critically evaluate their effectiveness (McCrory and Hobbs, 2001).

Inhibition of NH_3 production by the native microbial flora through the use of urease inhibitors has been applied to poultry, swine, and beef wastes (Kim and Patterson, 2003; Singh et al., 2009; Varel, 1997). In studies of beef and swine slurries, Varel (1997) found that urease inhibitors improved N retention by up to 92%. In this study, the N stabilizer Agrotain® Plus was applied to unamended litter. It contains the urease inhibitor *N*-(*n*-Butyl) thiophosphorictiramide (NBPT) and an N stabilizer. In the field, it is designed to minimize volatilization, denitrification, and leaching of N. In this study, its performance was similar to that of the BT, retaining significantly more total and organic N than the unamended control litter (Fig. 1A and B, respectively). A previous litter study using NBPT found that the inhibitor did not effect equilibrium NH_3 concentrations (Singh et al., 2009). Better performance in this study may be due to the significantly higher application rate (about 5 times greater) than in their study or to the fact that AG was re-applied on day 30. In that study and others, it has been found that re-application of NBPT is necessary to maintain function (Singh et al., 2009; Varel, 1997), however this may be cost-prohibitive for poultry production.

3.4. Adsorbents

Aluminum-based WTR were included in this study to evaluate N loss and the effect on the microbial population. Total N loss from

the WTR treatments was similar to that of the acidifiers (Fig. 1A); however, organic N loss was slightly higher (Fig. 1B). In this study, there was also no pH effect using WTR and no increase in fungal concentrations (Table 1; Fig. 3). Concentrations of urease and uricase producing bacteria in this treatment were very low (Fig. 2B and C) possibly due to binding of trace nutrients required for their growth. Inhibition may also be due to the presence of inhibitory residual compounds from the water purification process. Recent studies have allayed many of the concerns about the WTR composition, binding capacity and metal concentrations (Hyde and Morris, 2000; Lombi et al., 2010; Makris et al., 2010). Further studies are warranted given the low cost, availability and proven ability of the material to adsorb P and reduce N loss from poultry litter.

Microbial urease is a nickel containing enzyme which requires six Ni^{2+} atoms to be functional (Manunze et al., 1999). It has been shown that the addition of nickel increases urease activity in some soils, suggesting that nickel availability influences urease production (Dalton et al., 1985). In this study, chitosan was included as a low cost, non-specific adsorbent to evaluate its effect on urea hydrolysis. Chitosan was the most effective non-acidifier amendment. Both total and organic N losses in the CH treatments were similar to those in the most effective acidifier amendments (Fig. 1A and B). Additionally, both bacterial and fungal urease and uricase cell concentrations remained low (Figs. 2 and 3). In fact, chitosan was extremely inhibitory to bacterial urease and uricase producing bacteria (Fig. 2B and C). This suggests that chitosan is binding compounds that are important to the proliferation of these populations. It is possible that chitosan adsorbed nickel, reducing urease production. However, chitosan is a non-specific adsorbent so it is also possible that it affected the availability of other important trace minerals. The inhibition of fungi by chitosan, may make this a useful additive to acidifier amendments as a means to inhibit unwanted microbial growth. Further studies would be required to determine how these adsorbents compare to acidifiers in terms of pathogen inhibition and run-off prevention. Given the high performance, low cost, availability and ability to modify the compound, future research should focus on application rates and mechanisms of action of this compound within the litter.

4. Conclusions

Poultry use less than 30% of the N included in their feed; the remainder is excreted in manure and urine. Failure to re-capture this N lowers the efficiency of the livestock/crop production process and reduces the value of litter as a commodity. In this study, litter amendments consistently reduced N loss as compared to no amendment. While acidifiers consistently out-perform other litter amendments, the adsorbent compounds, chitosan and WTR, should be further evaluated. They are both readily available and cost-effective, chitosan chemistry can be modified to improve performance, and the environmental benefits of re-using WTR are significant.

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References

Awad, W.A., Böhm, J., Razzazi-Fazeli, E., Ghareeb, K., Zentek, J., 2006. Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. Poult. Sci. 85, 974–979.

Babel, S., Kurniawan, T.A., 2003. Low-cost adsorbents for heavy metals uptake from contaminated water: a review. J. Hazard. Mater. B97, 219–243.

Badaway, M.E.I., Rabea, E.I., 2009. Potential of the biopolymer chitosan with different molecular weights to control postharvest gray mold of tomato fruit. Postharvest Biol. Tech. 51, 110–117.

Brown, S., Christensen, B., Lombi, E., McLaughlin, M.J., McGrath, S., Colpaert, J., Vangronsveld, J., 2005. An inter-laboratory study to test the ability of amendments to reduce the availability of Cd, Pb, and Zn in situ. Environ. Pollut. 138, 34–45.

Chatterjee, S., Leeb, D.S., Lee, M.W., Woo, S.H., 2009. Nitrate removal from aqueous solutions by cross-linked chitosan beads conditioned with sodium bisulfate. J. Hazard. Mater. 166, 508–513.

Cole, K., Farnell, M.B., Donoghue, A.M., Stern, N.J., Svetoch, E.A., Eruslanov, B.N., Volodina, L.I., Kovalev, Y.N., Perelygin, V.V., Mitsevich, E.V., Mitsevich, I.P., Levchuk, V.P., Pokhilenko, V.D., Borzenkov, V.N., Svetoch, O.E., Kudryavtseva, T.Y., Reyes-Herrera, I., Blore, P.J., Solis De Los Santos, F., Donoghue, D.J., 2006. Bacteriocins reduce Campylobacter colonization and alter gut morphology in turkey poults. Poult. Sci. 85, 1570–1575.

Cook, K.L., Rothrock, M.J., Warren, J.G., Sistani, K.R., Moore Jr., P.A., 2008. Effect of alum treatment on the concentration of total and Ureolytic microorganisms in poultry litter. J. Environ. Qual. 37, 2360–2367.

Dalton, D.A., Evans, H.J., Hanus, F.J., 1985. Stimulation by nickel of soil microbial urease activity and urease and hydrogenase activities in soybeans grown in a low-nickel soil. Plant Soil 88, 245–258.

DeLaune, P.B., Moore Jr., P.A., Daniel, T.C., Lemunyon, J.L., 2004. Effect of chemical and microbial amendments on ammonia volatilization from composting poultry litter. J. Environ. Qual. 33, 728–734.

Hovsepyan, A., Bonzongo, J.-C.J., 2009. Aluminum drinking water treatment residuals (Al-WTRs) as sorbent for mercury: Implications for soil remediation. J. Hazard. Mater. 164, 73–80.

Hyde, J.E., Morris, T.F., 2000. Phosphorus availability in soils amended with dewatered water treatment residual and metal concentrations with time in residual. J. Environ. Qual. 29, 1896–1904.

Kelleher, B.P., Leahy, J.J., Henihan, A.M., O'Dwyer, T.F., Sutton, D., Leahy, M.J., 2002. Advances in poultry litter disposal technology - a review. Biodegrad. Tech. 83, 27–36.

Kim, W.K., Patterson, P.H., 2003. Effect of minerals on activity of microbial uricase to reduce ammonia volatilization in poultry manure. Poult. Sci. 82, 223–231.

Lin, S.-B., Lin, Y.-C., Chen, H.-H., 2009. Low molecular weight chitosan prepared with the aid of cellulase, lysozyme and chitinase: characterisation and antibacterial activity. Food Chem., 47–53.

Line, J.E., Bailey, J.S., 2006. Effect of on-farm litter acidification treatments on *Campylobacter* and *Salmonella* populations in commercial broiler houses in northeast Georgia. Poult. Sci. 85, 1529–1534.

Lombi, E., Stevens, D.P., McLaughlin, M.J., 2010. Effect of water treatment residuals on soil phosphorus, copper and aluminium availability and toxicity. Environ. Pollut. 158, 2110–2116.

Makris, K.C., O'Connor, G.A., Harris, W.G., Obreza, T.A., 2005. Relative efficacy of a drinking-water treatment residual and alum in reducing phosphorus release from poultry litter. Comm. Soil Sci. Plant Anal. 36, 2657–2675.

Makris, K.C., Sarkar, D., Salazar, J., Punamiya, P., Datta, R., 2010. Alternative amendment for soluble phosphorus removal from poultry litter. Environ. Sci. Pollut. Res. 17, 195–202.

Manunze, B., Deiana, S., Pintore, M., Gessa, C., 1999. The binding mechanism of urea, hydroxamic acid and N-(N-butyl)-phosphoric triamide to the urease active site. A comparative molecular dynamics study. Soil Biol. Biochem. 31, 789–796.

Martinez, J., Dabert, P., Barrington, S., Burton, C., 2009. Livestock waste treatment systems for environmental quality, food safety, and sustainability. Bioresour. Tech. 100, 5527–5536.

McCrory, D.F., Hobbs, P.J., 2001. Additives to reduce ammonia and odor emissions from livestock wastes: a review. J. Environ. Qual. 30, 345–355.

McWard, G.W., Taylor, M.A., 2000. Acidified litter amendment. J. Appl. Poult. Res. 9, 518–529.

Moore Jr., P.A., Daniel, T.C., Edwards, D.R., 1999. Reducing phosphorus runoff and improving poultry production with alum. Poult. Sci. 78, 692–698.

Moore Jr., P.A., Daniel, T.C., Edwards, D.R., 2000. Reducing phosphorus runoff and inhibiting ammonia loss from poultry manure with aluminum sulfate. J. Environ. Qual. 29, 37–49.

Moore Jr., P.A., Daniel, T.C., Gilmour, J.T., Shreve, B.R., Edwards, D.R., Wood, B.H., 1998. Decreasing metal runoff from poultry litter with aluminium sulfate. J. Environ. Qual. 27, 92–99.

Nagar, R., Sarkar, D., Makris, K.C., Datta, R., Sylvia, V.L., 2009. Bioavailability and bioaccessibility of arsenic in soil amended with drinking-water treatment residuals. Arch. Environ. Contam. Toxicol. 57, 755–766.

Patterson, J.A., Burkholder, K.M., 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82, 627–631.

Peters, J., Wolfe, N., 2003. Ammonium nitrogen. In: Peters, J. (Ed.), Recommended Methods of Manure Analysis. Univ. Wisconsin extension, Madison, pp. 23–29.
Pope, M.J., Cherry, T.E., 2000. An evaluation of the presence of pathogens on broilers raised on poultry litter treatment®-treated litter. Poult. Sci. 79, 1351–1355.
Ritz, C.W., Fairchild, B.D., Lack, M.P., 2004. Implications of ammonia production and emissions from commercial poultry facilities: a review. J. Appl. Poult. Res. 13, 684–692.
Rothrock Jr., M.J., Cook, K.L., Lovanh, N., Warren, J.G., sistani, K.R., 2008a. Development of a quantitative real-time polymerase chain reaction assay to target a novel group of ammonia-producing bacteria found in poultry litter. Poult. Sci. 87, 1058–1067.
Rothrock Jr., M.J., Cook, K.L., Warren, J.G., Sistani, K.R., 2008b. The effect of alum addition on poultry litter microbial communities. Poult. Sci. 87, 1493–1503.
Rothrock Jr., M.J., Cook, K.L., Warren, J.G., Eiteman, M.A., Sistani, K., 2010. Dynamics of nitrogen and associated organic nitrogen mineralizing microbes in acidified poultry litters. J. Environ. Qual. 39, 1–10.
Shah, S.B., Baird, C.L., Rice, J.M., 2007. Effect of a metabolic stimulant on ammonia volatilization from broiler litter. J. Appl. Poult. Res. 16, 240–247.
Singh, A., Casey, K.D., King, W.D., Pescatore, A.J., Gates, R.S., Ford, M.J., 2009. Efficacy of urease inhibitor to reduce ammonia emission from poultry houses. J. Appl. Poult. Res. 18, 34–42.
Terzich, M., Quarles, C., Goodwin, M.A., Brown, J., 1998. Effect of poultry litter treatment® (PLT®) on death due to ascites in broilers. Avian Dis. 42, 385–387.
Varel, V.H., 1997. Use of urease inhibitors to control nitrogen loss from livestock waste. Bioresour. Tech. 62, 11–17.
Vold, I.M.N., Vårum, K.M., Guibal, E., Smidsrød, O., 2003. Binding of ions to chitosan—selectivity studies. Carbohydr. Polym. 54, 471–477.
Warren, J.G., Phillips, S.B., Mullins, G.L., Keahey, D., Penn, C.J., 2006. Environmental and production consequences of using alum-amended poultry litter as a nutrient source for corn. J. Environ. Qual. 35, 172–182.
Watson, M.A., Wolfe, N., 2003. Total nitrogen. In: Peters, J. (Ed.), Recommended Methods of Manure Analysis. University of Wisconsin extension, Madison, pp. 18–24.