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The Impact of Peat Moss Amendments on the Microbial Load in Used Pine Shaving Poultry Litter

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Abstract: In addition to pine shavings, alternative litter sources for poultry bedding include sand, pine straw, or even peat moss. Peat moss has a high absorptive capacity and is naturally acidic, possibly making it a good poultry litter amendment. The objective of this study was to determine if microbial populations changed when different levels of peat moss were added to poultry litter. Experimental treatments included 0, 13 and 20% peat moss which were added to used pine shavings. A total of 216 male broilers (42 d) were separated into 18 pens (6 pens/3 treatments). Control litter samples (100 g) were collected prior to the addition of peat moss and birds (0 d); then litter samples from each pen were collected weekly thereafter for 3 wk. From each litter sample, 10 g was diluted in 90 ml of Buttersfield's Phosphate and then serially diluted. For Tryptic soy agar, MacConkey agar and Sabouraud Dextrose agar, 100 µl of inoculums was plated in duplicate to detect aerobic bacteria, total coliforms and yeasts/molds, respectively. Plates were incubated aerobically for 24 h at 37°C and then counted. The results indicated that there were no differences between treatments for total aerobic bacterial counts. Initially, an increase in coliforms was detected in treatments that had peat added. By the second week coliforms were reduced in the peat treatments and a treatment by week interaction was detected (P = 0.012). The level of coliforms in litter which had peat added (13 or 20%) was 3.92 and 4.04 log cfu/g, respectively. For the control litter where no peat was added, coliforms were 5.43 log cfu/g of litter. Also a treatment by week interaction was detected for yeast and molds (P = 0.0025). Over each week of the experiment a decrease in the number of yeast and molds occurred in litter where peat was added. In week 1, yeast and molds were at 5.22 log cfu/g of litter in the control and 4.42 and 4.54 log cfu/g of litter in the 13 and 20% peat treatments, respectively. Week 2 the yeast and molds were 5.43 log cfu/g of litter in the control and 4.0 and 3.88 log cfu/g of litter for the 13 and 20% peat treatments, respectively. For, week 3 the yeast and molds were 6.03 log cfu/g of litter in the control and 4.82 and 3.72 log cfu/g of litter for the 13 and 20% peat treatments, respectively. In conclusion, the data demonstrates that the addition of peat moss may be a useful amendment for reducing bacteria, yeasts and molds in poultry litter. Overall, future studies should test the absorptive capacity of peat moss for trapping ammonia and changing the litter pH which could demonstrate how peat moss is actually reducing bacteria and yeast/mold growth in poultry litter.

Key words: Sphagnum peat, litter, anaerobic, coliforms

INTRODUCTION

Although most poultry farms in the southern U.S. use pine shavings as bedding material (Malone, 1992), this material is becoming expensive and less available due to its use in other markets (Kline *et al*., 2010) such as biofuels. As a result producers are leaving used pine shavings in their houses for longer periods of time and some are trying to discover alternatives altogether. Used litter has been shown to harbor more microorganisms than fresh litter (Schefferle, 1965) which can result in colonization of the broilers by pathogenic bacteria leading to bird illness and potential food safety concerns. Fungi such as yeast and molds thrive in warm, moist and low light environments, the same environment which is created in used poultry litter. The pH of poultry litter ranges from 7.5-8.5 which is an ideal habitat for yeast, molds and bacteria to grow. Yeasts and molds produce metabolites called mycotoxins that can cause a decreased growth rate and a suppressed immune system if eaten by the broilers (Cook, 1990). Research has reported that broilers consume as much as 4% of their diet in bedding material (Malone *et al*., 1983). Many alternative litters have been tested for broiler production such as pelleted newspaper (Frame *et al*., 2002), leaves (Willis *et al*., 1997), sand (Arnould *et al*., 2004) and peat (Petherick and Duncan, 1989) but none have all the positive attributes needed to grow broilers compared to regular pine shavings.

Sphagnum peat is a material that has a naturally low pH which reduces bacterial growth but doesn't have an

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effect on yeast and molds (3.9-4.3; Cocozza *et al*., 2003). Also, peat is pathogen free and can absorb 20 times its weight in moisture (Trail, 2013). All of these traits make peat an ideal candidate for a poultry litter amendment. Peat is brown to black in color and is formed under water drenched conditions via partial decomposition of mosses and other trees, grasses or shrubs (Cocozza *et al*., 2003). Peat moss is naturally chemical free and readily available in areas that contain peat bogs; it can also be found at most garden supply stores. Peat has many uses in agriculture already, such as the production of protein concentrates for animal feeds (Trckova *et al*., 2005), medicinal products (Klocking *et al*., 1976) and fertilizers (Kruglov *et al*., 1975).

The objective of this study was to determine if total bacteria, coliforms and yeast/molds are prevented or decreased when peat moss is added as a litter amendment to used pine shavings.

MATERIALS AND METHODS

Bird husbandry: In both trials, 216 d old Ross 708 male broilers were obtained from a commercial hatchery. In each experiment, chicks were randomly placed in 18 total concrete floor pens (12 birds/pen) measuring 1.1 m^2 that contained a nipple watering system and a pan feeder. There were 6 replications per treatment. The total weight of litter in each pen, including treatment peat moss, was approximately 85 lbs. Treatment peat moss amendments were added to 12 of the 18 randomly selected treatment pens (11.05 lbs for 13% peat and 17.00 lbs for 20% peat). Used pine shavings were used as the control treatment during this experiment. All birds had *ad-libitium* access to water and feed. Daily lighting schedule consisted of 23 h of light and 1 h of darkness. Birds were treated in accordance with the Guide for the Care and Use of Laboratory animals.

Litter collection: Litter samples were collected from each of the 18 mini pens on D 0, 7, 14 and 21 following chick placement. Each sample consisted of 5, 20 g subsamples of litter. The subsamples were collected from the corners of each pen and from the center. All samples were collected by the grab sample method which included using a pair of sterile gloves within each pen. Litter samples from each pen were placed inside a sterile Whirl Pak ™ bag and transported back to the laboratory for processing. All samples were processed within 1 hr once back at the laboratory.

Microbial analysis: The 100 g samples were mixed thoroughly when they arrived to the lab. Ten grams of the 100 g samples were used for analysis. Analysis of litter was performed by placing 10 g of each sample in 90ml of sterile Butterfield's solution (0.00031 M KH_2PO_4, pH 7.2). Each 10 g sample was then placed in a Brinkmann/Seward 440C stomacher (Thermo Scientific, Marietta, OH) for 30 *s* at 130 rpm. Each sample was serially diluted to a final dilution of 1.0×10^9 using sterile Butterfield's solution and 0.1 mL of each dilution was spread in duplicate across the following media: Tryptic Soy Agar (TSA) for total bacteria count, MacConkey agar (MAC) for total coliform count and Sabouraud Dextrose agar (SDA) for yeast and molds (Difco, Sparks, MD.). Before plates were used, they were incubated for 24 h at 37°C for detection of contamination or growth. After all plates were spread with inoculum from the serial dilutions, they were placed inside a Precision Model 815 low-temperature incubator (Thermo Scientific, Marietta, OH) at 37°C for 24 h. After 24 h, colonies were counted and recorded using the standard plate counting method. All counts were log transformed after calculations.

Statistics: Data was analyzed using a randomized complete block design with a split plot over weeks using SAS analytical software (SAS Institute, 2003). The means were separated using Fishers Protected least significance difference and were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Because the effect of peat moss on the microbial populations in the litter was the main concern in this project, litter moisture and pH were not observed in this study. Also, so that baseline microbial counts could be determined, litter samples were taken from each pen before the addition of treatment peat moss. Additionally, in the present study, peat moss was added in percentages to used pine shavings because peat moss has a tendency to dry out and create a dusty environment that can present health problems to the broilers or to the poultry farm workers.

For our litter mixtures containing peat there were no significant differences for the total aerobic bacteria count ($P = 0.7$; Fig. 1) when compared to the control. However, there was a significant week x treatment interaction effect for coliforms ($P = 0.049$; Fig. 2) and yeast and molds ($P<0.0001$; Fig. 3). For the 0 % peat moss treatment, coliforms decreased at Wk 1, compared to all other sampling times (Fig. 2). A decrease in coliforms was also detected in the 13 and 20% peat moss treatments at Wk 2 of the experiment, compared to Day 0 and Wk 1 sampling times (Fig. 2). Also the peat moss treatments at Wk 2 yielded lower coliform counts than the control. The decrease in coliforms may be attributed to the natural microflora associated with sphagnum peat. These microorganisms rely on cellulose as a food source which may allow them to out compete the coliforms for nutrients, initially (Pankratov *et al*., 2011). Although the decrease in coliforms when peat was added to the litter only lasted for 7-14d, this could be very beneficial for food safety. It is hypothesized that if fewer coliforms enter the plant via broiler carcasses, there is

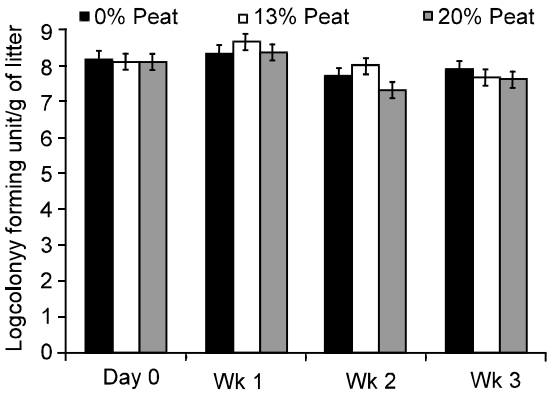

Fig. 1: Total aerobic count from 1 g of litter with different amounts of peat moss added. Data has been logged transformed (Trt x Week, P<0.7, SEM = 0.23). Black columns represent 0% peat moss. White columns represents 13% peat moss added to used litter. Gray columns represents 20% peat moss added to used litter.
[a-h]Means with different superscripts differ significantly at P<0.05

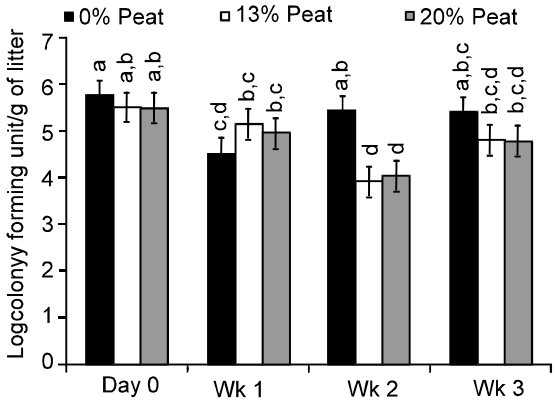

Fig. 2: Total coliform count from 1 g of litter with different amounts of peat moss added. Data has been logged transformed (Trt x Week interaction, P = 0.049, SEM = 0.32). Black columns represent 0% peat moss. White columns represents 13% peat moss added to used litter. Gray columns represents 20% peat moss added to used litter.
[a-d]Means with different superscripts differ significantly at P<0.05

less chance for bacteria to multiply and cross contaminate carcasses, as well as the end product that is presented to the consumer.

Unlike coliforms, yeast and mold counts from weeks 1 to 3 were reduced in litter that contained the 13% and 20% peat moss as compared to the control (Fig. 3). This is contrary to the finding by Lovett *et al.* (1971) that stated that yeast and mold populations increase with litter

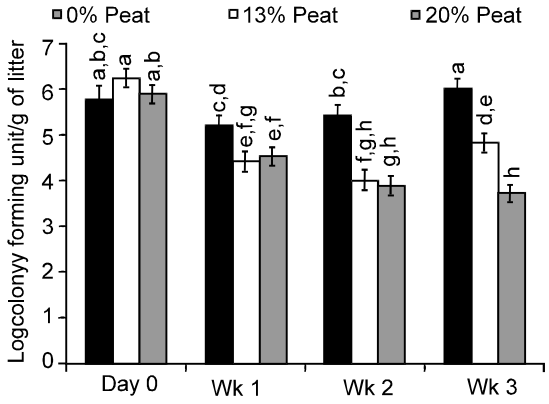

Fig. 3: Total yeast and mold count from 1 g of litter with different amounts of peat moss added. Data has been logged transformed (Trt x Week interaction P<0.0001, SEM = 0.21). Black columns represent 0% peat moss. White columns represents 13% peat moss added to used litter. Gray columns represents 20% peat moss added to used litter.
[a-h]Means with different superscripts differ significantly at P<0.05

usage for about 4 wk then decline slightly. The reduction of yeast and molds may have been due to the low acidic pH (4.3) of Sphagnum peat moss (Cocozza *et al*., 2003). Although, yeast and molds are known to survive in a pH range between 2.5-8.0 and 1.5-8.5, respectively (Donald, and Watkins, 2003), the addition of peat moss to the used pine shavings may have shifted the pH out of the optimal range for the particular yeast and molds growing in the litter. It is also possible that the Sphagnum peat moss may have absorbed some of the water normally available to the microorganisms, yeast and mold. Peat moss is capable of absorbing 20 times its weight in water (Trial, 2013). If less water is available to the microorganisms, yeast and molds, they may not have the ability to proliferate, leading to the deceased levels.

Conclusion: The results of this experiment confirm that peat moss has potential for being a chemical free poultry litter amendment. Sphagnum peat has been shown to decrease yeast, molds and coliforms in used poultry litter. By reducing the amount of yeast, molds and coliforms in used poultry litter, bird health, production and overall quality should be increased. The total aerobic bacteria population in used pine shavings is not affected by the addition of peat moss at any level. More research needs to be conducted to determine the overall advantages peat could provide to the poultry industry as a chemical free litter amendment. Therefore, future research should study how sphagnum peat moss shifts the pH and moisture content of used litter, as well as how it reacts with the nutrients in poultry litter to bind nitrogen and reduce ammonia production.

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