Bacterial Levels of Pine Shavings and Sand Used as Poultry Litter

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Primary Audience: Broiler Managers, Researchers

SUMMARY

Bacterial loads associated with sand and pine shavings litter were determined in 2 grow-out experiments with broilers that lasted 7 wk. These experiments consisted of 8 pens each of pine shavings and sand. Birds were placed with 1.16 ft^2/bird. Litter samples were collected weekly and plated to enumerate (cfu/g) aerobic, anaerobic, and enteric bacterial counts. In addition each sample was analyzed for water activity and percentage moisture levels. Overall, sand litter had lower bacterial counts, water activity, and moisture level compared with pine shavings litter. This work illustrates that, bacteriologically, sand can be a viable litter alternative to pine shavings if locally available.

Key words: pine shaving, sand, litter, bacterial count, water activity

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DESCRIPTION OF PROBLEM

The poultry industry uses diverse types of bedding materials, including pine shavings, sawdust, peanut hulls, rice hulls, sawdust, and hardwood shavings [1]. The type utilized is largely dependent upon local availability of the material and location of the farm. In the southeastern United States, pine shavings and peanut hulls are the preferred bedding materials. Unfortunately, the availability of pine shavings has steadily decreased due to competition from the composite board industry and horticulture and use as an energy source [2]. One possible alternative to pine shavings is mortar sand, which has been used outside the United States with no detrimental effect on bird performance or survivability [1, 3]. Work performed at Auburn University in recent years [1, 4] has confirmed the utility of sand as a bedding material.

The objective of this study was to profile the aerobic, anaerobic, and enteric bacterial counts on pine shavings and sand bedding material over a 7-wk grow-out trial with broilers. Water activity and percentage moisture were also measured to determine if there is a correlation between these 2 measurements and bacterial levels.

MATERIALS AND METHODS

For the first experiment, 1,600 1-d-old broiler chicks were placed evenly into 16 pens (1.16 ft^2/bird). Eight pens contained freshly placed pine shavings, and the remaining pens contained freshly placed sand. Litter was collected weekly, starting the day prior to chick placement, and continued for 8 wk. Collection was performed in each pen by using a clean 200-mL beaker and consisted of a 6-cm core sample of litter taken from 3 areas in each pen. Samples included litter from under the nipple drinkers, next to the feed troughs, and the middle of the

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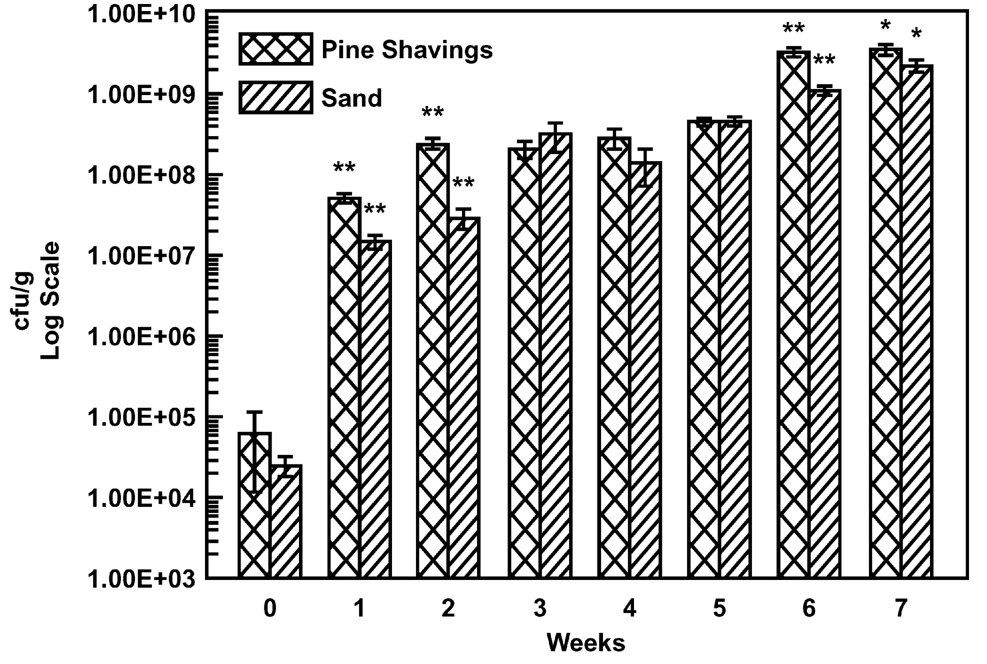

FIGURE 1. Aerobic bacterial counts from experiment 1. Statistical differences for that week between litter types are denoted by *≤ 0.05 or **≤ 0.01.

pen. The 3 collected samples were then thoroughly mixed together in a sterile stomacher bag.

Populations of aerobic, anaerobic, and enteric bacteria were enumerated (cfu/g) for each pen using plate count agar (PCA) [5], reduced blood agar (RBA) [5], and MacConkey agar (MA) [5], respectively. Dilutions were performed by adding 10 g of litter to 90 mL of sterile physiological saline (0.75% NaCl); this produced a 10^{-1} dilution. Further dilutions were performed by transferring 10 mL into another 90-mL sterile saline bottle; this process was repeated until dilutions ranging from 10^{-1} to 10^{-8} were made. The dilutions were then spiral plated [6] in triplicate onto their respective media types and incubated under appropriate conditions. PCA and MA were incubated aerobically at 37°C; RBA was incubated at 37°C in an anaerobic chamber [7] containing 5% CO_2, 5% H_2, and 90% N_2. After 18 h colonies were quantified on a digital plate reader [8], and average bacterial count for each medium and litter type was obtained with the plate reader's software. Counts were converted to a log scale and analyzed using a *t*-test to determine differences between the 2 litter types. To determine if there was a correlation between percentage moisture or water activity on bacterial counts, a bivariate correlation was performed, and the correlation coefficient was determined using Pearson's r [9]. Significant differences were at $P \leq 0.05$ unless otherwise stated.

The second experiment was performed on the same litter as was utilized in the first experiment. Day-old chicks were evenly placed onto used litter in 16 pens with 100 chicks per pen. Litter samples were collected weekly, as previously described, with the exception that the first sample was taken at chick placement. Aerobic, anaerobic, and enteric bacterial counts were determined as described previously. In this experiment, water activity (a measure of the amount of free water available for bacterial growth) and moisture level were also measured from the collected samples. Water activity of a 1-g sample was measured using an AquaLab Water Activity Meter [10]. Moisture level was determined for each sample by placing 1 g of litter into a drying oven (150°C) for 24 h. Dried samples were removed from the oven on the following day and placed immediately into des-

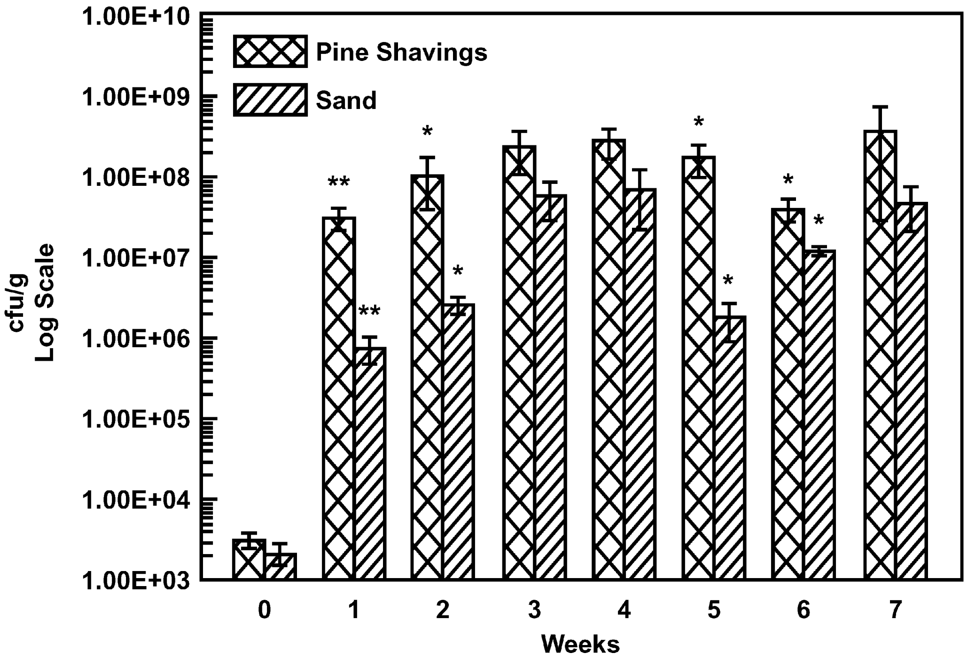

FIGURE 2. Enteric bacteria counts in experiment 1. Statistical differences for that week between litter types are denoted by *≤ 0.05 or **≤ 0.01.

iccators. The samples were then weighed, and percentage of litter moisture was calculated.

RESULTS AND DISCUSSION

In the first experiment, overall bacterial counts were lower in sand than in pine shavings. As expected, both litter types went through a dramatic increase in bacterial counts after the birds were placed. Figure 1 shows that the aerobic bacterial counts on pine shavings had, by the second week, reached a plateau of 10^8 cfu/g that lasted until wk 6. In contrast, sand reached the 10^8 cfu/g plateau on wk 3, which also lasted until wk 6. At wk 6, bacterial levels had approximately a 1-log increase, which lasted until the birds were removed. Even at this time, sand lagged slightly behind the pine shavings in the amount of bacteria that were recovered. Four of the 8 wk produced significant differences ($P <$ 0.05 at wk 1, 2, 6, and 7) with sand producing lower bacterial counts compared with pine shavings.

Enteric bacterial counts in experiment 1 showed more variation from week to week than the other 2 media types (Figure 2). Once chickens were placed, enteric bacteria ranged from 10^7 to 10^8 cfu/g with pine shavings and 10^5 to 10^7 cfu/g with sand. There was an overall trend for lower bacterial counts in sand litter compared with pine shavings. Sand had significantly lower enteric bacterial counts than pine shavings at wk 1, 2, 5, and 6.

Anaerobic bacteria in pine shavings reached a plateau of approximately 10^8 cfu/g by wk 2 (Figure 3). A plateau for anaerobes of 10^7 cfu/g was reached in sand by wk 2; however, by wk 6 it had reached the 10^8 cfu/g plateau as well. Sand produced significantly lower anaerobic bacterial counts in wk 1, 2, 4, 5, and 6.

Bacterial counts from the second experiment (Figures 4 to 6) were similar to those observed in the first experiment with pine shavings having higher overall bacterial counts than sand. Aerobic bacteria reached a plateau at 10^9 cfu/g by wk 4 with both litter types (Figure 4). Enteric bacterial levels (Figure 5) tended to fluctuate from week to week. Anaerobic bacterial counts reached a plateau by wk 3, with both litter types attaining 10^8 cfu/g (Figure 6). Interestingly, differences in colony-forming units per gram between the 2 litter types in this experiment were not as extreme as was observed in the initial

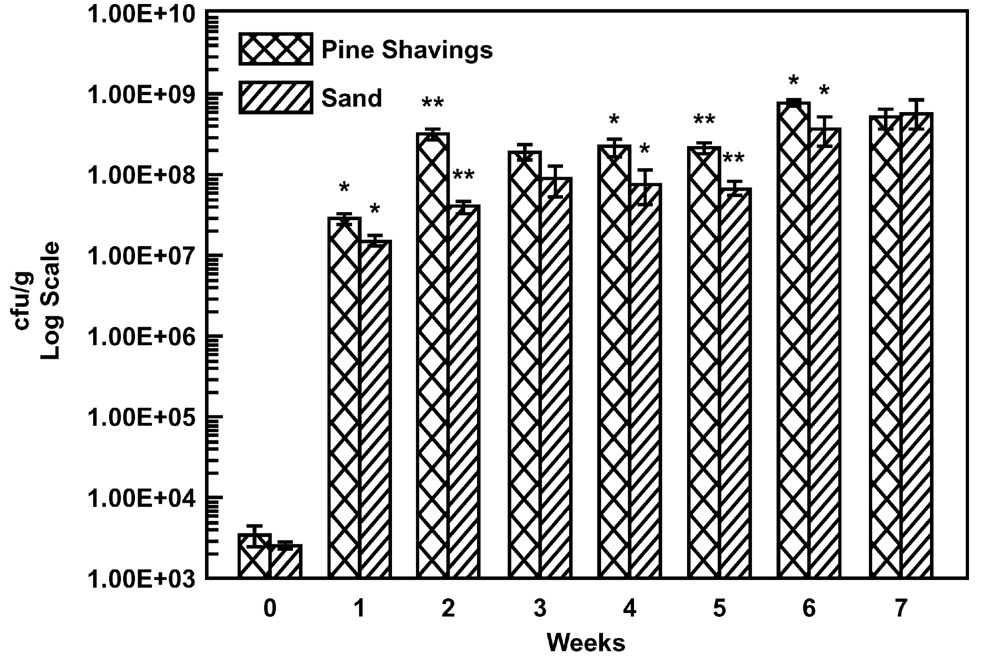

FIGURE 3. Anaerobic bacterial counts from experiment 1. Statistical differences for that week between litter types are denoted by *≤ 0.05 or **≤ 0.01.

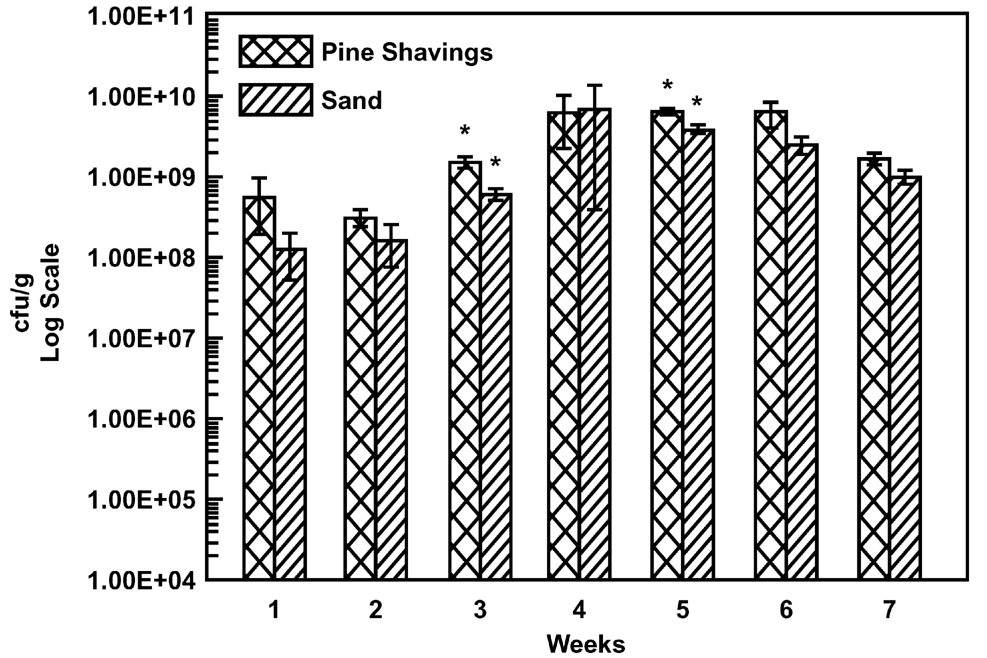

FIGURE 4. Aerobic bacteria counts in experiment 2. Statistical differences for that week between litter types are denoted by *≤ 0.05.

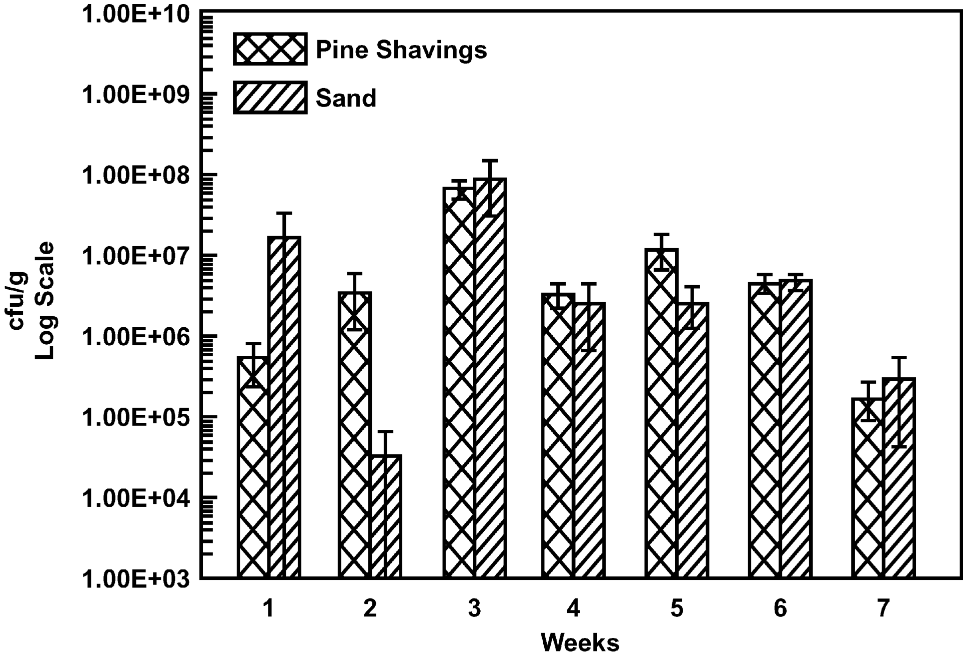

FIGURE 5. Amount of enteric bacteria from experiment 2.

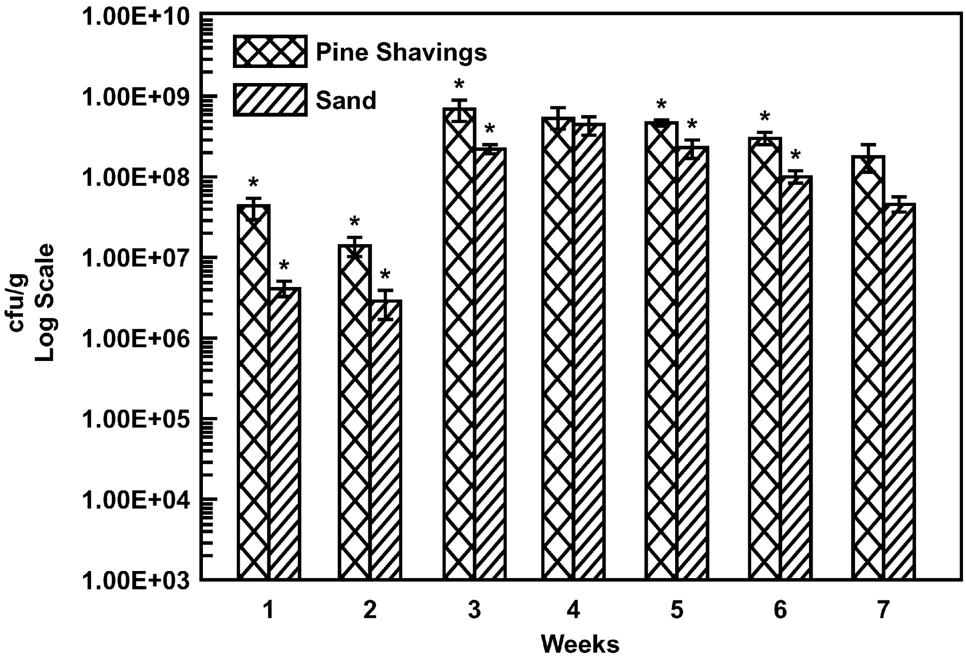

FIGURE 6. Number of anaerobic bacteria from experiment 2. Statistical differences for that week between litter types are denoted by *≤ 0.05.

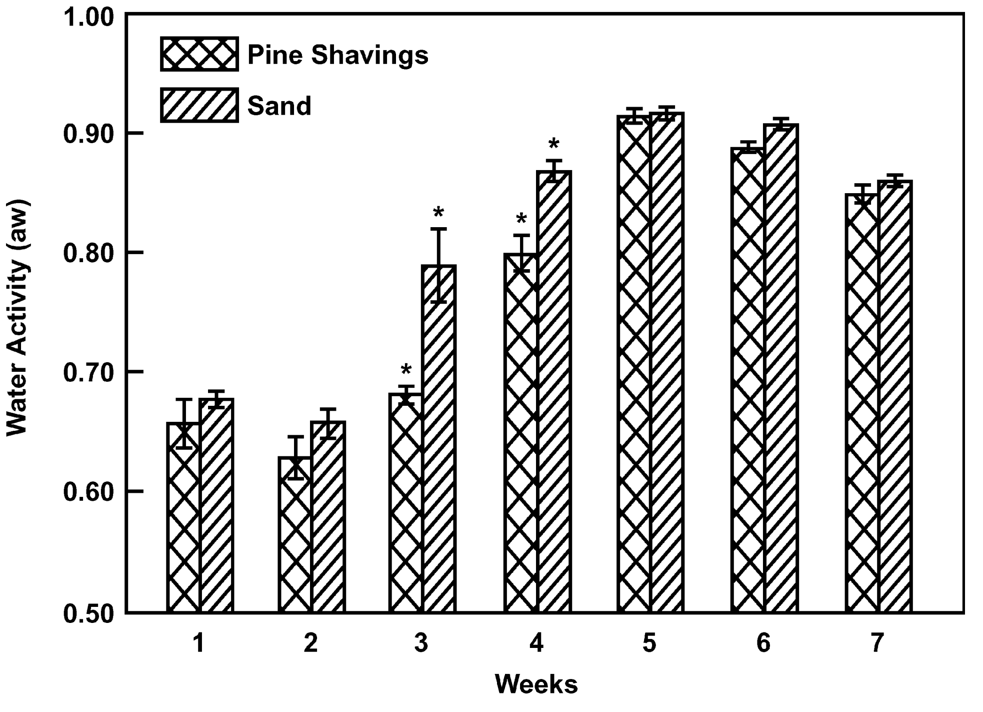

FIGURE 7. Water activity (aw) in experiment 2. Statistical differences for that week between litter types are denoted by *≤ 0.05.

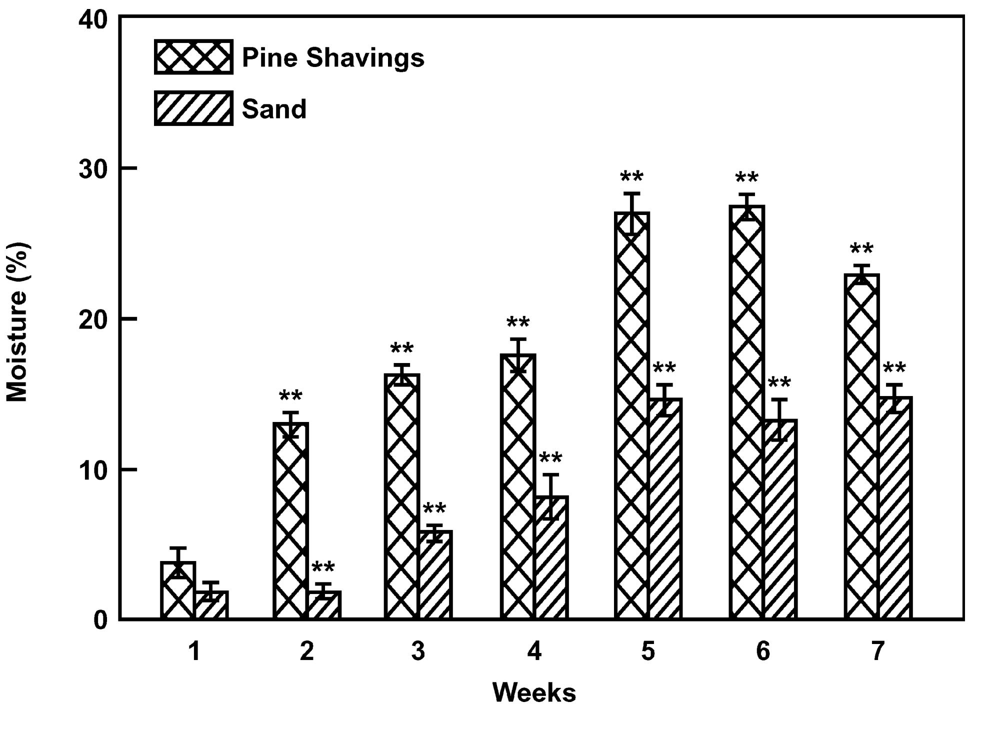

FIGURE 8. Percentage moisture content of pine shavings and sand litter in experiment 2. Statistical differences for that week between litter types are denoted by **≤ 0.01.

experiment. This result might be explained by the fact that the litter used in experiment 2 was not freshly placed, whereas the litter used in experiment 1 was freshly placed. Although bacterial levels were not determined for experiment 2 prior to chick placement, it is expected that the background microflora would be higher than what was observed in the freshly placed litter.

Previous work by Bilgili et al. [1, 4] has shown that aerobic bacterial counts on sand are lower than pine shavings [1] or are the same [4]. Those findings are similar to the results reported here for the aerobic bacterial counts. The enteric and anaerobic bacterial counts were also generally lower on sand than on pine shavings. The observed differences between sand and pine shavings could be associated with the lower surface area to weight ratio of sand when compared with pine shavings. Sand, being inorganic, contains few nutrients that could be utilized by bacteria and, thus, would tend to lead to lower bacterial numbers. Pine shavings, being organic, would contain nutrients that could be utilized by some bacterial species. Additionally, sand may lack binding sites for bacteria.

Water activity increased in both litter types until wk 5 when it reached a plateau of 0.90 (Figure 7). Interestingly, water activity in sand was significantly higher than in pine shavings for wk 3 and 4. This is probably due to the inability of sand to bind free water, whereas, conversely, pine shavings can bind free water. This inability of sand to bind water would lead to more free water being in the litter matrix, which would give a higher water activity reading. Typically, the majority of bacteria are inhibited by water activity less than 0.91. Water activity for the first 4 wk was below 0.90 and, hence, inhospitable to bacteria (Figure 7). The high bacterial counts observed in Figures 4 to 6 can be attributed to feces and mixing of the litter by the birds.

Moisture level is a measure of the amount of water bound to the litter. Moisture levels were significantly higher ($P < 0.01$) in pine shavings compared with sand in every sample other than the initial sample (Figure 8). Typically, pine shavings had 10% more bound water than sand.

Results from Pearson's correlation indicate that aerobic and anaerobic bacterial levels corresponded to both water activity and percent moisture (data not shown). Interestingly, this correlation was not observed with the enteric bacteria, which was due to the high standard error that was observed on the medium.

From the data reported here, as well as from other previous reports [1, 4, 11], it can be concluded that, bacteriologically, sand is a good alternative to pine shavings. Further work is needed to determine if there are differences in bacterial species present in each litter type, because it has been documented that poultry litter is a source of pathogenic bacteria for birds and humans [12].

CONCLUSIONS AND APPLICATIONS

1. After birds had been on the litter for 3 wk, bacterial levels for both litter types reached a plateau that was maintained until the trial was terminated.
2. Bacteriologically, sand is equivalent or slightly superior to pine shavings when used as poultry litter.
3. Percentage moisture was higher in pine shavings than sand, although water activity was the same or lower in pine shavings.

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