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SHORT COMMUNICATION



# Composting of laying hen manure with the addition of a yeast probiotic

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**ABSTRACT**

This study assessed the effect of inclusion of a yeast probiotic on the counts of *lactobacilli*, total aerobes (TA), total coliforms (TC), *E. coli* and *Salmonella* in the composting of laying hen manure. The yeast probiotic concentration (0, 7.5 and 15% wet basis) in the composting effect was defined as factor A and the fermentation time (FT) (0, 7 and 23 days) was defined as factor B. A completely randomised design with a factorial arrangement of $3 \times 3$ and four replicates was used. An interaction was found in *Salmonella* and pH ($p < .0001$). The lowest ($p < .05$) *Salmonella* counts were obtained at day 7 in the treatments with 7.5% and 15%, respect to the control. The lowest values ($p < .05$) for pH were obtained at day 23 in the treatments containing 0 and 15%. *Lactobacilli*, *E. coli*, TC, whereas TA counts were affected only by fermentation time ($p < .05$). In all treatments, lactobacilli increased ($p < .05$) by day 7. *E. coli* and TC counts decreased ($p < .05$) across the fermentation time and TA remained constant for the first seven days. ($p < .05$). Anaerobic fermentation of poultry litter over 23 days is sufficient to lower the pH and eliminate pathogenic microorganisms.

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## Introduction

Laying hen manure is mainly composed of excreta of egg-producing birds and the amount produced by laying birds is estimated to be approximately 120 kg per 1000 hens. Due to its high content of non-protein nitrogen and minerals, it can be included in the ruminant feed. The poultry litter fed to ruminants was briefly banned in December of 2003 by the FDA due to problems of bovine spongiform encephalopathy transmission but lifted in October 2005 (Daniel and Olson 2005). However, its high content of pathogenic bacteria, such as *colibacille* and *Salmonella,* poses the risk of microbial contamination and can cause gastrointestinal diseases in the animals that consume it and thus safe treatments are needed to eliminate the pathogens before feeding as a non-protein supplements to ruminants (Ruiz-Barrera et al. 2017). The present work is focussed on the biological treatment of fermentation. Investigations have observed that poultry excreta after undergoing *in vitro* fermentation, solid-state fermentation or composting show a reduction in the content of pathogenic bacteria such as *Salmonella* and *colibacille* (Berradre et al. 2009).

From a practical standpoint, the inclusion of yeast probiotics to chicken manure may adequately kill the pathogens (Sheffield et al. 2014) while preserves the nutritional value of the waste since microbial destruction of uric acid is avoided. Sheffield et al. (2014) added three types of microorganisms to chicken manure (*Saccharomyces cerevisiae*; Rhodopseudomonas *palustris* and *Lactobacillus casei*) and found total reduction of *Salmonella typhimurium* at 9 days post-inoculation. The yeast (*Candida norvegensis*) containing the probiotic under study was isolated and identified by Castillo-Castillo et al. (2016) which demonstrated a favourable effect as a non-*Saccaromyces* yeast on some fermentative parameters. Therefore, the objective of the present investigation was to evaluate the addition of the yeast probiotic at different concentrations (0%, 7.5% and 15%) and fermentation durations (0, 7 and 23 days), on hen manure silage to reduce its

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pathogenic microbiological load and thus make it more suitable for consumption by domestic animals.

## Materials and methods

### Experimental design

Approximately, 240 kg of hen manure and molasses in a ratio of 80:20 were mixed with water and the yeast probiotic ($3 \times 10^6$ CFU/mL yeast). Depending on the treatment, the mixture was distributed in 24 nylon bags with a capacity of 10 kg. All bags were hermetically sealed and incubated at room temperature.

Following treatments were used: A control (C) consisting of a mixture of hen manure-molasses +15% water; Treatment 1 (T1), a mixture of hen manure-molasses +7.5% Yeast +7.5% water; and Treatment 2 (T2), a mixture of hen manure-molasses +15% yeast. The sampling was performed at day 0, 7 and 23. At each sampling time, four bags were removed per treatment, except for time zero where the samples were taken directly from the initial mixtures of each treatment. Chicken manure temperature was measured at each sampling time by inserting a digital thermometer to a depth of 6 cm at three different locations in the bag. The contents of each bag were collected and homogenised and 200 g of the sample was placed in plastic bags. From a sample, 10 g was taken from the homogenised substrate of each bag, placed in a sterile 250-mL Erlenmeyer flask and 90 mL of sterile distilled water was added, stirred for 30 min and filtered through sterile gauze.

### pH, lactic acid and microbial counts

Of the supernatant, pH was determined with a HANNA pH metre, lactic acid according to the colorimetric method described by Madrid et al. (1999), total aerobics and total coliforms/*E. Coli* counts were determined in a Petrifilm (3M, Minneapolis, MN) following the procedure described in the manual. The plating of lactobacilli was done in MRS agar at 37 °C for 24 h. *Salmonella* spp. was enumerated via viable cell counts in bright green agar added with 0.25 μg $mL^{-1}$ novobiocin and incubated at 37 °C for 24 h. Novobiocin was added to prevent the growth of other enterobacteria and to promote the growth of *Salmonella* since this bacterium is naturally resistant to this antibiotic. The colonies of *Salmonella* spp. were subjected to serological identification (Antiserum Poly A-I and VI).

### Chemical analysis

Dry matter (DM), ash and crude protein (CP) were determined in the rest of the fermented product collected in the plastic bags (190 g) according to AOAC (1995). Lipid content (L) was determined in an Ankom XT10 extraction system. Total energy content (TE) was measured with a PARR 6100 calorimeter.

### Statistical analysis

A completely randomised design with a factorial arrangement of $3 \times 3$ and four replicates was used to test the effects of fermentation time (FT). The effect of the yeast probiotic concentration (0%, 7.5% and 15% on a wet basis) in the ensiling was defined as factor A and the fermentation time (FT) (0, 7 and 23 days) was defined as factor B. The statistical analysis of the measured variables was performed by the PROC GLM method of SAS (2002) to carry out ANOVA and comparing the means between treatments. The comparison of means was made using the predicted difference procedure of SAS (2002).

## Results and discussion

### Temperature

The changes in the temperature of the incubation bags showed that there was an effect of the fermentation time factor ($p < .0001$; SE = 0.15) but not of the 7.5% and 15% yeast treatments ($p = .7108$; SE = 0.15) or its interaction ($p = .5387$; SE = 0.26) during the incubation. The increase in temperature from 14.9 °C at the beginning of incubation to 28.8 °C and 42.5 °C at day 7 and 23 of the incubation, respectively, suggest that the heat produced by the exothermic reactions due to the microbial activity in the fermentation was trapped in the substrate.

### pH, lactic acid and microbial counts

For pH, an interaction effect ($p < .0001$) was found between the concentration of yeast and fermentation time (Table 1). The pH decreased as the fermentation time elapsed in all three treatments, showing the lowest values on day 23 of fermentation. A reduction in the pH values at day 7 of fermentation in the treatments with 7.5% and 15% of yeast could be related to the accumulation of organic acids such as lactic acid and acetic acid produced by the microorganisms involved in the fermentation and also due to the consumption of $NH_4$ ions (Elías and Herrera 2008). Prior et al. (1992) mentioned that the pH of a substrate may

**Table 1.** Effect of the yeast probiotic concentrations and fermentation time on pH, *Salmonella* counts, ash, total energy and lipid content of laying hen manure.

| Item | Yeast concentration, % | Sampling time | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | | day 0 | day 7 | day 23 | | |
| pH | 0 | 8.49[a] | 6.84[b] | 5.65[d] | 0.30 | .0001 |
| | 7.5 | 8.58[a] | 6.18[c] | 5.85[e] | | |
| | 15 | 8.63[a] | 6.52[c] | 5.69[d,e] | | |
| *Salmonella*, $Log^{10}$ CFU/mL | 0 | 4.50[a] | 3.86[a] | 0[b] | 0.31 | .0001 |
| | 7.5 | 4.01[a] | 0.5[b] | 0[b] | | |
| | 15 | 4.47[a] | 0.83[b] | 0[b] | | |
| Ash | 0 | 43.14[d] | 42.29[c,d] | 43.04[d] | 0.52 | .02 |
| | 7.5 | 41.88[c,d] | 39.68[a] | 41.09[a,c] | | |
| | 15 | 40.66[a,c] | 42.22[c] | 41.28[b,c] | | |
| Total energy | 0 | 2566.7 | 2503.9 | 2555.1 | 33.19 | .28 |
| | 7.5 | 2544.7 | 2643.0 | 2572.3 | | |
| | 15 | 2540.1 | 2448.5 | 2613.5 | | |
| Lipid content, % | 0 | 0.94 | 1.23 | 0.86 | 0.16 | .17 |
| | 7.5 | 0.95 | 0.98 | 1.40 | | |
| | 15 | 1.08 | 1.05 | 1.23 | | |

[a,b,c,d,e]Means with different superscripts between rows and columns are significantly different at $p < .0001$; SEM: standard error of means.

decrease due to the presence of organic acids, such as lactic and acetic acid, produced by an incomplete oxidation or the use of ammonium ions, depending on the metabolic activity the substrate used and the buffering capacity of the medium.

There was no interaction between the addition of yeast and fermentation time in the lactic acid production (Table 2). The lactic acid content was higher on day 23 ($p < .05$) than day 0 of the fermentation and no effect of the yeast concentration was found. The increase of lactic acid by the fermentation time can be attributed to the catabolism of carbohydrates by lactic acid producing bacteria.

The bacterial counts for *Salmonella* indicated that some interaction occurred between the treatments and the duration of fermentation ($p < .001$) (Table 1). The *Salmonella* count decreased significantly on day 7 with respect to day 0 of the fermentation in the treatments with 7.5% and 15% of yeast unlike the treatment without yeast (0%), which was maintained with high counts of *Salmonella* until day 7. No *Salmonella* was noticeable by day 23 of fermentation in the three treatments. The elimination of *Salmonella* in the treatments with 7.5% and 15% of yeast after day 7 of fermentation could be related to the fact that yeast is a product rich in organic acids such as lactic acid and acetic acid (Castillo-Castillo et al. 2016), which under certain conditions induce cell death (Ludovico et al. 2001). The increase in these metabolites possibly inhibited the growth of this and other microorganisms present in the fermentation medium generating an antagonistic environment for undesirable putrefactive and pathogenic bacteria (Ludovico et al. 2001). These acids cause toxicity that is closely related to the pH of the medium, as at a low pH, the acids exist in their non-dissociated forms, which enter the bacterial cells by means of passive diffusion (Geros et al. 2000) and within the bacterial cytoplasm they dissociate due to the neutral pH releasing large amount of protons to the medium, causing acidification of the bacterial cytoplasm, altering bacterial metabolism, and causing cell death (Schüller et al. 2004). Sheffield et al. (2014) added three types of microorganisms to chicken manure and found total reduction of *Salmonella typhimurium* at 9 days post-inoculation, conforming to the results found in the present study.

**Table 2.** Effect of the yeast probiotic concentration and fermentation time on microorganism counts, crude protein and lactic acid of laying hen manure.

| Item | *Lactobacilli* | *E. coli* | *T.C* | *T.A* | C.P | L.A, µg/g MS |
|---|---|---|---|---|---|---|
| Yeast inclusion level, % WB | | | | | | |
| 0 | 8.72 | 2.91 | 2.91 | 7.93 | 26.30[a] | 2.049 |
| 7.5 | 8.76 | 3.24 | 3.12 | 7.77 | 28.29[b] | 2.054 |
| 15 | 8.88 | 3.39 | 3.10 | 7.76 | 27.88[b] | 2.047 |
| SEM | 0.15 | 1.85 | 0.35 | 0.10 | 2.49 | 0.003 |
| Time, Days | | | | | | |
| 0 | 8.40[a] | 5.92[a] | 4.63[a] | 8.55[a] | 26.01[a] | 2.042[a] |
| 7 | 9.64[b] | 3.99[b] | 2.79[b] | 8.46[a] | 27.84[b] | 2.050[a,b] |
| 23 | 8.63[a] | 0.33[c] | 1.71[c] | 6.46[b] | 28.62[b] | 2.057[b] |
| SEM | 0.15 | 1.09 | 0.35 | 0.10 | 0.86 | 0.003 |

[a,b,c,d,e]Means with different superscripts within rows are significantly different at $p < .05$; SEM - standard error of means.

As shown in Table 2, only the effect of the fermentation time was observed on other microbial counts studied. In the case of *lactobacilli*, an increase in the bacterial count was found on day 7 of the fermentation with respect to day 0 and then decreased on day 23 ($p < .05$). The increase in lactobacilli count was accompanied with an optimum growth pH throughout the fermentation time, and the addition of molasses as an energy source served as a substrate. In the case of the total aerobic microorganisms ($p < .05$), there was a decrease in the counts on day 23 with respect to the

previous durations of fermentation. Similarly, the elimination of *Salmonella, E. coli* and total coliforms could also be attributed to the acidification of the medium due to the accumulation of lactic acid produced by the lactic acid flora. *Lactobacilli* are known to produce high bacteriocins and organic acids which have a positive effect on the elimination of pathogenic bacteria such as *Salmonella* and coliforms (Coventry et al. 1997). In addition to the change in pH, the influence of soluble carbohydrates (molasses) as an energy source caused the *lactobacilli* to thrive in the acidic environment that eliminated the pathogenic bacteria. *Lactobacilli* promote the accumulation of organic acids such as lactic acid, which reduces the pH of the medium and inhibit the growth of gram-positive and gram-negative bacteria. These acids in their non-dissociated form penetrate the microbial cells in an alkaline environment leading to microbial cell death or growth inhibition (Ramirez et al. 2011).

### *Chemical analysis*

Table 1 shows ash, total energy and the lipid contents in chicken manure silage. An interaction effect with the yeast concentration and the fermentation time ($p < .0001$) was noted only for ash content. Energy and lipids contents were not affected by the yeast concentration nor by the fermentation time. The lowest values of ash were found in the 7.5% treatment at day 7 and 23 of the fermentation and in the treatment with 15% of probiotic at day 0. The reduction in the ash content could be related to the use of minerals by the microorganisms for the synthesis of microbial protein.

There was no interaction effect on CP, but its values were increased through fermentation time ($p < .05$) and yeast addition ($p < .05$) (Table 2). These results may be related to the lowering of pH in the medium, where ammonia ($NH_3$) produced in the gastrointestinal tract of the hen was transformed to ammonium ions ($NH_4^+$) (non-volatile form) and thus retained in the manure. It could also serve as the nitrogen source to the bacteria and yeasts and other microorganisms for the synthesis of protein (Calderon et al. 2005).

## Conclusions

Fermentation of laying hen manure over 23 days is sufficient to lower the pH and to eliminate *E. coli* and *Salmonella*. The addition of the probiotic yeast at 7% and 15% resulted in the reduction of *Salmonella* to undetectable levels by day 7 and preserved the nitrogen content of manure. The treatment of laying hen manure compost rendered it a pathogen-free, safe and nitrogen- and mineral-rich ruminant ration.

## Ethical approval

All experimental procedures were in accordance with Autonomous University of Chihuahua Bioethical and Welfare Comitee.

## Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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