mTurmaric Powder (*Curcuma longa*) Affected on Microbial Dynamics, Ammonia Emission Rate and Some Chemical Properties of Layer Litter

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Abstract

Turmeric (*Curcuma longa*) (TM) is a natural antiseptic showed good antimicrobial activity against microbes. Study was attempted to compare the microbial dynamics and ammonia emissions of turmeric amended layer litter by wiping out conditions that favor microbes at several application levels. Three months old well managed layer litter was subjected for the study. A litter sample (2kg) was taken and well mixed in a black poly bag and 4 samples were taken for the determination of initial pH and moisture (%). Turmeric powder was used to mix with litter at 5 different levels; 0, 1, 3, 5 and 8% (w/w). After mixing, 150g of mixed litter was placed in a container for each level of the 4 replicated samples. Then samples were incubated for 5h, and the emitted NH_3 was trapped with boric acid and then titrated with *HCL* to determine the NH_3 emissions. The emission rate was calculated as milligrams of NH_3 emitted/ kilogram of fresh litter/hour. One hundred litter samples (5 samples from each replicate) were incubated for 5h and analyzed for total plate count (TPC), yeast and mould count (YMC), nematode count (NEM), litter pH and moisture. TPC and YMC were determined by using microbial cultures on PCA media and PDA media, respectively. Nematode count was determined using Mc Master Chamber. Irrespective of the application level of TM, significant ($p<0.05$) reduction of bacteria count and YMC were found. Reduction of bacteria were 42.42, 60.6, 95.15 and 98.18%, for 1, 3, 5 and 8% application levels respectively. Significant reduction of YMC (approximately 100%) was observed at 8% application of TP compared to 0% application resulted numerous count. The reduction of total nematodes was 21.87, 34.37, 50 and 53.12% for 1, 3, 5 and 8% applications respectively. The pH was significantly ($p<0.05$) reduced with the increment of TM level showing highest (9.34) at 0% and the lowest (9.07) at 8% application level. Moisture (%) was significantly increased in 1% (44.53%) and 8% (45.18%) applications. There was a trend in reducing NH_3 emission with all applications but not significantly different among treatments. Application of TM (>3%) was effective in reducing pH (towards acidic). It was concluded that the bacterial and fungal counts of layer litter can be effectively reduced with the application of 8% TP there by a possible reduction in ammonia emission by altering favourable conditions for microbes in the litter such as pH and moisture.

Keywords: *Ammonia Emission, Nematode Count, Plate Count, Turmeric, Yeast and Mould Count*

INTRODUCTION

Poultry litter has been shown to harbor bountiful numbers of bacteria. A byproduct of commercial layer chicken grow out is thousands of tons of litter. This litter is the material on which the chickens spend their entire life. The quality of the in-house environment is highly dependent upon litter quality. The litter environment is ideal for bacterial growth and ammonia production. The two factors that influence litter conditions are manure and moisture. The presence of microbes in litter may leads to contaminated processed carcasses or by increasing the microbial load of skin and feathers or by providing a source for upper gastrointestinal contamination during pre-harvest feed withdrawal both broilers and layers (Bennett *et al.*, 2003).

Use of Turmeric (*Curcuma longa*) [TM] as a disinfectant was seen in many cultures especially throughout Asia. Several research studies suggested that turmeric can help to combat microbial activities. Aqueous extracts of turmeric showed good antimicrobial activity against common bacteria, fungi, viruses, yeast, and round worms (J. of Pharmacology and Toxicology, 2009; Natural Products Report, 2008). Curcumin is the main active ingredient responsible for numerous activities. Researchers also noted another active component called curcumoids which gives its signature yellow color. (J. of Biochemical Pharmacology, 2008). The antimicrobial activity of this versatile compound often seems to depend on the type of extract, and the type of infection. Different studies reveal different degrees of antimicrobial activity, with essential oils and aqueous extracts of TM showing the greatest potential. Overall, however, the scientific literature offers promising information for the use of TM in the prevention and treatment of microbes. The antimicrobial activity of TM was used from thousands of years in civilizations over the world to disinfect diverse substances in various ways. Despite its anti inflammatory activities, commonly used as a foodstuff, cosmetic and medicine.

Total litter bacteria concentrations fall within the range of 10^{10} to 10^{11} colony forming units (cfu) per gram of litter (Scheffererle, 1965; Terzich *et al.*, 2000). Total aerobic bacteria counts are lower at 10^8 to 10^{10} (Macklin *et al.*, 2005; Lu *et al.*, 2003). These numbers can vary with the age of the litter and age of the birds, as shown by Macklin *et al.*, 2005. Fresh litter and bedding were found to have 10^5 cfu/g, as soon as birds were placed on the litter, the numbers of bacteria increased by several orders of magnitude to 10^8. The bacterial numbers peaked at 10^{10} during 6th week for new bedding and 4th week for litter used for more than one flock. The researchers found that after aerobic bacteria peaked at 10^{10} cfu/g of litter bacteria numbers either remained steady or declined until the end of each poultry flock grow-out.

Breakdown of nitrogenous compounds in litter materials is another task of litter microbes. Through this breakdown, they produce Ammonia. Factors that directly control NH_3 formation are pH, temperature and moisture level of the litter (Elliot and Collins, 1982; Carr *et al.*, 1990). Ammonia emissions from poultry litter not only cause environment problems, but also detrimental to performance, health, behavior and welfare of birds. Health and welfare problems associated with high NH_3 concentrations include damage to the respiratory tract (Nagaraja *et al.*, 1983), increased susceptibility to Newcastle disease (Anderson *et al.*, 1964), incidence of airsacculitis (Oyetunde *et al.*, 1976), increased Mycoplasma gallisepticum (Sato *et al.*, 1973), and incidence of keratoconjunctivitis (Bullis *et al.*, 1950). High NH_3 concentrations in poultry houses reduce growth rate of the birds (Reece *et al.*, 1979, 1981; Moore *et al.*, 1999), feed efficiency (Caveny and Quarles, 1978; Caveny *et al.*, 1981) and egg production (Deaton *et al.*, 1984). Atmospheric NH_3 pollution plays an important role in acid rains and the dominant source of NH_3 in Europe is livestock waste (Ap Simon *et al.*, 1987). Consequences of high NH_3 concentrations in poultry facilities on human health are also a matter of concern (Miles *et al*, 2004).

The overall objective of the study was to investigate the applicability of TM as a litter amendment to diminish microbial populations, ammonia emission rates from poultry litters by antimicrobial effects and wiping out conditions that favor microbes such as pH, moisture *etc.*

MATERIALS AND METHOD

Three months old well managed layer litter was subjected for the study. A representative sample of a litter (±2kg) was obtained from the layer unit of the Faculty of Agriculture, University of Ruhuna, put into a black polythene bag, sealed and brought to lab. Then the litter sample was mixed well keeping inside the black polythene bag. TM powder available in the local market was used to mix with litter (w/w) at 5 different levels; 0, 1, 3, 5 and 8% (1g TM powder /100g litter (1:99). After mixing with TM powder, 150g of mixed litter was placed in a container for each level of the replicated sample and incubated at 30°C for 5h and analyzed for total plate count (TPC), yeast and mould count (YMC), total nematode count (NEM), litter pH (Brake *et al.*,1992), litter moisture and litter ammonia emission rate (Moore *et al.*, 1996). TPC and YMC were determined by using microbial cultures on PCA and PDA media respectively. The PCA and PDA were prepared according to standard procedure and were sterilized at 121°C for 20 min in autoclave. The 10^{-1} dilution was prepared by mixing the 1ml of litter sample of each treatment with 9ml of MRD. Using separate sterile pipettes, serial decimal dilutions of 10^{-2}, 10^{-3} and 10^{-4} were prepared by transferring 1ml of previous dilution to 9ml of diluents. Then 15ml of media (cooled to 45±1°C) were poured into to each plate and the plates were allowed to solidify. Then the appropriately marked Petri dishes were inoculated with 1ml of each dilution separately and in duplicates. Then the inoculated Petri dishes were incubated at 37°C for 48hrs (TPC) and at 28°C for 5 days (YMC). Colonies were counted using the colony counter. Then the TPC and YMC were calculated.

Nematode count was determined using Mc Master Chamber. Ground litter sample of 3g was dissolved in saturated 47ml of NaCl solution for each replicate. Then a drop of solution was observed under dissecting microscope for Nematodes. Litter NH_3 emissions were determined as described by Moore *et al.*, (1996), with slight modifications. The containers used to put litter samples were equipped with air inflow and outflow. Samples were incubated at 30°C for 5h. Air was continuously passed through each flask, and NH_3 volatilized from litter samples in the containers were trapped in 100ml of 0.32N H_3BO_4 solutions. The trap was titrated with 0.1N HCl to determine the NH_3 emission. The emission rate was calculated as milligrams of NH_3 emitted /kilogram of fresh litter/hour. Complete randomize design was used with 4 replicates. Data were analyzed using GLM procedure of SAS (SAS Institute, 1985). Effects were considered significant when $p<0.05$. Means were compared using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

It was visually observed that TM powder mixed litter samples were turned in to yellowish color at each level of mixing. This typical color gives by the active ingredient curcumoids.

Comparison of microbial dynamics: Very significant removal of total bacteria and YMC were found after treatment with different level of application of TM powder (Table 1). The reduction of total bacteria were 42.42, 60.6, 95.15 and 98.18%, for 1, 3, 5 and 8% applications of TM powder respectively. The significant reduction of YMC was observed when treated with 8% of turmeric powder compared to numerous counts present in no treatment of TM powder.

Significant reduction of nematode count also observed where 21.87, 34.37, 50 and 53.12% were recorded for 1, 3, 5 and 8% applications levels. According to many past research findings, the volatile oil of TM reported for the antibacterial and antifungal activities.

Comparison of ammonia emission rates and litter parameters: Basically NH_3 in litter is formed through the microbial breakdown of undigested proteins and excretory uric acid which is present in feces of birds. Conditions that favor microbial growth will result in increased ammonia production. These conditions include warm temperature, moisture, pH in the neutral range or slightly higher (7.0–8.5) and the presence of organic matter (Pokharel, 2010). Effect of turmeric at different level of applications with poultry litter for NH_3 emission, moisture % and pH values are shown in Table 1.

The pH of all application levels were significantly different with the control. Increasing the level of application of TM shows a significant reduction of pH value. Pokharel (2010) also found that the NH_3 emissions depend on how much of the ammonia-nitrogen in solution reacts to form ammonia (NH_3) versus ionized ammonium (NH_4^+), which is nonvolatile and lowering the pH in litter can reduce the NH_3 emission by directing the equilibrium between NH_3 and NH_4^+ towards the NH_4^+ ions. Vitharana *et al.* (2012) reported that high compactness of the layer type litter may also be a reason for lower NH_3 emission from layer litters due to its higher bulk density. This higher bulk density is a result of low moisture content in the litter. Levels 1% and 8% have significant increase of moisture and the 3% level has significant decrease in moisture than the control. Practically lowering of moisture in a litter may cause lowering of NH_3 production (Pokharel, 2010). The emission of NH_3 is lowered with the increase of turmeric levels in samples but not significant. High bulk density in the litter samples may cause this. It was observed that there is a trend of decreasing of NH_3 emission within the treatments.

Jingrang *et al.* (2003) reported that total aerobic bacteria in poultry litter were detected by culture at 10^9 CFU/g (Colony Forming Units per Gram) of material. Enteric bacteria such as *Enterococcus* spp. and coliforms composed 0.1 and 0.01%, respectively, of the total aerobic cultivatable bacteria in poultry litter; no *Salmonella* strains were detected by culture. Twelve families or groups were identified with lactobacilli and *Salinococcus* spp. forming the most abundant groups. In addition Jingrang *et al.* (2003) detected many bacterial sequences for organisms, such as *Globicatella sulfidofaciens*, *Corynebacterium ammoniagenes*, *Corynebacterium urealyticum*, *Clostridium aminovalericum*, *Arthrobacter* sp., and *Denitrobacter permanens*, which may be involved in the degradation of wood and cycling of nitrogen and sulfur by producing NH_3 and SO_2.

However Vitharana *et al.* (2012) also reported that emission of NH_3 from broiler litter is three times higher than that of layer litter. Due to this lower emission from this layer litter samples we can suggest that the application of turmeric powder to broiler type litters is more effective to reduce NH_3 emission. Ammonia emission in the poultry farms can be controlled by reducing litter moisture, dietary manipulations such as use of low crude protein diet high fibrous diet and dietary additives to appropriate NH_3, use of bio-filters and litter treatment with acidifiers such as alum, sodium bisulfate, calcium sulfate, magnesium chloride (Pokharel, 2010). Further researches in this topic is much needed to apply this techniques in large scale operations thus to reduce the environmental pollution.

CONCLUSION

The bacterial and fungal counts of layer litter can be effectively reduced with the application of 8% turmeric powder (w/w). The pH values of treatments were significantly high and the moisture % was significantly lower compared with the control. But the NH_3 emissions within the treatments were not significantly different but having a reducing trend. It was concluded that application of turmeric higher than 3% (w/w) to poultry litter as an amendment is effective in lowering pH and the NH_3 emission.

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Table

Table 1: Effect of turmeric at different levels of application with poultry layer litter for NH3 emission, moisture%, and pH values microbial properties

Parameter	Level of Turmeric application				
	0%	1%	3%	5%	8%
pH	9.34a±0.01	9.25b±0.01	9.13c±0.01	9.12c±0.01	9.07d±0
Moisture	44.06c±0	44.53b±0.05	43.61d±0.02	44.10c±0.01	45.18a±0.02
NH3	0.59±0.26	0.21±0.22	0.29±0.18	0.19±0.1	0.07±0.14
TPC	3.3×10^4	1.9×10^4	1.3×10^4	1.6×10^3	6.0×10^2
YMC	TNC	2.4×10^4	1.3×10^4	2.1×10^3	1.1×10^2
NEM	400	312	262	200	187