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Microbial and Oligosaccharides Treatments of Feces and Slurry in Reducing Ammonia of the Poultry Farm

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ABSTRACT

This study was conducted to investigate the effectiveness of *Lactobacillus* sp and fructooligosaccaride (FOS) to reduce the volatile ammonia formation from chicken excreta and layer slurry. For each treatment-replication, 150 g of fecal material were collected from the poultry farm and placed in 500 ml beaker glass. The fecal sample was then treated with 2% *Lactobacillus* sp ($2.6x10^6$) cfu/g and 2% FOS and covered with plastic wraps. The volatile ammonia contents and pH were measured after one hour of standing (0 d) and repeated at 48 h intervals for 6 d. For the dropping slurry study, 300 g of each layer dropping slurry sample were used. Results indicated that 2% *Lactobacillus* sp or FOS supplementations in the feces and dropping slurry after 1 h up to 6 d reduced the ammonia odor formation, fecal pH, and moisture content. The *Lactobacillus* sp and FOS treated manure resulted in increasing *Lactobacillus* sp count and reducing in *E. coli, Salmonella,* and *Campylobacter* in 6 days for both feces and layer dropping slurry. In addition, reducing moisture content was observed in treated manure. It is concluded that *Lactobacillus* sp and FOS reduced the volatile ammonia formation and pathogenic bacteria from chicken excreta and layer slurry.

Key words: Lactobacillus, fructooligosaccaride, ammonia, feces, poultry farm

ABSTRAK

Penelitian ini bertujuan untuk mempelajari penggunaan *Lactobacillus* sp dan fruktooligosakarida (FOS) dalam menurunkan ammonia feses dan limbah feses ayam (slurry). **Feses sebanyak 150 g dikumpulkan dari kandang ayam dan ditempatkan pada 500 ml** *beaker glass* **untuk masing-masing perlakuan. Feses tersebut dicampur dengan 2% *Lactobacillus* sp ($2,6 x10^6$) cfu/g dan 2% dari berat feses FOS, permukaan *beaker glass* ditutup dengan plastik. Kadar ammonia dan pH diukur setelah 1 jam perlakuan (0 hari) dan diulang setiap 48 jam selama 6 hari pada beaker yang sama. Penelitian limbah feses ayam** *(slurry)* **menggunakan 300 g untuk masing-masing perlakuan. Hasil penelitian menunjukkan bahwa pemberian *Lactobacillus* sp dan FOS pada feses dan limbah feses sampai 6 hari perlakuan dapat menurunkan kadar ammonia, pH, dan kadar air feses. *Lactobacillus* sp dan FOS dapat meningkatkan jumlah *Lactobacillus* sp dan menurunkan jumlah *E. coli, Salmonella,* dan *Campilobacter* selama 6 hari perlakuan baik pada feses maupun pada limbah feses. Perlakuan juga berpengaruh terhadap penurunan kadar air pada feses dan slurry. Dapat disimpulkan bahwa perlakuan *Lactobacillus* sp dan FOS dapat menurunkan kadar ammonia dan bakteri patogen pada feses dan limbah *(slurry)* feses ayam**.

Kata kunci: Lactobacillus, fruktooligosakarida, amonia, feses

INTRODUCTION

Currently, there is increased concern related to ammonia emission from poultry operations (Singh *et al*., 2009). Ammonia is the aerial pollutant from livestock operations, with domestic animals being the largest global contributor of atmospheric NH_3 emissions (Aneja *et al*., 2006) and poultry (including laying hens) being the largest contributor among domestic animal in the United State (EPA, 2004). Therefore, controlling ammonia in poultry house is essential to ensure a better environment, better health, performance of the birds and operators.

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Concern about ammonia in poultry production is not new. Traditionally the concern has been with the level of ammonia in the poultry house. High ammonia concentrations affect bird performance, including poor feed efficiency, blindness, and respiratory problem (Pescatore *et al*., 2005). Beker *et al*. (2004) also reported that gain to feed ration was depressed at 60 ppm ammonia and indicated that ammonia in poultry houses lowers performance and may increased diseased susceptibility. Li *et al*. (2008) mentioned that ammonia emission from animal feeding operations not only decrease the fertilizer N value of the manure but also lead to environmental pollution.

Concern regarding ammonia (NH_3) concentrations within poultry housing, ammonia emissions from poultry operation, and subsequent negative environmental effects of excessive ammonia emission have emphasized the need for research to find ways to reduce the volatilization of ammonia from poultry facilities (Coufal *et al*. 2006). Several direct applications of chemicals have been tested for their ability to control or reduce ammonia release from poultry manure. Li *et al.* (2008) reported that application zeolite, AL$^+$Clear (aluminium sulfat, Ferix-3 (ferric sulfate, Poultry Litter Treatment (PLT, sodium bisulfate) onto nearly 1-d-old laying hen manure led to considerable reduction in ammonia (NH_3) emission from store manure. Singh *et al.* (2009) mentioned that various abatement method, including dietary manipulation, chemical amendment of litter, and improvement in ventilation system management have been used to control ammonia concentrations in the housing, however these method are perceived to be expensive, to impair bird growth, or to add pollution in some other form.

The probiotics can be used as growth promoter in the poultry diet: (Azis *et al*., 2010). Moreover, *Lactobacilli* and fructooligosaccharide (FOS) have been used in poultry diets to reduce urease activity in poultry intestines, which in turn may reduce fecal ammonia production. Yusrizal & Chen (2003) mentioned that these microorganisms produce short chain fatty acids, creating an acidic environment which suppresses the growth of putrefactive proteolytic bacteria. Fructooligosaccharide also have shown the ability to decrease colonization of bacteria such as *E. coli* and *Salmonella,* while increasing the growth of non-pathogenic microorganisms.

However, no report is available on the effect of *Lactobacillus* and FOS on ammonia formation and microbial count in both the feces and dropping slurry of laying hens. Therefore, the objective of this research was to study the effectiveness of *Lactobacillus* and FOS in reducing the volatile ammonia formation and microflora on layer excreta and dropping slurry.

MATERIALS AND METHODS

Experimental

Fresh fecal and slurry samples were collected from poultry farm. One hundred and fifty gram of fecal were collected from the same sources and put into 500 ml beakers, covered with plastic wraps and randomly assigned to three treatment as follows: 1) feces (control), 2) feces + 2% *Lactobacillus* (2.6 x10^6) cfu/g, 3) feces + 2% FOS. The samples having three replications were covered with plastic wraps and incubated at 24 ^{o}C for 6 d. The volatile ammonia contents and pH were measured every 2 d for 6 d on the same beakers. For the dropping slurry study, 300 g each of layer dropping slurry samples were used. The beakers were incubated at 37 ^{o}C for 60 min prior to the first measurement of ammonia on day 0 and repeated every 2 d for 6 d on the same beakers. Total plate count (TPC), *Lactobacillus*, *E. coli*, *Salmonella*, and *Campylobacter* count also were measured between 1 h and 144 h of the experiment.

Laboratory Analysis

Volatile ammonia. Volatile ammonia contents of the fecal material and slurry were measured by using a Kitagawa Toxic Gas Detector (Kitagawa, Matheson, NJ) and ammonia detecting tubes (0-20 ppm and 0-130 ppm capacities, Matheson, NJ). One hundred milliliter air samples were used for each measurement. One hundred fifty grams of each fecal material and 300 g of each layer dropping slurry were collected and placed into a 500 ml beaker and covered with wrapping polyethylene. The beakers were incubated at 37 ^{o}C for 60 min prior to the first ammonia measurement. The volatile ammonia contents were measured after one hour of standing (0 d) and then were repeated at 48 h intervals for 6 d that incubated in the room temperature during measurement.

Fecal pH. The pH of fecal and slurry content was measured by using a pH meter (Model LS, Sargent-Welch Co., Springfield, NJ). One hundred fifty grams of each fecal material and 300 g of each layer dropping slurry were collected and placed into a 500 mL beaker for pH measurement. The pH was measured after one hour (0 d) and repeated at 48 h intervals for 6 d.

Fecal moisture content. Fecal moisture contents were determined according to the method as described in AOAC (AOAC, 2005).

Microbiological analysis. Fresh fecal samples were collected by placing a 54 x 81 cm sterile aluminum foil on the bottom of each cage, 12 h prior to the fecal collection, and slurry samples were collected from the pit on the bottom of cages. One gram of fecal material and slurry were taken from each treatment for microbial analysis. Total aerobe, *Coliform/E. coli*, and *Salmonella* were enumerated by using plate count agar (Difco, Detroit, MI), 3 M *E. coli* Petri-Film (3M, St. Paul, MN) and Brilliant Green Agar (Difco, Detroit, MI), respectively. Plates were incubated at 30 ^{o}C for 24 h. *Lactobacilli* count was enumerated by using MRS Agar (Difco) and incubated in an anaerobic chamber at 37 ^{o}C for 48 h. *Campylobacter* agar kits with Blaser supplement (Difco, Detroit, MI) in a microaerophilic system (5% oxygen, 10% carbon-dioxide, balance nitrogen, Bacton Dickenson, Cockeysville, MD) were used to enumerate the *Campylobacter* count after incubating the plates at 42 ^{o}C for 24 h.

Statistical analysis. The experiment applied a completely randomized design (CRD) with three replications. Data were analyzed by analysis of variance (ANOVA) . When significant differences (P<0.05) among treatments were detected, the least significant difference (LSD) test was used to separate the mean values (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Volatile Ammonia for Feces and Dropping Slurry

Use of *Lactobacillus* and FOS to feces and dropping slurry reduced (P<0.05) the volatile ammonia formation (Table 1). Liu *et al*. (2007) reported that supplementation of the wheat-based diet with transformed *Lactobacillus reuteri* Pg4 decreased excreta ammonia concentrations, and increased the caecal total volatile fatty acid (VFA) and lactic acid concentrations. Canh *et al*. (1998) and Panetta *et al*. (2005) said that the pH of slurry and NH_3 emission are inversely related.

More effectiveness on volatile ammonia reduction was observed for FOS than *Lactobacilus*. Roberts *et al*. (2007) reported that inclusion of 10% corn dried distillers grains (DDGS), 7% wheat middlings (WM), or 5% soybean hulls (SH) in laying-hen diets lower total manure NH_3 emission and the NH_3 emission rate by up to 50%. This effect was mainly trough a decrease in manure pH. In addition, Mobley & Hausinger (1989) reported that pH feces is a major determinant of the rate and extent of NH_3 volatilization from animal waste because lower manure pH shift the NH_3 equilibrium toward NH_4^+ which more water soluble and therefore less volatile than ammonia (NH_3)

The reduction of volatile ammonia by the treatment becomes less effective after 6 d for *Lactobacilli*. This probably was due to the reduction of *Lactobacilli* count during the treatment. *Lactobacilli* reduction resulted in less short chain fatty acid (SCFA) production and increased the ammonia volatilization. However, the effectiveness of FOS in reducing ammonia formation is consistent compared to other treatments during 6 d observation period. These probably were due to the FOS as colonic food may increase the metabolism and growth of bacterial population in the feces and slurry and produce SCFA in long period of time. These SCFA cause a lower feces and slurry pH and result in the extend of decreasingly ammonia (NH_3) volatilization. In addition, bacterial enzymes that involved in the breakdown of uric acid to NH_3 have relatively high optimum pH and are therefore less active when manure pH is decreased (Mobley & Hausinger, 1989).

Fecal pH and Moisture

In general, fecal pH values were lower (P<0.05) for *Lactobacillus* and FOS reated feces and dropping slurry of laying hens during the observation period (Table 2). This probably was due to the activity from the lactic acid bacteria such as lactic acid and acetic acid in the fecal coming from *Lactobacillus sp.* and FOS fermentation. Yusrizal & Chen (2003) reported that the fecal pH values are lower for the the fructooligosaccaride treated broiler. Moreover, Roberts *et al*. (2007) reported that compared with manure from hens fed corn dried distillers grains (DDGS) or wheat middlings (WM) was lower, whereas the manure from soybean hulls (SH)-fed hens tended to have lower pH. Regardless of treatment, it seems that reducing the ammonia formation was closely related with reducing fecal pH. Meda *et al*. (2011) mentioned that manure pH is a major factor that influences NH_3 emissions from poultry manure, the pH influences enzymatic reaction involved in the degradation of uric acid and undigested protein.

Moisture levels were significantly reduced (P<0.05) in both feces and dropping slurry of the treatments (Table 2). This reduction was probably due to direct application of *Lactobacillus* and FOS in the fecal and slurry resulted in increasing bacteria activity in the fecal that use up moisture content in the fecal and slurry. The results were similar to Yusrizal & Chen (2003) reported that during the 4th and 5th week experiment, inulin and oligofructose treated broilers had lower fecal moisture content. Furthermore Chang & Chen (2003) also reported that the moisture content can be reduced when broilers are fed a diet with lactobacilli.

Fecal Microbial Count

There was no difference (P>0.05) in fecal microbial count at 1 h of application of *Lactobacillus* and FOS. However, increasing (P<0.05) *Lactobacilli* count and reduction count (P<0.05) were observed for *E. coli*, *Salmonella*, and *Campylobacter* at 144 h (6 d) of fecal treatment (Table 3). There were differences (P<0.05) in reduc-

Table 1. Volatile ammonia (ppm) of feces and dropping slurry from laying hen as affected by *Lactobacillus* sp and fructooligosaccaride (FOS)

Stored time (h)	Feces				Slurry			
	Control	*Lactobacillus*	FOS	SEM	Control	*Lactobacillus*	FOS	SEM
1 (37 °C)	20.00^a	5.83^b	4.33^c	0.124	17.33^a	5.33^b	3.83^b	0.385
48 (24 °C)	68.33^a	22.00^b	0.00^c	3.105	3.50^a	0.83^b	1.17^b	0.124
96 (24 °C)	90.00^a	66.67^b	0.00^c	3.514	6.33^a	2.08^b	0.67^b	0.329
144 (24 °C)	100.00^a	95.00^a	1.33^b	3.968	8.00^a	4.83^b	2.00^c	0.261

Note: Means in the same row with different superscript differ significantly (P<0.05).

Table 2. Mean of pH and moisture content (%) of feces and dropping slurry from laying hen as affected by *Lactobacillus* sp and fructooligosaccaride (FOS)

Stored time (h)	Feces				Slurry			
	Control	*Lactobacillus*	FOS	SEM	Control	*Lactobacillus*	FOS	SEM
pH value								
1 (37 °C)	6.83[a]	6.60[b]	6.51[c]	0.018	6.90[a]	6.84[b]	6.78c	0.006
48 (24 °C)	7.37[a]	7.12[b]	5.25[c]	0.025	7.78[a]	6.73[ab]	5.50[b]	0.303
96 (24 °C)	7.73[a]	7.20[b]	5.30[c]	0.029	7.08[a]	7.11[a]	5.43[b]	0.009
144 (24 °C)	7.56[a]	7.28[b]	5.40[c]	0.016	7.42[a]	7.23b	5.63c	0.009
Moisture (%)								
1	76.81[a]	76.17[b]	76.17[b]	0.094	93.63[a]	92.48[b]	92.80[b]	0.133
144	80.20[a]	79.49[b]	77.90[c]	0.063	95.07[a]	93.46[b]	93.63[b]	0.183

Note: Means in the same row with different superscript differ significantly (P<0.05).

Table 3. Microflora of feces and dropping slurry from laying hen as affected by *Lactobacillus* sp and fructooligosaccaride (FOS)

Microbial groups (Log cfu/g)	1 h				144 h			
	Control	*Lactobacillus*	FOS	SEM	Control	*Lactobacillus*	FOS	SEM
Feces								
MRS media (Lactobacilli)	9.51[a]	9.79[ab]	9.88[b]	0.049	9.89[a]	9.59[a]	10.43[b]	0.051
Total aerobes	8.91[a]	8.96[a]	8.85[a]	0.356	9.69[ab]	9.13[a]	10.25[b]	0.111
E. coli/ Coliform	8.58[a]	8.60[a]	8.63[a]	0.037	7.58[b]	6.82[a]	7.39[ab]	0.097
B.G. media (for S*almonella*)	8.53[a]	8.22[a]	8.18[a]	0.087	5.19[a]	4.68[b]	3.63[c]	0.036
Campylobacter	6.85[a]	6.63[a]	6.77[a]	0.054	7.09[a]	6.81[b]	6.05[c]	0.029
Slurry								
MRS media (Lactobacilli)	9.11[a]	8.88[b]	8.89[ab]	0.033	7.54[a]	7.67[a]	8.01[b]	0.039
Total aerobes	10.37[a]	8.13[b]	8.69[b]	0.132	7.94[a]	7.77[a]	7.91[a]	0.103
E. coli/ Coliform	9.58[a]	7.86[b]	7.61[b]	0.122	5.44[a]	4.83[b]	4.72[b]	0.092
B.G. media (for S*almonella*)	9.40[a]	6.17[a]	5.89[b]	0.113	5.20[a]	4.99[a]	4.42[b]	0.062
Campylobacter	6.62[a]	6.36[a]	6.32[a]	0.056	6.02[a]	5.70[b]	5.71[b]	0.045

Note: Means in the same row with different superscript differ significantly (P<0.05).

ing of the bacterial pathogen count for *E. coli*, *Salmonella*, and *Campylobacter* both in the 1 h and 144 h of dropping slurry. Liu *et al*. 2007 reported that supplementation of the wheat-based diet with transformed *L. reuteri* Pg4 decreased caecal coliform population from 0 to 21 d of age. It also decreased excreta ammonia concentrations, and increased the caecal total volatile fatty acid (VFA) and lactic acid concentrations from 0 to 21 d and 22 to 37 d of age. Furthermore Islam (2012) mentioned that the mode of action of organic acid on bacteria is the undisociated from of acid diffuse across cell membrane of pathogens, destroying their cytoplasm or inhibiting growth. Akyurek *et al*. (2011) reported that addition of organic acid in ileal digesta caused lactic acid bacteria count to be significantly increased, whereas *E. coli* was significantly decreased.

Increasing (P<0.05) *Lactobacillus* count also was observed in the 6 d treatment of dropping slurry. Little or no reports concerning the effect of Lactobacillus and FOS to slurry on the reducing pathogenic bacteria have been reported. Reduction of harmful bacteria by using *Lactobacillus* and FOS should be of useful in food safety improvement. However, Yusrizal & Chen (2003) reported that significant total reductions *E. coli* count recorded between the oligofructose treatment and control at the 4th wk for male bird. They also reported that among the microflora tested, the *Campylobacter* count of the male bird and the *Salmonella* count of the female birds were lower in the cecal contents for the oligofructose supplemented bird.

It is noted that there was an increase (P <0.05) in *Lactobacilli* count in both the feces and the dropping

slurry for FOS treatment as compared to the other treatments. It proved that the FOS as colonic food can stimulate the growth of *Lactobacilli* in the feces or dropping slurry. Yusrizal & Chen (2003) report that supplementing diets with FOS increased Lactobacilli count in the gizzard, and small intestine contents for female broiler. Rebole *et al*. (2010) also reported that dietary prebiotic inulin had a positive and significant effect in increasing on bifidobacteria and lactobacilli count in both ileal and cecal contents.

CONCLUSION

Direct application of 2% *Lactobacillus* ($2.6x10^6$cfu/g) and 2% FOS in the feces and dropping slurry reduced the volatile ammonia formation, fecal pH, increased *Lactobacilli* count and decreased *E. coli, Salmonella,* and *Campylobacter* count.

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