Dried *Bacillus subtilis* Culture Reduced Ammonia Gas Release in Poultry House

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ABSTRACT : The present study showed the advantages of dried *Bacillus subtilis* culture (DBSC) supplementation on reducing ammonia gas release in the poultry house. In Experiment 1, 65-week-old Hyline W-36 hens were raised in individual wire-floor cages in a windowless house, and divided into two groups of 180 hens each. One group was fed diets without DBSC as the control and another group was fed a diet supplemented with 2% DBSC. In Experiment 2, 2-week-old broiler chicks were divided into 3 treatment groups of 20 chicks each and maintained in individual floor cages. One group was fed the diet without DBSC and other two groups were fed the diet supplemented with 1 or 2% DBSC, respectively. In Experiment 1, DBSC consistently reduced ammonia gas release in the laying house ($p<0.01$) and manure storage facilities ($p<0.01$). Incubation of feces for 1, 2, 3, 4, 5, 6, 24 or 48 hours showed that DBSC consistently reduced ammonia gas release. In Experiment 2, DBSC reduced ammonia gas release in the broiler house; however, DBSC had no effect on total N, urate-N and ammonia-N contents of feces, but it improved cumulative N utilization and decreased serum urea-N concentration when chicks were fed 1% DBSC. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 5 : 806-809*)

Key Words : Dried *Bacillus subtilis* Culture, Ammonia Gas Release, Poultry House, N Utilization

INTRODUCTION

The nitrogen compounds originating from the animal production industry constitute one of the most serious environmental contaminants because microbial action converts them into ammonium gas (Hothuijen, 1993). Ammonia, which is a component resulting from the rapid microbiological breakdown of large amounts of uric acid in bird excreta (Whyte, 1993), is a well-known irritant and the most common and noxious gas in the animal house. It caused histological changes in the respiratory system, increased mycoplasmal infections in laboratory animals (Broderson et al., 1976), and depressed growth. Furthermore, ammonia in the ground is converted into nitrates by nitrifying bacteria, and the result is much reduced ground water pH and high concentrations of nitrates in drinking water.

Growth promoters added to poultry feed, such as some antibiotics and yucca saponin, are thought to reduce the rate of ammonia production from litter (Carlile, 1984). Yucca saponin is believed to have an inhibiting action on urease as well as containing two glycoproteins that bind ammonia. Reduction in the ammonia concentration from 18 to 11 ppm in broiler houses in summer following the addition of yucca extract to a poultry feed has been reported (Rocklifte, 1991). Crober and Seftan (1994) reported that ammonia levels in the laying house in winter were significantly lower in all years when yucca extract was supplemented to the diet compared to the year without yucca extract. However, a reduction in ammonia production is not always evident (Chrlile, 1984). Wacharonke (1994) found that yucca extract reduced ammonia concentration in seawater and improved survival rate of tiger prawns. The use of zeolites as feed additives was considered to have little value (Carile, 1984). However, Xia et al. (1992) showed that inclusion of zeolite in the diet consistently reduced ammonia release from feces of broiler chickens. Furthermore, Xia et al. (1992) found that serum ammonia was also decreased by zeolite supplementation. It was proven that certain microorganisms added to the diet reduced ammonia production from feces in layers (Tanabashi, 1994, personal communication) as well as in pigs and cattle (Santoso, 1994, unpublished data). A possible mechanism of reduced ammonia release caused by dried *Bacillus subtilis* culture (DBSC) was evaluated in the present study.

MATERIALS AND METHODS

Experiment 1

Sixty five-week-old Hyline W-36 were raised in individual wire-floor cages in a windowless house. Feed and water were available at all times. Hens were weighed individually and divided into two groups of 180 hens each. One group was fed diets supplemented with 2% DBSC for 8 weeks. DBSC (obtained from Nippon Biotec Co. Ltd., Tokyo, Japan) is fermented

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products of *Bacillus subtilis* which was cultured in mainly wheat bran and rice bran.

Feces from front, middle and back of the windowless house were collected in plastic bags and ammonia release in plastic bags was meaured at 2, 4, 6 and 8 weeks of feeding using Kitagawa Gas Kenchikan AP-1. At the last experimental period (8 weeks) 75g of feces of two replicates in each group was placed Erlenmyer flask and closed. All flasks were incubated at 38℃, and ammonia release was measured at 0, 1, 2, 3, 4, 5, 6, 24 and 48 hours. After the experiment was finished, all plastic bags were placed in a manure storage house and ammonia release was measured at 2, 4, 7, 11 and 14 weeks of storage.

Experiment 2

Two-week-old broiler chicks were weighed individually and divided into three groups of 10 chicks each. One group was fed a diet without DBSC, and the other two groups were fed diets with either 1 or 2% DBSC supplementation. Five broiler chicks of each group were then raised in individual wire-floor cages, and the remainder of each group was raised as a group for measuring ammonia gas release. In this period, house temperature was maintained at 25±3℃ with a photoperiod of 14 hours, and feed and water were provided *ad libitum*. Composition of the experimental diet was published elsewhere (Santoso et al., 1995).

For the 28 day experimental period, a mixture of feces and urine was collected and weighed daily. Feces from five chicks of each group in individual wire-floor cages was sprayed with 5% HCl to prevent fermentation of the urine and loss of ammonia, and dried in an electric oven at 55℃ for uric acid and ammonia analysis. Feces from another five chicks of each group were collected daily and stored in a plastic bag for measurement of ammonia release using Kitagawa Gas AP-1. Uric acid, total N, and ammonia contents of feces and ammonia release were measured weekly. Feed intake was recorded daily and chicks were weighed weekly.

Uric acid and Ammonia Analysis

Uric acid content in a poultry excreta was determined by the methods of Dubbs (1956) and Pudelkiewcz et al. (1968) with further modification as follows. One gram samples of finely ground excreta were quantitatively tranferred to 200 ml volumetric flasks. The neck of each flask was washed down with 100 ml of 0.4% Li_2CO_3 solution. After extracting for 1.5 hours in an ice bath with frequent swirling of the samples, the flasks were made up to volume with deionized water and mixed by inversion. A portion was centrifuged at 3,300 rpm for 5 minutes to remove solids, and 1:2 dilution of an aliquot of each samples was made with glycine buffer. The 0.27 ml aliquots of each of the diluted samples were pipetted into 15 ml tubes. A blank was prepared using 0.27 ml of glycine buffer and 18 ml uricase solution. Cell corrections for possible changes in absorbance of uricase solution at 292 nm before and after incubation were determined. Extinction readings on test samples were made by adding 18 ml uricase solution. After all the samples were read, they were incubated at 45℃ for 16 hours. After the incubation period, cell corrections were again made, using the incubated blank solution followed by the terminal extinction reading of the samples. Ammonia content of excreta was determined using a kit (ammonia-test Wako, Cod No. 277-14401, Wako Pure Chemical Industries, Ltd., Japan).

All data were statistically analyzed using the one-way analysis of variance (Yoshida, 1975). In Experiment 2, significant differences among treatments were determined by Duncan's multiple range test (Duncan, 1955).

RESULTS

Experiment 1

Effect of DBSC on ammonia gas release in layer house and manure storage house are shown in table 1 and 2.

Table 1. Effect of dried *Bacillus subtilis* culture (DBSC) on ammonia production in laying house (Experiment 1)

Length of feeding	Control	2% DBSC	P[1]
(week)	(ppm)		
2	3.3±0.1[2]	1.5±0.2	<0.01
4	17.7±0.5	6.4±1.0	<0.01
6	82.0±5.0	32.0±6.5	<0.01
8	140.0±9.1	80.0±10.1	<0.01

[1] Probability of significance.
[2] Values reported represent means of 3 replicates (front, middle and back of the house)±SE.

Table 2. Effect of dried *Bacillus subtilis* culture (DBSC) on ammonia production in manure storage (Experiment 1)

Length of feeding	Control	2% DBSC	P[1]
(week)	(ppm)		
2	140.2±8.1[2]	50.0±7.5	<0.01
4	223.3±11.2	130.0±10.7	<0.01
7	203.2±6.8	110.0±6.5	<0.01
11	180.0±14.3	87.6±15.3	<0.01
14	153.3±9.1	63.3±9.4	<0.01

[1] Probability of significance.
[2] Values reported represent means of 3 replicates (front, middle and back of the house)±SE.

Ammonia release in the laying house and manure storage house were lower in the supplemented group than in the control group at all weeks. Ammonia gas released in the laying house was decreased by 55, 64, 61 or 43% for 2, 4, 6 or 8 weeks of feeding DBSC, respectively.

In order to evaluate effect of DBSC on rate of ammonia gas release, feces of hens were incubated at 38℃ for 0, 1, 2, 3, 4, 5, 6, 24 or 48 hours, and the results are shown in table 3. The DBSC-supplemented group had a consistently lower rate of ammonia gas release than the control group. Longer incubation increased the rate of ammonia gas release in both groups.

Table 3. Effect of dried *Bacillus subtilis* culture (DBSC) on ammonia in feces of hens incubated at 38℃ (Experiment 1)

Length of incubation	Control	2% DBSC	P[1]
(hours)	(ppm)		
0	8.9±8.1[2]	1.0±0.2	<0.01
1	23.0±11.2	1.0±0.7	<0.01
2	24.0±6.8	1.1±0.6	<0.01
3	25.0±14.3	1.3±0.7	<0.01
4	30.5±9.1	1.4±0.8	<0.01
5	29.5±1.0	2.3±1.0	<0.01
6	38.5±1.1	2.7±0.9	<0.01
24	62.5±3.2	3.2±1.5	<0.01
48	153.5±2.4	3.9±1.4	<0.01

[1] Probability of significance.
[2] Values represent means of 3 replicates (front, middle and back of the house)±SE.

Experiment 2

Effect of DBSC on body weight gain, feed and protein intakes, feed conversion ratio and feces weight are shown in table 4. DBSC supplementation did not influence body weight gain, feed intake, and feces moisture. However, feces weight (wet basis) and feed conversion ratio were significantly decreased ($p<0.05$) when DBSC was supplemented to the diet at 1% level.

Table 5 showed effect of DBSC on ammonia gas release, ammonia-N, urate-N and total N excretion, N utilization and serum urea-N in broiler chicks. There was a reduction in ammonia gas release by 33 or 67% for the groups fed DBSC at the level of 1 or 2%, respectively, at the 1st week of feeding (data not shown). Feeding DBSC for 4 weeks, either 1 or 2 %, resulted in consistent differences from the control. Total N excretion was significantly decreased in chicks fed 1% DBSC, whereas N utilization was improved as compared with the control. Urate-N excretion, however,

Table 4. Effect of dried *Bacillus subtilis* culture (DBSC) on growth characteristics of broiler chicks (Experiment 2)

Measurements	Control	1% DBSC	2% DBSC	P[3]
Body weight gain (g/d)	52.4 ±0.7[1]	53.9 ±0.8	53.6 ±0.6	NS
Protein intake (g/d)	8.6 ±0.2	8.7 ±0.3	8.7 ±0.1	NS
Feed intake (g/d)	100.7 ±5.0	96.5 ±6.0	97.6 ±5.5	NS
Feed conversion ratio	1.92±0.05	1.79±0.06	1.82±0.04	<0.05
Feces weight[2] (g/wk/100g BW)				
wet	81.0 ±5.0	67.0 ±6.0	77.0 ±4.0	<0.05
dry matter	15.5 ±0.7	14.5 ±0.6	14.7 ±0.8	NS
Moisture in feces (%)	80.9 ±0.9	78.4 ±1.0	80.8 ±1.3	NS

[1] Values represent means of 10 chicks±SE. [2] Values represent means of 5 chicks±SE. [3] Probability of significance.
[a,b,c] Means in the same row with no common superscript differ significantly ($p<0.05$). NS = not significant.

Table 5. Effect of dried *Bacillus subtilis* culture (DBSC) on ammonia gas release, ammonia-N, urate-N and total N excretion, N utilization and serum urea-N in broiler chicks (Experiment 2)

Measurements	Control	1% DBSC	2% DBSC	P[1]
Ammonia gas release (ppm)	277.0[1]	177.0	77.0	
Ammonia-N (Nmg/gBW/wk)	0.78±0.01[ab]	0.75±0.02[b]	0.84±0.05[a]	<0.05
Total N (Nmg/gBW/wk)	10.3 ±1.0[a]	9.3 ±1.1[c]	9.8 ±0.5[b]	<0.05
Urate-N (Nmg/gBW/wk)	6.0 ±0.5	5.6 ±0.6	5.6 ±0.4	NS
N utilization (%)	56.0 ±0.5[b]	59.0 ±0.4[a]	58.0 ±0.4[ab]	<0.05
Serum urea-N (Nmg/100ml)	1.73±0.04[a]	1.59±0.03[c]	1.67±0.06[b]	<0.05

[1] Values represent means of 5 chicks±SE. [2] Probability of significance.
[a,b,c] Means in the same row with no common superscript differ significantly ($p<0.05$). NS = not significant.

was not significantly different. Serum urate-N concentration was significantly lower in 1% DBSC than in the control. There was a negative relationship between urate-N and N utilization.

DISCUSSION

DBSC did not affect feed intake, feed conversion ratio, egg production, egg weight and Haugh units of laying hens (data not shown), which was in agreement with the observation of Tanabashi (1994, personal communication). No changes in body weight gain, protein intake, and an improved feed conversion ratio in chicks fed 1% DBSC was in agreement with our previous results (Santoso et al., 1995). A decrease in feces weight would reduce pollutants from poultry.

The present study showed that level of ammonia gas release in the poultry house was decreased by feeding DBSC. It seems that length of feeding DBSC influenced release of ammonia from feces of layer and broiler chicks. One possible mechanism of the decreased ammonia release in chicks is decreased urate-N excretion. However, the present results showed that a decrease in ammonia release was not accompanied by a decrease in urate N excretion. Furthermore, ammonia content in feces was not significantly changed (table 5). Lower serum urate N in chicks fed 1% DBSC supplemented diet also could not explain lower ammonia gas release. Higher rate of ammonia production in treatment groups before ammonia measurement is not a possible mechanism, because there was a consistent decrease in ammonia gas release during 1, 2, 3, 4, 5, 6, 24 or 48 hours when feces were incubated at 38℃. It seems that improved N utilization could not fully explain this phenomenon. It is known that *Bacillus subtilis* produces subtilin (a kind of antibiotic) which may reduce urease-producing microflora in the gastrointestinal lumen, thereby reducing release of ammonia in gastrointestinal tract (Visek et al., 1978). Furthermore, ammonia produced from degradation of uric acid or urea in feces might also be bound by an unknown substance produced by DBSC, because the ammonia content of feces was not decreased. Further study is needed to elucidate the mechanism of decreased ammonia release from feces.

Ammonia liquid on feces will be converted into nitrates by nitrifying bacteria resulting in reduced water pH, and high concentrations of nitrates in drinking water (Holthuijen, 1993). Thus, the inclusion of DBSC could not solve the problem of ammonia completely. The combination of several microorganisms as a supplement (Tanabashi, 1994, personal communication) and/or zeolite as a feed additive (Xia et al., 1992) may be useful to solve this problem.

In conclusion, dietary inclusion of DBSC improved N utilization and feed conversion ratio, and decreased ammonia gas release but not ammonia-N, urate-N and total-N of feces.

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