

# Effects of Dietary Fiber and Reduced Crude Protein on Ammonia Emission from Laying-Hen Manure<sup>1</sup>

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**ABSTRACT** Ammonia (NH<sub>3</sub>) emission is a major concern for the poultry industry. The objective of this research was to determine whether inclusion of dietary fiber and a reduced dietary CP content would decrease NH<sub>3</sub> emission from laying-hen manure. A total of 256 Hy-Line W-36 hens were fed diets with 2 levels of CP (normal and reduced) and 4 fiber treatments in a 2 × 4 factorial arrangement. The fiber treatments included a corn and soybean meal-based control diet and diets formulated with either 10.0% corn dried distillers grains with solubles (DDGS), 7.3% wheat middlings (WM), or 4.8% soybean hulls (SH) to contribute equal amounts of additional neutral detergent fiber. The CP contents of the reduced-CP diets were approximately 1 percentage unit lower than those of the normal-CP diets. All diets were formulated on the basis of digestible amino acid content and were formulated to be isoenergetic. Fresh manure was collected

such that pH, uric acid, and Kjeldahl N contents could be measured. The NH<sub>3</sub> emission from manure was measured over 7 d by placing pooled 24-h manure samples in NH<sub>3</sub> emission vessels. Data were analyzed by ANOVA with Dunnett's multiple-comparisons procedure to compare results from the fiber treatments with the control, whereas the main effect of protein was used to compare the normal- and reduced-CP treatments. Dietary corn DDGS, WM, or SH lowered ( $P \leq 0.01$ ) the 7-d cumulative manure NH<sub>3</sub> emission from 3.9 g/kg of DM manure for the control to 1.9, 2.1, and 2.3 g/kg of DM manure, respectively, and lowered ( $P < 0.05$ ) the daily NH<sub>3</sub> emission rate. Results of this study showed that dietary inclusion of 10.0% corn DDGS, 7.3% WM, or 4.8% SH lowered NH<sub>3</sub> emission from laying-hen manure; however, reducing the CP content by 1 percentage unit had no measurable effect on NH<sub>3</sub> emission.

**Key words:** ammonia emission, corn dried distillers grains with solubles, reduced-protein diet, soybean hull, wheat middlings

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## INTRODUCTION

Ammonia is a major aerial pollutant lost from livestock operations, with domestic animals being the largest global contributor of atmospheric NH<sub>3</sub> emissions (Aneja et al., 2006) and poultry (including laying hens) being the largest contributor among domestic animals in the United States (EPA, 2004). Not only can NH<sub>3</sub> adversely affect health and production by contributing to corneal ulcers, deciliation of the trachea, impaired macrophage function, decreased lung function, lower egg production, and lower BW gains (Kling and Quarles, 1974; Nagaraja et al., 1983; Carlile, 1984; Deaton et al., 1984; Omland, 2002), but it

may also cause eutrophication of surface water resources and nuisance odors (De Boer et al., 2000; Angus et al., 2003; Ritz et al., 2004). The Federal Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and Emergency Planning and Community Right to Know Act (EPCRA) require reporting of NH<sub>3</sub> releases above 45 kg/d per animal-housing site. The Occupational Safety and Health Administration (OSHA) has set the NH<sub>3</sub>-exposure threshold at 50 ppm averaged over an 8-h workday, whereas the National Institute of Occupational Safety and Health (NIOSH) has established a threshold limit of 25 ppm averaged over 8 h. The United Egg Producers 2006 Animal Husbandry Guidelines state that atmospheric concentrations of NH<sub>3</sub> should ideally be below 25 ppm and should not exceed 50 ppm (United Egg Producers, 2006). Maintaining air NH<sub>3</sub> concentrations below these maximum allowable amounts is difficult in high-rise laying-hen houses, especially during winter when ventilation is decreased (Liang et al., 2005). To comply with government and industry-group regulations, minimize health risks, and optimize egg-production po-

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tential, egg producers need to either decrease hen numbers at each production site or develop methods to lower  $\text{NH}_3$  emission with current hen populations.

Adjusting the diet composition may decrease the amount of  $\text{NH}_3$  that is lost from laying-hen facilities. Inclusion of feed ingredients with high concentrations of fiber has been shown to lower  $\text{NH}_3$  emission from pigs, and reduced-CP diets have been shown to decrease N excretion from pigs, broilers, and laying hens (Summers, 1993; Canh et al., 1997, 1998b; Bregendahl et al., 2002; Shriver et al., 2003). We hypothesized that reducing the dietary CP content and including high-fiber feed ingredients would lower  $\text{NH}_3$  emission from laying-hen manure. The objectives of this research were to feed diets with a reduced-CP content and additional high-fiber ingredients to laying hens and measure manure  $\text{NH}_3$  emission, egg production, and N balance. The effects of the dietary treatments on  $\text{NH}_3$  emission are presented here, with production and N balance data presented separately (Roberts, 2007).

## MATERIALS AND METHODS

### *Animals and Diets*

A total of 256 Hy-Line W-36 laying hens, 17 wk of age, were obtained from a commercial facility and placed in completely enclosed, fan-ventilated, light (12:12 L:D) and temperature (20°C) controlled room. Hens were housed 2 per cage, corresponding to 619  $\text{cm}^2$  (96  $\text{in}^2$ ) per hen, in wire-bottomed cages (Chore-Time, Milford, IN), each equipped with a plastic self-feeder and a nipple drinker. Feeders were fitted with a wire grate to minimize waste of feed but not restrict feed consumption. The photoperiod was increased incrementally according to the W-36 Commercial Management Guide (Hy-Line International, Des Moines, IA) to 16:8 L:D at 25 wk of age. Pre-lay and pre-peak diets were formulated according to recommendations in the W-36 Commercial Management Guide for 17 to 18 and 19 to 22 wk of age, respectively, and fed to all hens before commencement of the trial. At 23 wk of age (corresponding to 95% production and denoted as wk 1 of the study) hens were reassigned to cages according to a randomized complete block design, with BW and cage location within the barn as blocking criteria, and were fed the treatment diets. Hens were phase-fed to account for changes in nutrient requirements and feed consumption, with 3 phases from 23 to 31 (phase 1), 32 to 44 (phase 2), and 45 to 58 (phase 3) wk of age. Manure was collected only during phase 3, so phases 1 and 2 will not be discussed in this paper. The 8 dietary treatments were arranged in a  $2 \times 4$  factorial arrangement with 2 contents of CP and 4 fiber diets. The normal-CP diet simulated a diet typically used in industry and the reduced-CP content was achieved by lowering CP by approximately 1 percentage unit with supplemental crystalline amino acids. The 4 fiber diets included a corn- and soybean meal-based control diet and 3 higher fiber diets formulated with 10.0% corn dried distillers grains with solubles (DDGS),

7.3% wheat middlings (WM), or 4.8% soybean hulls (SH). Corn DDGS, WM, and SH were chosen for their high neutral detergent fiber content and availability in the Midwest. Diets were formulated using corn, soybean meal, and meat and bone meal to contain equal amounts of  $\text{ME}_n$  and digestible TSAA and Lys (Table 1). All diets contained Celite (Celite Corporation, Lompoc, CA) as a source of acid-insoluble ash (AIA), which was used as an indigestible marker, and  $\text{NaHCO}_3$  (American Soda, Parachute, CO) to equalize the calculated dietary electrolyte balance. The Iowa State University Institutional Animal Care and Use Committee approved all techniques and procedures involving birds.

### *Sample Collection and Chemical Analysis*

Manure was collected during 2 separate periods; the first collection, during wk 27 of the study (birds at 50 wk of age), was used for determination of manure pH, N, uric acid, and short-chain fatty acids (SCFA), and the second collection, during wk 33 (birds at 56 wk of age), was used for determination of manure  $\text{NH}_3$  emissions. For the first collection, manure was collected on plastic trays 15 cm below each cage. Within 2 min after excretion, droppings were collected from the tray, placed into capped 50-mL polypropylene centrifuge tubes, and stored on ice until analysis later the same day. To facilitate mixing, manure from each cage was stirred with 5 mL of double-distilled water using a glass rod. A 1-g subsample was used for N analysis and the remainder was frozen at  $-20^\circ\text{C}$ . Samples were analyzed for total N using the micro-Kjeldahl method (method 990.03, AOAC, 2006) on a Kjeltex 1028 distilling unit (US Tecator Inc., Herndon, VA). The frozen manure samples were later thawed at  $4^\circ\text{C}$  for 24 h, and pH was subsequently measured (Accumet AR-15, Fisher Scientific, Pittsburgh, PA) after mixing 1 part manure (approximately 1 g) with 10 parts double-distilled water with a vortex mixer. The manure remaining after pH measurement was oven-dried at  $70^\circ\text{C}$  for 24 h before further analysis. Uric acid content in the dried manure was measured using methods modified from Adeola and Rogler (1994). Briefly, 0.1 g of pulverized, dried manure was homogenized in 15 mL of ice-cold 1 M sodium carbonate (Fisher Scientific, Pittsburgh, PA) at 20,000 rpm for 1 min. Samples were then centrifuged at  $16,100 \times g$  for 30 min at  $2^\circ\text{C}$ . Five milliliters of the supernatant were transferred to a 15-mL polypropylene centrifuge tube with 5.525 mL of 3 M Tris hydroxymethyl aminomethane-HCl (Fisher Scientific) and the mixture was diluted 5 to 10 times with double-distilled water to obtain a uric acid concentration readable by the kit. Uric acid was quantified using a commercial kit (Uric Acid Reagent Set, catalog no. U7580-150, Pointe Scientific, Inc., Canton, MI) and a microplate reader. The contents of AIA in diets and manure were measured using the method of Vogtmann et al. (1975).

Contents of SCFA, phenol, and indole were measured using GLC on 1 fresh manure sample per diet, pooled from 16 cages. Briefly, 4 g of manure was transferred into

**Table 1.** Diet composition and calculated chemical analyses<sup>1</sup>

Item	Normal CP				Reduced CP			
	Control	Corn DDGS	WM	SH	Control	Corn DDGS	WM	SH
Ingredient (%)								
Corn DDGS	—	10.00	—	—	—	10.00	—	—
WM	—	—	7.30	—	—	—	7.30	—
SH	—	—	—	4.80	—	—	—	4.80
Corn	62.82	51.90	54.92	56.55	66.40	57.28	57.78	59.21
Soybean meal (48% CP)	18.52	18.37	17.62	18.43	15.27	13.50	15.09	16.04
Calcium carbonate	8.96	9.00	8.98	8.90	8.95	8.97	8.97	8.88
Meat and bone meal (50% CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Vegetable oil	2.27	3.65	3.85	3.88	1.69	2.78	3.37	3.44
Celite <sup>2</sup>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.27	0.14	0.21	0.28	0.30	0.19	0.23	0.33
Sodium bicarbonate	0.20	—	0.11	0.12	0.37	0.25	0.24	0.25
Trace-mineral premix <sup>3</sup>	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix <sup>4</sup>	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Sodium chloride (iodized)	0.19	0.21	0.24	0.25	0.11	0.10	0.15	0.16
Alimet	0.17	0.13	0.17	0.19	0.20	0.18	0.19	0.21
L-Lys·HCl	—	—	—	—	0.11	0.15	0.08	0.08
Calculated chemical composition (%)								
CP	16.94	18.37	17.05	16.77	15.79	16.64	16.16	15.92
ME <sub>N</sub> , kcal/kg	2,840	2,840	2,840	2,840	2,840	2,840	2,840	2,840
Ether extract	4.72	6.07	6.25	6.15	4.24	5.33	5.85	5.78
Linoleic acid	1.47	1.69	1.43	1.34	1.54	1.79	1.49	1.39
Neutral detergent fiber	12.58	14.55	14.80	15.11	12.55	14.51	14.78	15.09
Acid detergent fiber	3.04	3.89	3.55	5.04	2.96	3.77	3.49	4.99
Crude fiber	1.61	2.06	2.14	3.41	1.56	1.98	2.10	3.37
Ca	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
P (nonphytate)	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
K	0.64	0.67	0.67	0.68	0.59	0.59	0.63	0.64
Na	0.18	0.18	0.18	0.18	0.20	0.21	0.18	0.18
Cl	0.19	0.21	0.21	0.22	0.16	0.18	0.18	0.18
Dietary electrolyte balance <sup>5</sup> (mEq)	191	191	191	191	191	191	191	191
Ile (digestible)	0.60	0.64	0.60	0.59	0.55	0.55	0.55	0.55
Lys (digestible)	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
TSAA (digestible)	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Thr (digestible)	0.54	0.56	0.53	0.53	0.49	0.50	0.50	0.50
Trp (digestible)	0.18	0.19	0.18	0.18	0.16	0.16	0.17	0.17
Val (digestible)	0.70	0.75	0.70	0.69	0.65	0.67	0.66	0.65

<sup>1</sup>DDGS = dried distillers grains with solubles; WM = wheat middlings; SH = soybean hulls.

<sup>2</sup>Celite was included as an indigestible marker (Celite Corporation, Lumpoc, CA).

<sup>3</sup>Trace-mineral premix supplied (per kilogram of diet): Mn (MnSO<sub>4</sub>), 70 mg; Zn (ZnO), 90 mg; Fe (FeSO<sub>4</sub>), 60 mg; Cu (CuSO<sub>4</sub>), 12 mg; Se (Na<sub>2</sub>SeO<sub>3</sub>), 0.15 mg; NaCl, 2.5 g.

<sup>4</sup>Vitamin premix supplied (per kilogram of diet): vitamin A (retinyl acetate), 8,065 IU; cholecalciferol, 1,580 IU; vitamin E (DL- $\alpha$ -tocopheryl acetate), 15 IU; vitamin B<sub>12</sub>, 16  $\mu$ g; vitamin K (menadione sodium bisulfite), 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline, 509 mg; folic acid, 1.62 mg; biotin, 270  $\mu$ g.

<sup>5</sup>Dietary electrolyte balance was calculated as K + Na - Cl.

a tared 15-mL polypropylene centrifuge tube, after which 1 mL of HPLC-grade water (Fisher Scientific) and 5 mL of HPLC-grade acetone (Fisher Scientific) were added, and each tube was sonicated for 15 s. After sonication, 100  $\mu$ L of 85% *o*-phosphoric acid (Fisher Scientific) was added and the contents of the tube were mixed using a vortex mixer. Tubes were then centrifuged at 21,000  $\times$  g for 23 min at 4°C. The supernatant was filtered through a 0.2- $\mu$ m syringe filter and analyzed on an Agilent 6890 gas chromatograph equipped with a flame-ionization detector and DB-FFAP column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m; Agilent Technologies, Wilmington, DE). The following gas chromatograph parameters were used: split mode, 20:1; inlet temperature, 220°C; initial inlet pressure, 168 kPa; injection volume, 1  $\mu$ L; constant column flow (He), 1.4 mL/min; and detector temperature, 250°C. The oven temperature program was initial temperature 35°C, 0.5

min hold; 90°C, 2.0 min hold; increase by 10°C/min; final temperature 230°C, hold for 6 min; increase by 12°C/min.

For the second collection, manure was collected during wk 33 (birds at 56 wk of age) over 3 consecutive 24-h periods on plastic trays placed 15 cm below each cage. To obtain sufficient quantities for NH<sub>3</sub> emission analysis, manure from each of the 24-h periods was pooled within diet and stored at -20°C until analysis. Manure was thawed at 4°C and analyzed for contents of DM by oven drying a 2-g subsample at 70°C for 24 h and for NH<sub>3</sub> emission and NH<sub>3</sub> emission rate by placing 2.5 kg of manure in NH<sub>3</sub> emission vessels. The 8 NH<sub>3</sub> emission vessels were made of 19-L (5-gal) plastic containers, which were lined with Teflon FEP100 film (200A, DuPont Teflon Films, Wilmington, DE). The air inlet and outlet were located in the airtight Teflon-lined lid. Teflon tubing (0.64 cm diameter) and manifold, along with poly(vinyl chlo-

ride) compression fittings, were used in constructing the emission vessel system. The vessels were operated under positive pressure with a diaphragm pump (model DOA-P104-AA, Gast Manufacturing, Inc., Benton Harbor, MI) to supply fresh air to the vessels. Flow rate of the fresh air was controlled and measured using an air mass-flow controller (0 to 30 L/min, stainless steel wetted part, Aalborg Instruments and Control Inc., Orangeburg, NY). The supply air was connected to a distribution manifold in which air was divided via 8 identical flow meters (0.2 to 4 L/min, stainless steel valve, VFB-65-SSV, Dwyer Instruments, Inc., Michigan City, IN). A flow rate of 3 L/min was provided to each vessel, resulting in an air-exchange rate of 11 air changes/h. Each vessel was equipped with a stirring fan (12 V DC, Radio Shack, Fort Worth, TX) located 6 cm below the lid for uniform mixing of the headspace. Gas exhausted from the vessels was connected to a common 5-cm plastic pipe that was routed to the building vent outlet. Exhaust air from the headspace of each of the 8 vessels, incoming air, and room air were sampled sequentially at 6-min intervals, with the first 4 min for stabilization and the last 2 min for measurement. This yielded a measurement cycle of 1 h. A photoacoustic infrared analyzer (Chillgard RT Refrigerant Monitor, MSA, Pittsburgh, PA) was used to measure  $\text{NH}_3$  concentration. The analyzer uses an internal pump to draw air at a flow rate of approximately 1.0 L/min. Eight solenoid valves (Type 6014, 24 V, stainless steel valve body, Burkert Contromatic USA, Irvine, CA) controlled the sequential sampling. A Teflon filter was placed in front of each solenoid valve. Analog outputs from the  $\text{NH}_3$  analyzer and mass-flow controller were recorded at 20-s intervals with a measurement and control module (model CR10, Campbell Scientific, Inc., Logan, UT).

### Calculations

The AIA contents of feed and manure were used to calculate total DM manure excretion as:

$$\text{DM}_{\text{manure}} = (\text{AIA}_{\text{feed}} \times \text{DM}_{\text{feed}}) / \text{AIA}_{\text{manure}}$$

where  $\text{DM}_{\text{manure}}$  is the DM manure excreted (g/d),  $\text{AIA}_{\text{feed}}$  and  $\text{AIA}_{\text{manure}}$  are the analyzed contents (%) of AIA in feed and manure, respectively, and  $\text{DM}_{\text{feed}}$  is the DM feed consumption (g/d) of the hen. The  $\text{NH}_3$  emission per kilogram of manure was determined in the  $\text{NH}_3$  emission vessels and the  $\text{NH}_3$  emitted from the hens was calculated as:

$$A_{\text{hen}} = (A_{\text{manure}} \times \text{DM}_{\text{manure}})$$

where  $A_{\text{hen}}$  is the  $\text{NH}_3$  emitted (g/d) from the hens and  $A_{\text{manure}}$  is the  $\text{NH}_3$  emitted (g/g) from manure on a DM basis. The total N excretion of each hen was calculated as:

$$N_{\text{excretion}} = N_{\text{manure}} \times \text{DM}_{\text{manure}}$$

where  $N_{\text{excretion}}$  is the total N excretion (g/d) and  $N_{\text{manure}}$  is the N content (%) of the manure on a DM basis. Uric acid excretion was calculated as:

$$\text{UA}_{\text{excretion}} = \text{UA}_{\text{manure}} \times \text{DM}_{\text{manure}}$$

where  $\text{UA}_{\text{excretion}}$  is the uric acid excreted (g/d) and  $\text{UA}_{\text{manure}}$  is the uric acid content (%) of the manure on a DM basis. The proportion of N in manure from uric acid was calculated as:

$$\% \text{UAN}_{\text{excretion}} = 100 \times (\text{UA}_{\text{excretion}} \times 1/3) / N_{\text{excretion}}$$

where  $\% \text{UAN}_{\text{excretion}}$  is the uric acid N excreted, expressed as a percentage of total N excretion and 1/3 is the N content of uric acid by weight.

### Statistical Analyses

Statistical analyses were performed with JMP (version 5.1.2, SAS Institute, Inc., Cary, NC). Data were analyzed by ANOVA appropriate for a randomized complete block design with 16 blocks and 8 dietary treatments in a  $2 \times 4$  factorial arrangement (Morris, 1999). The ANOVA model included effects of block, protein, fiber, and the interaction of protein and fiber. Dunnett's multiple-comparisons procedure (Dunnett, 1955) was used to compare the results from each of the fiber treatments with the results from the control; the main effect of protein was used to compare the reduced- and normal-CP diets. The experimental unit for analysis of fresh manure was a cage with 2 hens, whereas the experimental unit for  $\text{NH}_3$  emission was the pooled manure sample for each diet;  $P$ -values  $\leq 0.05$  were considered significant and  $P \leq 0.10$  considered a trend.

## RESULTS AND DISCUSSION

Including high-fiber ingredients in pig diets has been shown to decrease  $\text{NH}_3$  emission because dietary fiber increases the metabolism and growth of bacterial populations in the large intestine (Kirchgessner et al., 1994). In addition to the energy provided by the fiber, the bacteria also require N, part of which may be acquired from N that would otherwise be excreted as uric acid, thereby shifting the N excretion from uric acid to bacterial protein. Indeed, when Shriver et al. (2003) fed SH or beet pulp to pigs, N excretion was repartitioned from the urine to the feces. Bacterial enzymes in manure readily degrade uric acid to  $\text{NH}_3$ , which is volatilized (Mackie et al., 1998). In contrast, bacterial protein in the feces is more stable compared with uric acid such that the N will remain in the manure for a longer period of time, thereby lowering  $\text{NH}_3$  volatilization and improving the fertilizer value of the manure (Canh et al., 1998c).

In the present study,  $\text{NH}_3$  emission from manure was lowered ( $P < 0.01$ ) by dietary inclusion of 10.0% corn DDGS, 7.3% WM, or 4.8% SH when measured per kilogram of manure over 7 d compared with manure of hens fed the control diet, with the diet containing corn DDGS

**Table 2.** Ammonia emission, N excretion, manure uric acid, and manure pH from hens fed diets varying in CP and fiber

Item	Fiber <sup>1</sup>					Protein		
	Control	Corn DDGS	WM	SH	Pooled SEM	Normal	Reduced	Pooled SEM
NH <sub>3</sub> emission <sup>2</sup> (g/kg per 7 d)	3.86	1.93***	2.10***	2.32**	0.29	2.57	2.53	0.20
NH <sub>3</sub> emission <sup>2</sup> (g/hen per 7 d)	0.13	0.08**	0.08**	0.09*	0.01	0.10	0.09	0.01
N excretion <sup>3</sup> (g/kg per d)	1.61	1.68	1.49	1.54	0.07	1.66 <sup>a</sup>	1.49 <sup>b</sup>	0.05
Uric acid <sup>3</sup> (g/hen per d)	3.40	3.26	3.03	3.11	0.17	3.32	3.07	0.12
Uric acid N <sup>3</sup> (% of total N)	71.16	65.10	67.78	69.32	2.63	67.07	69.60	1.86
pH	7.08	6.77*	6.80*	6.85†	0.04	6.79	6.95	0.05

<sup>a,b</sup>Means within a row lacking a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>DDGS = dried distillers grains with solubles; WM = wheat middlings; SH = soybean hulls.

<sup>2</sup>Means of 6 observations for the fiber factor and 12 observations for the protein factor.

<sup>3</sup>Means of 32 observations for the fiber factor and 64 observations for the protein factor.

† $P < 0.10$ , \* $P \leq 0.05$ , \*\* $P < 0.01$ , \*\*\* $P \leq 0.001$ ;  $P$ -value from Dunnett's multiple-comparisons procedure comparing the control with corn DDGS, WM, or SH.

resulting in a 50% decrease in NH<sub>3</sub> emission (Table 2). Canh et al. (1998b) found that pigs fed high-fiber diets excreted more DM manure than pigs fed a control diet. Inclusion of high-fiber feed ingredients in laying-hen diets may also cause an increase in DM manure excretion because of the possibility of lower DM digestibility (Jaroni et al., 1999; Hogberg and Lindberg, 2004; Holt et al., 2006). Although the DM digestibilities of the diets were not affected by the inclusion of fiber in the present study (Roberts, 2007), NH<sub>3</sub> emission was calculated on a per hen basis to account for any potential differences in manure excretion between control- and fiber-fed hens. Regardless of this adjustment, NH<sub>3</sub> emission was lower ( $P \leq 0.05$ ) from hens fed the fiber diets compared with emission from hens fed the control diet (Table 2).

The corn DDGS and WM diets each resulted in a lower ( $P < 0.05$ ) NH<sub>3</sub> emission rate from manure compared with the control diet each day during the entire 7-d measuring period (Table 3). The SH diet caused a lower ( $P \leq 0.05$ ) NH<sub>3</sub> emission rate during d 3 through 7, but not during d 1 and 2 ( $P < 0.10$ ). The top layer of a manure stack, such as is found in a high-rise laying-hen house, is principally responsible for NH<sub>3</sub> emission (Xin and Liang, 2005). Because manure is continually added to the stack, the NH<sub>3</sub> emission rate during the first few days after excretion and before manure becomes buried is therefore extremely important. In manure-belt houses, where manure is not allowed to build up over time, the NH<sub>3</sub> emission rate determines the amount of N lost during the 1 to 3 d after manure excretion and before removal of the manure from the house. Once the manure is removed to a storage building, it can be treated to minimize further NH<sub>3</sub> loss by lowering the surface area to volume ratio or applying chemical treatments (McCrorry and Hobbs, 2001; Panetta et al., 2005; Xin and Liang, 2005). The lower daily NH<sub>3</sub> emission rate indicates that, in a commercial setting, feeding high-fiber diets such as those fed in this study would cause more N to be retained in the manure from hens managed in either a high-rise or manure-belt house, where manure is most susceptible to NH<sub>3</sub> volatilization the first few days after excretion.

The N excretion in manure consists mainly of uric acid and bacterial protein with some urea, NH<sub>3</sub>, and endogenous N. To determine whether the additional dietary fiber caused a repartitioning of N excretion from uric acid to bacterial protein, the uric acid content of the manure was measured. A decrease in uric acid N as a percentage of total N excretion would suggest an increase in the bacterial-protein N contents of the manure. However, there were no differences ( $P > 0.10$ ) in uric acid N as a percentage of total N excretion for the manure from fiber-fed hens compared with the manure from hens fed the control diet (Table 2), suggesting that N was not repartitioned from uric acid to bacterial protein. This result contradicts the results of Kreuzer and Machmuller (1993), Tetens et al. (1996), and Canh et al. (1997), who found less urinary N and more fecal N when diets with up to 30% sugarbeet pulp were fed to pigs or rats. Considering the relatively lower inclusion rate of the high-fiber feed ingredients in the present study, the repartitioning of N may not have been large enough to be detected. Additionally, hens have a relatively short large intestine in relation to body size compared with that of the pig or rat, and this provides a short period of time for bacterial protein synthesis.

The bacterial fermentation of fiber produces SCFA, which are readily absorbed across the mucosal membranes of the digestive tract (Bergman, 1990). However, when pigs are fed a high-fiber diet, the slurry contains considerably more SCFA compared with the slurry from pigs fed a common grain-based diet, and these SCFA cause a lower slurry pH (Canh et al., 1997, 1998c; Hogberg and Lindberg, 2004; Kerr et al., 2006). The pH of slurry is a major determinant of the rate and extent of NH<sub>3</sub> volatilization from animal waste because lower manure pH shifts the NH<sub>3</sub> equilibrium ( $pK_a = 9.2$ ) toward NH<sub>4</sub><sup>+</sup>, which is more water soluble and therefore less volatile than NH<sub>3</sub>. Additionally, bacterial enzymes that are involved in the breakdown of uric acid to NH<sub>3</sub> have a relatively high optimum pH and are therefore less active when manure pH is decreased (Mobley and Hausinger, 1989). Indeed, Canh et al. (1998a) and Panetta et al. (2005) showed that the pH of slurry and NH<sub>3</sub> emission are in-

**Table 3.** Daily ammonia emission rate, calculated on a DM basis, of manure from hens fed diets varying in CP and fiber

Treatment (g/kg per d)	Day						
	1	2	3	4	5	6	7
<b>Fiber<sup>1</sup></b>							
Control	0.0517	0.1348	0.3635	0.7112	0.8428	0.8739	0.8743
Corn DDGS <sup>2</sup>	0.0325	0.1055	0.1696	0.3627	0.4809	0.5521	0.6313
Wheat middlings	0.0245	0.0844	0.1287	0.2912	0.4521	0.5320	0.5949
Soybean hulls	0.0295	0.0663	0.1763	0.2828	0.3772	0.4585	0.5240
Pooled SEM	0.0056	0.0088	0.0344	0.0594	0.0725	0.0752	0.0671
<b>Protein<sup>3</sup></b>							
Normal CP	0.0341	0.0923	0.2034	0.4251	0.5547	0.6136	0.6656
Reduced CP	0.0349	0.1032	0.2157	0.3988	0.5217	0.5947	0.6467
Pooled SEM	0.0040	0.0062	0.0243	0.0420	0.0512	0.0532	0.0474
<b>P-value<sup>4</sup></b>							
Corn DDGS <sup>4</sup>	0.0307	0.0001	0.0032	0.0002	0.0007	0.0029	0.0046
Wheat middlings <sup>4</sup>	0.0080	0.0021	0.0004	0.0003	0.0036	0.0129	0.0228
Soybean hulls <sup>4</sup>	0.0657	0.0755	0.0024	0.0017	0.0067	0.0192	0.0503
Protein <sup>5</sup>	0.8883	0.2333	0.7262	0.6645	0.6555	0.8050	0.7821
Protein × fiber <sup>5</sup>	0.1405	0.0016	0.1219	0.2894	0.4780	0.5756	0.5196

<sup>1</sup>Values are means of 6 observations.

<sup>2</sup>DDGS = dried distillers grains with solubles.

<sup>3</sup>Values are means of 12 observations.

<sup>4</sup>P-values from Dunnett's multiple-comparisons procedure comparing the control with corn DDGS, wheat middlings, or soybean hulls.

<sup>5</sup>Main effect of protein or protein × fiber.

versely related. Compared with manure from hens fed the control diet, the pH of manure from hens fed corn DDGS or WM was lower ( $P < 0.05$ ), whereas the manure from SH-fed hens tended ( $P = 0.10$ ) to have a lower pH (Table 2). Because the manure N was not repartitioned from uric acid to bacterial protein, the lower pH was probably responsible for the lower  $\text{NH}_3$  emission that was observed from the manure of the fiber-fed hens. Higher contents of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids were observed in the manure from hens fed each of the 3 high-fiber diets compared with manure from the control-fed hens (Table 4). Although only 1 manure sample was measured from each diet, the observed higher contents of these SCFA indicate that the

lower pH was caused by increased contents of SCFA in the manure.

Fiber content of the diets in this study was measured as neutral detergent fiber, an analysis that measures the total contents of cellulose, hemicellulose, lignin, and silica in a feed sample. The proportions of each cell-wall component in a given feedstuff may partially determine the extent of fermentation by microbes in the large intestine, and therefore the suitability of that feedstuff to lower  $\text{NH}_3$  emission from the manure. Hens were fed the treatment diets during phases 1, 2, and 3, with manure samples collected only during phase 3. Further studies are warranted to determine the length of time necessary for bacterial populations and  $\text{NH}_3$  emission to adjust to the high-

**Table 4.** Contents of volatile compounds in manure from hens fed diets varying in CP and fiber<sup>1</sup>

Volatile compound ( $\mu\text{mol/g}$ , as-is manure)	Fiber <sup>2</sup>				Protein <sup>3</sup>	
	Control	Corn DDGS	WM	SH	Normal	Reduced
Acetic acid	8.570	11.127	9.410	9.918	10.126	9.386
Propionic acid	1.041	1.528	1.234	1.351	1.388	1.189
Isobutyric acid	0.039	0.054	0.043	0.062	0.049	0.050
Butyric acid	0.401	0.642	0.532	0.513	0.572	0.472
Isovaleric acid	0.062	0.090	0.075	0.079	0.079	0.074
Valeric acid	0.084	0.138	0.133	0.119	0.116	0.111
Isocaproic acid	0.027	0.034	0.005	0.057	0.006	0.055
Caproic acid	0.171	0.175	0.164	0.227	0.201	0.168
Heptanoic acid	0.040	0.026	0.031	0.037	0.031	0.036
Phenol	0.067	0.062	0.062	0.056	0.057	0.067
<i>p</i> -Cresol	0.084	0.068	0.056	0.061	0.061	0.078
Indole	0.050	0.046	0.055	0.045	0.050	0.047
3-Methyl indole	0.003	0.020	0.033	0.019	0.028	0.010

<sup>1</sup>One sample from each diet was analyzed; therefore, no statistical analysis is available.

<sup>2</sup>Means of 2 observations. DDGS = dried distillers grains with solubles; WM = wheat middlings; SH = soybean hulls.

<sup>3</sup>Means of 4 observations.

fiber diet as well as the effects of different fiber types and fermentability on  $\text{NH}_3$  emission.

Standard corn-soybean meal diets, balanced for the first- and second-limiting amino acids (TSAA and Lys, respectively), contain contents of the other amino acids above hen requirements. Hens do not have the ability to store excess dietary amino acids, which are instead excreted, mainly as uric acid. Formulating a diet based on amino acid requirements with no CP minimum and including supplemental amino acids lowers the CP and N contents of the diet. We hypothesized that reduced dietary CP content would cause a decrease in total N and uric acid excretion, and therefore have less potential for microbial conversion of uric acid to  $\text{NH}_3$ . Typically, when the dietary CP content is lowered by using crystalline amino acids, N excretion is lowered by 8 to 10% for each 1 percentage unit decrease in dietary CP (Kerr and Easter, 1995; Canh et al., 1998c). Indeed, in the present study, the reduced-CP diets caused a 10% decrease ( $P < 0.05$ ) in total N excretion compared with the normal-CP diet (Table 2). In addition, lower uric acid excretion, and therefore lower  $\text{NH}_3$  emission, was expected from hens fed the reduced-CP diets. However,  $\text{NH}_3$  emission was not lower from the manure of hens fed the reduced-CP diets, contrary to previous studies, which showed approximately 7 to 10% lower  $\text{NH}_3$  emission for each 1 percentage unit lower dietary CP in pigs (Canh et al., 1998b). Not only was  $\text{NH}_3$  emission higher than expected from the hens fed the reduced-CP diets, but egg production and egg mass also were lower compared with hens fed the normal-CP diets (Roberts, 2007), indicating a possible amino acid deficiency in the reduced-CP diets. If the reduced-CP diets were indeed deficient in 1 or more individual amino acids, the other dietary amino acids, now in excess, would have been deaminated and the N would have been excreted as uric acid, which could explain both the higher than expected uric acid excretion and  $\text{NH}_3$  emission from the reduced-CP fed hens.

Results of this study showed that inclusion of 10% corn DDGS, 7% WM, or 5% SH in laying-hen diets lowered total manure  $\text{NH}_3$  emission and the  $\text{NH}_3$  emission rate by up to 50%. This effect was mainly through a decrease in manure pH. When corn DDGS, WM, or SH are included in a commercial laying-hen diet, it is typically because of their contribution of nutrients to the diet or their relatively low cost in least-cost feed formulations. However, this study showed that, in addition to the essential amino acids, minerals, and other nutrients provided by the corn DDGS, WM, and SH, these ingredients also function to lower  $\text{NH}_3$  emission.

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