

Characterisation and quantification of changes in odorants from litter headspace of meat chickens fed diets varying in protein levels and additives

Nishchal K. Sharma,* Mingan Choct,† Mark W. Dunlop,‡ Shu-Biao Wu,* Hardy Z. Castada,§ and Robert A. Swick*,¹

*School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia; †Poultry Cooperative Research Centre, University of New England, Armidale, New South Wales 2351, Australia; ‡Department of Agriculture and Fisheries, Queensland Government, Toowoomba, Queensland 4350, Australia; and §Thomson Environmental Systems Pty Ltd, Caringbah, New South Wales 2229, Australia

ABSTRACT The effect of dietary crude protein (CP) and additives on odor flux from meat chicken litter was investigated using 180 day-old Ross 308 male chicks randomly allocated to five dietary treatments with three replicates of 12 birds each. A 5 × 3 factorial arrangement of treatments was employed. Factors were: diet (low CP, high CP, high CP+antibiotic, high CP+probiotic, high CP+saponin) and age (15, 29, 35 days). The antibiotic used was Zn bacitracin, the probiotic was a blend of three *Bacillus subtilis* strains and the saponin came from a blend of *Yucca* and *Quillaja*. Odorants were collected from litter headspace with a flux hood and measured using selective ion flow tube mass spectrometry (SIFT-MS). Litter moisture, water activity (A_w), and litter headspace odorant concentrations were correlated. The results showed that low CP group produced lower flux of dimethyl amine, trimethyl amine, H₂S, NH₃, and phenol in litter compared to high CP group ($P < 0.05$). Similarly, high CP+probiotic group produced lower flux of H₂S

($P < 0.05$) and high CP+saponin group produced lower flux of trimethylamine and phenol in litter compared to high CP group ($P < 0.05$). The dietary treatments tended ($P = 0.065$) to have higher flux of methanethiol in high CP group compared to others. There was a diet × age interaction for litter flux of diacetyl, 3-hydroxy-2-butanone (acetoin), 3-methyl-1-butanol, 3-methylbutanal, ethanethiol, propionic acid, and hexane ($P < 0.05$). Concentrations of diacetyl, acetoin, propionic acid, and hexane in litter were higher from low CP group compared to all other treatments on d 35 ($P < 0.05$) but not on d 15 and 29. A high litter moisture increased water activity ($P < 0.01$) and favored the emissions of methyl mercaptan, hydrogen sulfide, dimethyl sulfide, ammonia, trimethyl amine, phenol, indole, and 3-methylindole over others. Thus, the low CP diet, *Bacillus subtilis* based probiotic and the blend of *Yucca/Quillaja* saponin were effective in reducing the emissions of some key odorants from meat chicken litter.

Key words: additive, broiler, crude protein, meat chicken, odor

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INTRODUCTION

Odor emissions have been identified as a potential threat for the sustainable development of the chicken meat industry. The expansion of existing farms or the establishment of new farms thus depends on effective control of dust and odor emissions. Emissions from meat chicken farms contain a large number of odorants (Murphy et al., 2014). Some of these odorants can be reduced through dietary manipulation (Chavez et al., 2004; Sharma et al., 2015). Feeding meat chickens a low protein diet reduces the concentration of putrefactive metabolites in the ceca such as ammonia, amines,

phenols, indoles, 3-methylindole (**skatole**), cresol, and branched chain fatty acids (Qaisrani et al., 2015). Some of these metabolites are toxic and odorous (Mackie et al., 1998). A low protein diet formulated to provide all the required amino acids without excesses may reduce putrefaction of animal waste and hence the production of toxic and odorous metabolites in the litter.

Feed additives such as in-feed antibiotics, probiotic and saponin are commonly used in meat chicken diets. In-feed antibiotics reduce the microbial load in the gut and decrease the production of microbial metabolites (Dibner and Richards, 2005). *Bacillus* based probiotics improved performance, decreased pathogen load in the gut and reduced the headspace air concentration of ammonia and hydrogen sulfide (H₂S) in excreta (Jeong and Kim, 2014; Zhang et al., 2013). A blend of *Yucca* and *Quillaja* saponin improved growth and feed efficiency in meat chickens (Cheeke, 2009) and reduced

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¹Corresponding author: rswick@une.edu.au

ammonia emissions in laying hens (Chepete et al., 2012). Thus, these in-feed additives may have the potential to reduce odor emissions. However, no information in the literature is available to determine whether this is the case. The objective of this experiment was to measure the effect of dietary crude protein (CP) level and additives on odor flux from litter at different ages of meat chickens using selective ion flow tube mass spectrometry (SIFT-MS).

MATERIALS AND METHODS

The experimental procedures were in accordance to the animal ethics committee guidelines of University of New England, Australia. Ethics approval was granted before the studies were conducted.

Bird Husbandry, Experimental Design, and Diets

A total of 180 one-day-old male Ross 308 chicks were allocated to five dietary treatments, each of which was replicated three times with 12 birds per replicate and measured at three ages. The replications were allocated in a completely randomized design. The chicks (35 ± 1 g) were weighed before placements to ensure consistency in pen weights. Each pen measuring 1.2 m \times 0.76 m represented one replicate consisting of a feeder and a double outlet cup drinker. A litter collection tray measuring 0.46 m \times 0.29 m \times 0.065 m was placed in each pen away from the feeder and drinker before litter was spread over the pens to cover the tray. Fresh pine shavings (Hysorb wood shavings, ECW, Australia) were used as litter and added at 10.35 kg/pen. The experiment was designed according to a 5 \times 3 factorial arrangement of treatments, and the main factors consisted of diet (low CP, high CP, high CP+antibiotic, high CP+probiotic, high CP+saponin) and age (15, 29, and 35 d). Two birds from each pen were culled at days 10 and 24 for other experimental purposes.

The composition of experimental diets and their calculated and analyzed nutrients are presented in Tables 1 and 2. High CP and low CP diets differed by 5% CP in starter phase and 4.5% CP in grower and finisher phases. These were formulated to contain the same ratio of soybean, canola, and meat meals in all phases and similar levels of metabolizable energy and digestible amino acid contents. Low protein diets were supplemented with L-valine, L-isoleucine, L-arginine, L-lysine, D,L-methionine, and L-threonine. Zinc bacitracin (ALBAC 150, a registered trademark of Zoetis Australia, Sydney) was used as the in-feed antibiotic at the rate of 330 g/t of feed (50 ppm active Zn bacitracin), a blend of three *Bacillus subtilis* strains (ENVIVA PRO, a registered trademark of Dupont Animal Nutrition, supplied by Feedworks Pty Ltd. Victoria, Australia) was used as a probiotic at 500 g/t to provide 150,000 cfu/g of feed and saponin came from a blend of *Yucca schidigera*

and *Quillaja saponaria* (NUTRAFITO PLUS, a registered trademark of Desert King International, supplied by Feedworks Pty Ltd. Victoria, Australia) and used at 150 g/t of feed. These additives were added by replacing equal amounts of wheat in the formulation. Feed was mixed and pelleted at 65°C at the University of New England, Australia. Feed was provided in three phases: starter (0 to 10 d), grower (10 to 24 d), and finisher (24 to 35 d). All feeds were in crumble form to 10 d and in pellet form thereafter until at 35 d. Feed and water were provided *ad libitum* throughout the study. The lighting program followed the recommendations set forth in the Ross 308 breed management manual (Aviagen, 2014).

Flux Hood

A modified United States Environmental Protection Agency type flux hood (Figure 1) was constructed using a 560 mL, dome-shaped, stainless steel bowl that had an internal diameter of 156 mm that covered a surface area of 0.0191 m² (Kienbusch, 1986). Teflon tubes (1/8" outer diameter) and stainless steel fittings (Swagelock Eastern Australia, Melbourne VIC) were used to construct all the inlet and outlet lines in the flux hood. For uniform incoming airflow distribution inside the flux hood, a 1/8" teflon tube, approximately 295 mm long, was positioned around the inner circumference of the hood and had four holes drilled in it. A short outlet tube (1/8" teflon tube) 260 mm long passed from the hood and connected to the SIFT-MS. A vent was constructed on top of the hood with a 55 mm length and 1/8" teflon tube to prevent pressure fluctuations within the hood.

Litter Collection Trays

The collection trays were carefully removed from each pen without disturbing the litter surface. The trays were covered with aluminium foil and immediately transferred to the laboratory under controlled conditions of 21°C ($\pm 1^\circ\text{C}$) with continuous air ventilation. Immediately before odorant analysis, the aluminium foil was removed and the sample litter tray was covered with the fabricated flux hood. The flux hood was purged with ultra-high purity Nitrogen gas (Nitrogen 99.99%, BOC Limited, Australia) at 500 mL/min until the gases under it reached the equilibrium concentration. The other end of the fluxhood was connected to the SIFT-MS, which drew the gas sample at 14 mL/min. Measurements were taken at two different points in the tray and averaged to get a single value. The trays were put back to the respective pens immediately after odorant measurements without disturbing the litter surface. After each measurement, the SIFT-MS was flushed with the ultra-high purity N₂ gas for 8 min to prevent cross contamination within the sampling lines or instrument. Background measurements were done using the same N₂ gas.

Table 1. Ingredient composition and calculated nutrients of experimental diets (as-fed basis).

Ingredients, %	High CP			Low CP		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Wheat	50.2	52.8	58.4	66.9	69.7	73.4
Soybean meal	30.2	23.7	23.1	18.6	13.9	13.4
Canola meal	9.45	13.1	8.16	5.84	7.69	4.73
Meat meal	5.40	4.08	4.26	3.34	2.40	2.47
Canola oil	3.42	4.95	4.67	1.71	2.95	3.05
Limestone	0.37	0.40	0.37	0.70	0.71	0.69
Dicalcium phosphate	0.03	0.19	0.05	1.03	0.81	0.68
Salt	0.21	0.15	0.15	0.23	0.17	0.17
Na bicarbonate	0.20	0.20	0.20	0.19	0.20	0.20
D,L-methionine	0.15	0.13	0.14	0.27	0.25	0.24
L-Lysine HCl	0.004	0.03	–	0.43	0.43	0.37
L-Threonine	0.03	0.04	0.08	0.20	0.21	0.20
L-Arginine	–	–	0.18	0.10	0.15	0.12
L-Valine	–	–	–	0.02	0.03	–
L-Isoleucine	–	–	–	–	0.02	–
Vitamin-mineral premix ¹	0.20	0.20	0.20	0.20	0.20	0.20
Choline Cl, 60%	0.06	0.06	0.04	0.09	0.09	0.07
Xylanase powder ²	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin ³	0.05	0.05	–	0.05	0.05	–
Calculated nutrients						
ME, MJ/kg	12.3	12.8	13.0	12.3	12.8	13.0
Crude protein	26.0	24.0	23.0	21.0	19.5	18.4
Crude fibre	2.97	3.08	2.82	2.69	2.74	2.58
dLysine	1.20	1.10	1.00	1.20	1.10	1.00
dM+C	0.84	0.80	0.76	0.84	0.80	0.76
dArginine	1.57	1.41	1.52	1.26	1.19	1.10
dIsoleucine	0.99	0.90	0.87	0.78	0.74	0.69
dThreonine	0.77	0.73	0.72	0.77	0.73	0.70
dValine	1.14	1.05	1.00	0.92	0.85	0.79
Ca	0.95	0.85	0.80	0.99	0.85	0.80
Total P	0.79	0.76	0.71	0.81	0.72	0.68
Av. P	0.47	0.43	0.40	0.52	0.43	0.40
Na	0.21	0.18	0.18	0.21	0.18	0.18
K	1.04	0.94	0.90	0.80	0.73	0.70
Cl	0.20	0.16	0.16	0.30	0.25	0.24
dEB ⁴ , mEq	300	273	263	211	194	189

¹Vitamin-Mineral concentrate supplied per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 5,000 IU; tocopheryl acetate, 75 mg; menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.

²Feedzyme XBC 1000 (Feedworks, Australia).

³Sacox 120 (coccidiostat).

⁴dEB (dietary electrolyte balance) = Na⁺+K⁺-Cl⁻.

SIFT-MS Measurement of Odorants

Emissions of odorants from the sample litter headspace were measured at 15, 29, and 35 days using SIFT-MS (Figure 2, Voice 200 SYFT technologies, Christchurch, New Zealand). A method containing 27 compounds, previously identified as key odorants in poultry litter (Murphy et al., 2014), was developed using the selected ion method scan mode and method development software (LabSyft) of the Voice 200. The targeted compounds were: 2,3-butanedione (diacetyl), 3-methyl-1-butanol, total butanol (1-butanol + 2-butanol), 3-methylbutanal, 3-methylindole (skatole), 3-hydroxy-2-butanone (**acetoin**), acetic acid, benzene, butanoic acid, 2-butanone, dimethyl disulfide (**DMDS**), dimethyl sulfide (**DMS**), dimethyl trisulfide (**DMTS**), ethyl mercaptan (**EM**), hexane, hydrogen sulfide (H₂S), indole, methylamine, methyl mercaptan (**MM**), naphthalene, total cresol (p-cresol + m-cresol), phenol, propionic acid, trimethylamine, am-

monia, methylamine, and dimethylamine. The scan duration was 130 s. The scans were repeated until the measured concentration of the compounds reached equilibrium. Prior to each analysis, the SIFT-MS was run with standard gases which included ethylbenzene, tetrafluorobenzene, toluene, hexafluorobenzene, ethylene, octafluorotoluene, benzene, and isobutene to ensure the instrument's mass-calibration and quantification for these compounds was consistent for each measurement session. A reconfigured sample inlet system was designed to allow a bypass flow system to let the sample gas flow continuously through the inlet. This inlet design was to ensure a minimal sample loss and to retain sample integrity.

Litter Water Activity and Moisture Content

At d 29, approximately 10 g of litter was sampled from the tray before odorant measurements to measure

Table 2. Analyzed nutrients of experimental diets (as-fed basis).

Analyzed nutrients, %	High CP			Low CP		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Dry matter	91.4	90.3	91.0	91.2	91.4	91.1
Gross energy, MJ/kg	17.6	17.7	17.8	17.0	17.4	17.5
Crude protein	27.0	25.7	23.3	22.9	20.3	19.5
Crude fibre	2.99	3.32	2.99	2.97	2.68	2.85
Lysine	1.41	1.35	1.15	1.38	1.26	1.12
M+C	0.90	0.85	0.82	0.90	0.84	0.81
Threonine	0.96	0.93	0.84	0.92	0.83	0.78
Arginine	1.58	1.48	1.47	1.32	1.18	1.12
Valine	1.25	1.21	1.04	1.04	0.90	0.84
Isoleucine	1.08	1.03	0.89	0.87	0.77	0.70
Leucine	1.86	1.80	1.56	1.55	1.31	1.18
Phenylalanine	1.20	1.15	1.00	1.00	0.84	0.78
Tyrosine	0.65	0.61	0.54	0.53	0.44	0.37
Glycine	1.45	1.37	1.25	1.15	0.95	0.93
Alanine	1.19	1.13	0.99	0.94	0.79	0.68
Tryptophan	0.35	0.36	0.33	0.29	0.26	0.21
Ca	1.00	0.93	0.92	1.09	0.95	0.89
Total P	0.76	0.79	0.69	0.78	0.73	0.71
Na	0.19	0.18	0.18	0.19	0.18	0.18
K	1.11	1.05	0.93	0.84	0.75	0.73
Cl	0.15	0.15	0.15	0.30	0.25	0.25
Mg	0.23	0.24	0.21	0.20	0.20	0.19
S	0.31	0.32	0.28	0.28	0.28	0.28
dEB ¹ , mEq	324	304	274	213	199	194
dEB ² , mEq	320	302	273	203	189	176

¹dEB (dietary electrolyte balance) = Na⁺+K⁺-Cl⁻.

²dEB = Na⁺+K⁺+Mg²⁺-Cl⁻-S²⁻.

**Figure 1.** Flux hood outer view (left) and inner view (right).

litter water activity (A_w) using tuneable diode laser water activity meter (AquaLab-TDL, Decagon Devices Inc, Pullman, WA, USA). After A_w measurements, the sample was stored in an airtight container and refrigerated for 8 hours before measuring the moisture content. At day 35, immediately after odorant measurements, a sample of the same surface litter was placed in a tightly sealed plastic container and stored refrigerated for 8 hours before measuring the moisture content. Correlation between the moisture content of litter and the odorant concentration values was analyzed. Litter moisture and pH were also measured separately at d 35 from the pens housing birds fed the high CP and low CP diets.

Chemical and Gross Energy Analysis

Dry matter contents of diets and litter were determined by subjecting samples to forced air at 105°C for 48 hours. Litter pH was determined by mixing litter and de-ionised water in a ratio of 1:5 with a pH meter (EcoScan 5/6 pH meter, Eutech Instrument Pte Ltd; Singapore). Nitrogen contents of feeds were determined on a 0.25 g sample with a combustion analyzer (Leco model FP-2000 N analyzer, Leco Corp., St. Joseph, MI) using EDTA as a calibration standard, with CP being calculated by multiplying percentage N by a correction factor (6.25). Analysis of feed for crude fibre, amino acids and Cl was conducted at Experimental

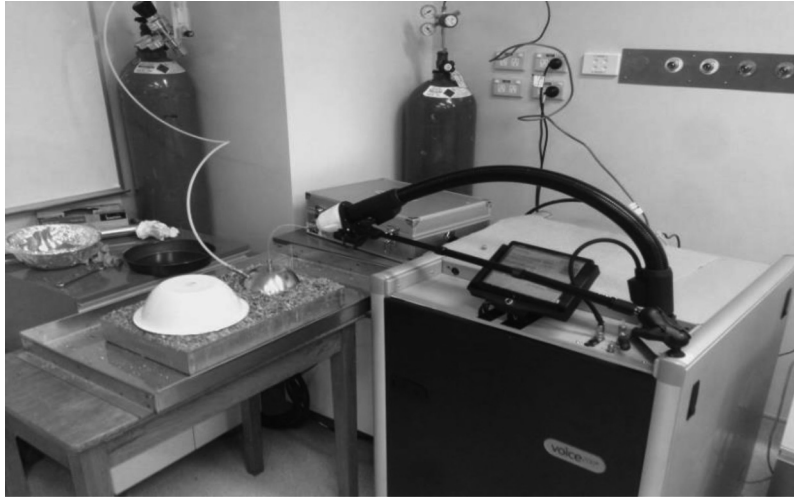


Figure 2. Measurement of odorants from litter headspace using flux hood and SIFT-MS.

Research Station, University of Missouri, USA. Minerals in the feed were analysed using inductively coupled plasma optical emission spectrometer (ICP-OES, Model- 725 radial viewed). Gross energy contents of feeds were determined on a 0.5 g sample using an adiabatic bomb calorimeter (IKA Werke, C7000, GMBH and Co., Staufen, Germany) with benzoic acid as standard.

Statistical Analysis

Odorant concentrations were analyzed following a 5×3 factorial arrangement using JMP statistical software version 8 (SAS Institute Inc, Cary, NC) to test the main effects of diet, age and their interactions. Odorant concentrations were not normally distributed and thus were transformed to a base 10 logarithm before analysis. Data were subjected to two-way ANOVA with repeated measures, and means were separated by Tukey's HSD test at a probability level of 0.05. Pearson correlation coefficients and associated significance were generated using JMP software to determine the relationship between litter moisture content and odorants. The relationship between litter moisture content and A_w was investigated by non-linear (exponential) regression analysis using JMP software.

RESULTS

Feed Analysis

The nutrient contents of finished feeds are presented in Tables 1 and 2. The analyzed CP was 1 to 2% higher than the calculated values but the trend was similar across all the treatments. The analyzed total amino acid contents in the high CP diets were higher than the low CP diets but the calculated digestible amino acid contents were nearly identical. The dietary electrolyte balance (dEB) calculated as $\text{Na}^+ + \text{K}^+ - \text{Cl}^-$ and dEB with Mg and S calculated as $\text{Na}^+ + \text{K}^+ + \text{Mg}^{2+} - \text{Cl}^-$

S^{2-} were nearly identical within each diet but varied across diets. The high CP diets had both forms of dEB approximately 100 higher than the low CP diet in all starter, grower and finisher phases. The S content in the high CP diet was 0.03/0.04 percentage points higher in starter/grower phases but similar in the finisher phase.

Odorants from Litter Headspace

The odorants under the fluxhood in the litter headspace reached equilibrium concentration after 7 to 8 scans in 15 to 20 mins.

a) Sulfur Compounds, Phenolic Compounds, Amines, and Ammonia. The effect of diets on concentrations of odorants belonging to the group of sulfur compounds, phenolic compounds, amines, and ammonia are presented in Table 3. Dimethyl amine, trimethyl amine, H_2S , ammonia, and phenol were produced at lower levels in litter of the pens housing birds fed the low CP diet compared to those fed the high CP diet ($P < 0.05$). Birds fed the high CP diet with probiotic produced lower concentration of H_2S ($P < 0.05$) in litter than those fed the high CP diet but the level was similar to those fed the low CP diet. Antibiotic addition to the high CP diet had no effect on the release of odorants compared to the high CP only diet ($P > 0.05$). Similarly, birds fed the high CP diet with saponin produced a lower concentration of trimethylamine and phenol in litter compared to those fed the high CP diet ($P < 0.05$) but the concentrations of these odorants were similar to those fed the low CP diet. The dietary treatments tended to have a significant effect on the concentration of MM ($P = 0.065$) and the highest value was observed in the litter of the birds fed the high CP diet. Dietary treatments had no effect on the concentrations of DMS, DMDS, DMTS, methyl amine and total cresol in litter ($P > 0.05$).

There was an effect of age on concentrations of sulfur compounds, phenolic compounds, amines, and ammonia in litter (Table 3). Among the amines, the

Table 3. Main effect of diet or age on the concentration of odorants belonging to the group of sulfur compounds, phenolic compounds, amines, and ammonia ($\log_{10} \mu\text{gm}^{-3}$).¹

Treatments	Odorants										
	Dimethyl sulfide	Dimethyl disulfide	Dimethyl trisulfide	Hydrogen Sulfide	Methyl mercaptan	Methyl amine	Dimethyl amine	Trimethyl amine	Ammonia	Phenol	Total cresol ⁵
Diet											
Low CP	1.059	0.289	0.654	0.714 ^b	0.757 ^b	0.823	0.859 ^b	1.536 ^b	2.067 ^b	0.683 ^b	0.746
High CP	1.186	0.361	0.670	1.241 ^a	0.932 ^a	0.854	1.028 ^a	1.819 ^a	2.517 ^a	0.757 ^a	0.776
High CP+ antibiotic ²	1.214	0.349	0.704	1.167 ^a	0.879 ^{a,b}	0.845	1.047 ^a	1.853 ^a	2.421 ^a	0.713 ^{a,b}	0.809
High CP+probiotic ³	1.150	0.306	0.635	0.870 ^b	0.832 ^{a,b}	0.846	0.960 ^{a,b}	1.687 ^{a,b}	2.329 ^{a,b}	0.712 ^{a,b}	0.802
High CP+saponin ⁴	1.075	0.302	0.641	1.027 ^{a,b}	0.717 ^b	0.826	0.892 ^{a,b}	1.569 ^b	2.211 ^{a,b}	0.666 ^b	0.732
SEM	0.063	0.025	0.020	0.137	0.051	0.013	0.050	0.065	0.111	0.021	0.027
Age											
15 d	0.744 ^b	0.302	0.811 ^a	0.525 ^b	0.526 ^c	0.941 ^a	0.781 ^c	1.088 ^c	1.373 ^c	0.771 ^a	0.800
29 d	1.286 ^a	0.327	0.602 ^b	0.609 ^b	0.872 ^b	0.829 ^b	0.972 ^b	1.896 ^b	2.412 ^b	0.682 ^b	0.762
35 d	1.381 ^a	0.336	0.571 ^b	1.877 ^a	1.133 ^a	0.747 ^c	1.119 ^a	2.094 ^a	3.142 ^a	0.665 ^b	0.757
SEM	0.049	0.019	0.016	0.106	0.040	0.023	0.039	0.051	0.086	0.017	0.021
P-value											
Diet	0.369	0.188	0.214	0.045	0.065	0.333	0.045	<0.01	0.047	0.041	0.265
Age	0.001	0.444	0.001	0.001	0.001	0.001	0.001	0.001	0.001	<0.01	0.311
Diet × age	0.625	0.501	0.314	0.475	0.829	0.732	0.364	0.590	0.700	0.291	0.091

^{a-c}within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$).

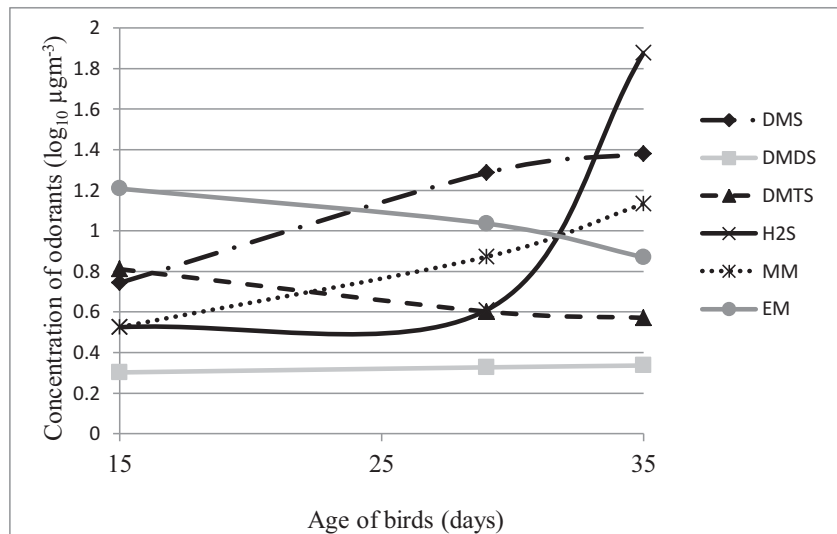
¹concentrations were measured in a flux hood placed on meat chicken litter and flushed with 500 mL/min N_2 .

²zinc bacitracin, ALBAC 150 (Zoetis).

³a combination of three *Bacillus subtilis* strains, ENVIVA PRO (Dupont Animal Nutrition).

⁴a blend of *Yucca schidigera* and *Quillaja saponaria*, NUTRAFITO PLUS (Desert King International).

⁵p-cresol+m-cresol.

**Figure 3.** Comparison of litter headspace concentration of sulfur odorants at different ages.

concentrations of dimethyl amine and trimethyl amine increased ($P < 0.01$) whereas the methylamine concentration decreased ($P < 0.01$) with age. Among the phenolic compounds, the concentrations of phenol in litter decreased as the birds aged ($P < 0.01$) whereas total cresol remained constant throughout the study period ($P > 0.05$). The concentration of ammonia in litter increased with age and the highest concentration was observed at d 35 ($P < 0.01$). The comparison of concentrations of sulfur odorants in litter at three different ages are presented in Figure 3. The concentration of DMS increased at d 29 and then remained constant at d 35. Hydrogen sulfide concentration remained similar

at d 15 and 29 but increased at d 35. The concentration of MM increased in litter as the birds aged from 15 to 35 days. The concentrations of DMTS and EM decreased with age ($P < 0.01$) and DMDS did not change with age ($P > 0.05$).

b) Compounds Belonging to the Group of Alcohols, Aldehydes, Ketones, Indoles, Short Chain Fatty Acids, and Benzene. There was no effect of diet on the emissions of total butanol, 2-butanone, indole, skatole, acetic acid, and butanoic acid (Table 4). In contrast, the birds fed the high CP diet with saponin produced a lower concentration of benzene in litter ($P < 0.05$) than those fed the high CP diet but the

Table 4. Main effect of diet or age on the concentration of odorants belonging to the group of alcohols, ketones, indolic compounds, SCFAs, and others ($\log_{10} \mu\text{gm}^{-3}$).¹

Treatments	Odorants						
	Total butanol ⁵	2-butanone	Indole	Skatole	Acetic acid	Butanoic acid	Benzene
Diet							
Low CP	1.398	1.529	0.308	0.361	2.260	1.709	0.531 ^{b,c}
High CP	1.472	1.631	0.396	0.424	1.909	1.608	0.578 ^{a,b}
High CP+antibiotic ²	1.250	1.421	0.372	0.406	2.002	1.708	0.552 ^{a-c}
High CP+probiotic ³	1.409	1.502	0.376	0.359	1.948	1.663	0.633 ^a
High CP+saponin ⁴	1.199	1.375	0.325	0.351	2.122	1.681	0.468 ^c
SEM	0.103	0.110	0.037	0.037	0.121	0.061	0.034
Age							
15 d	1.118 ^b	0.965 ^b	0.409 ^a	0.450 ^a	2.512 ^a	1.914 ^a	0.518 ^b
29 d	1.500 ^a	1.781 ^a	0.288 ^b	0.324 ^b	2.164 ^b	1.698 ^b	0.524 ^b
35 d	1.418 ^a	1.728 ^a	0.369 ^{a,b}	0.365 ^{a,b}	1.469 ^c	1.408 ^c	0.614 ^a
SEM	0.080	0.086	0.029	0.029	0.091	0.048	0.026
<i>P</i> -value							
Diet	0.319	0.523	0.361	0.525	0.207	0.749	0.020
Age	0.004	0.001	0.012	0.010	0.001	0.001	0.035
Diet × age	0.356	0.466	0.244	0.366	0.444	0.176	0.305

^{a-c}within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$).

¹concentrations were measured in a flux hood placed on meat chicken litter and flushed with 500 mL/min N₂.

²zinc bacitracin, ALBAC 150 (Zoetis).

³a combination of three *Bacillus subtilis* strains, ENVIVA PRO (Dupont Animal Nutrition).

⁴a blend of *Yucca schidigera* and *Quillaja saponaria*, NUTRAFITO PLUS (Desert King International).

⁵1-butanol+2-butanol.

concentration was similar to that in the low CP diet group. The concentrations of total butanol and 2-butanone increased at d 29 and then remained constant until d 35. The concentrations of indole and skatole were similar at d 15 and 35 but their concentrations were lower at d 29 compared to d 15 ($P < 0.05$). Acetic acid and butanoic acid concentrations in litter decreased with age ($P < 0.01$) whereas the benzene concentration increased at d 35 compared to days 15 or 29 ($P < 0.05$).

Table 5 shows the interaction between diet and age on the concentrations of 2,3-butanedione, acetoin, 3-methyl-1-butanol, 3-methylbutanal, ethyl mercaptan, propionic acid, and hexane ($P < 0.05$). Concentrations of 2,3-butanedione, acetoin, propionic acid, and hexane in litter were higher from the birds fed the low CP diet compared to all other treatments on d 35 ($P < 0.05$) but not on days 15 and 29. The low CP diet and the high CP diet with saponin produced higher levels of 3-methyl-1-butanol and ethyl mercaptan in litter at d 35 compared to other diets ($P < 0.05$).

Litter Moisture Content and Odorants

The correlation between litter moisture and odorants is presented in Table 6. Of the sulfur compounds, only methyl mercaptan ($r = 0.453$, $P < 0.01$), hydrogen sulfide ($r = 0.482$, $P < 0.01$), and dimethyl sulfide ($r = 0.621$, $P < 0.01$) had a significant positive correlation with litter moisture content. Dimethyl disulfide tended to be positively correlated with litter moisture ($r = 0.316$, $P = 0.061$). Of the amines, only trimethyl amine had a positive correlation with litter moisture ($r = 0.526$, $P < 0.01$). Similarly, only phenol ($r =$

0.409, $P < 0.05$) of the phenolic group, indole ($r = 0.503$, $P < 0.01$) and skatole ($r = 0.344$, $P < 0.05$) had a significant positive correlation with litter moisture. Methyl amine ($r = -0.309$, $P = 0.086$), propionic acid ($r = -0.318$, $P = 0.072$), and butanoic acid ($r = -0.318$, $P = 0.072$) tended to be negatively correlated with the litter moisture content. No correlation was observed between the litter moisture content and odorants belonging to the group of alcohols, aldehydes and ketones.

Litter Moisture Content and Water Activity

There was an exponential relationship between the litter moisture content and A_w values at d 29 ($r^2 = 0.938$, $P < 0.01$) which is presented in Figure 4. At d 29, A_w increased until it reached the value of 1.0 at a moisture content of approximately 50%.

Litter Moisture Content and pH

The observations of litter moisture and pH with high and low CP diets are presented in Table 7. Reduction in dietary CP content lowered the moisture content in litter ($P < 0.01$). The low CP diet also tended to lower litter pH ($P = 0.05$).

DISCUSSION

Diet can play a significant role in controlling odor issues from meat chicken farms (McGahan et al., 2002). It has recently been reported that meat chicken diets containing high levels of soybean meal produce a higher concentration of methyl mercaptan, a sulfur containing

Table 5. Interaction effect of diet and age on the concentration of odorants belonging to the group of alcohols, aldehydes, ketones, indolic compounds, SCFAs, and others ($\log_{10} \mu\text{gm}^{-3}$).¹

Treatments	Odorants							
	2-3, butanedione	Acetoin	3-methyl-1-butanol	3-methyl-butanol	Ethyl mercaptan	Propionic acid	Hexane	
Diet								
Low CP	1.762	2.989	1.533	0.965	1.091	1.125	1.889	
High CP	1.490	2.688	1.547	0.882	1.000	0.938	1.723	
High CP+antibiotic ²	1.527	2.666	1.529	0.973	1.025	0.969	1.768	
High CP+probiotic ³	1.578	2.750	1.578	0.918	1.032	0.995	1.717	
High CP+saponin ⁴	1.703	2.836	1.525	0.857	1.042	1.016	1.831	
SEM	0.049	0.061	0.041	0.052	0.039	0.069	0.051	
Age								
15 d	1.641	2.637	1.371	1.131	1.208	1.289	1.760	
29 d	1.643	3.072	1.739	0.879	1.036	1.074	1.782	
35 d	1.552	2.648	1.516	0.748	0.870	0.663	1.814	
SEM	0.038	0.047	0.032	0.041	0.030	0.054	0.036	
Diet × Age								
Low CP	15 d	1.700 ^{b,c}	2.688 ^{c-e}	1.361 ^g	1.148 ^{a,b}	1.155 ^{a,b}	1.171 ^{a,b}	1.813 ^{b-d}
	29 d	1.611 ^{b-e}	3.056 ^a	1.603 ^{c-f}	0.744 ^{d-f}	1.050 ^{b-d}	1.121 ^{a,b}	1.731 ^{b-d}
	35 d	1.975 ^a	3.222 ^a	1.636 ^{b-d}	1.002 ^{a-c}	1.068 ^{b-d}	1.082 ^b	2.124 ^a
High CP	15 d	1.553 ^{c-f}	2.471 ^{d-f}	1.308 ^g	1.127 ^{a,b}	1.167 ^{a,b}	1.259 ^{a,b}	1.641 ^{c,d}
	29 d	1.527 ^{b-f}	2.962 ^{a-c}	1.771 ^{a-c}	0.847 ^{c-e}	0.971 ^{c-e}	0.981 ^{b,c}	1.703 ^{b-d}
	35 d	1.489 ^{c-f}	2.632 ^{d-f}	1.462 ^{e,f}	0.671 ^{e,f}	0.860 ^{e,f}	0.573 ^d	1.823 ^{b,c}
High CP+ Zn antibiotic ²	15 d	1.650 ^{b-d}	2.679 ^{c-e}	1.440 ^{e-g}	1.244 ^a	1.271 ^a	1.304 ^{a,b}	1.849 ^{b,c}
	29 d	1.634 ^{b-d}	3.086 ^a	1.802 ^{a,b}	0.866 ^{c-e}	1.027 ^{b-e}	1.123 ^{a,b}	1.767 ^{b-d}
	35 d	1.297 ^{e,f}	2.234 ^f	1.343 ^{f,g}	0.809 ^{b-f}	0.778 ^{e,f}	0.480 ^d	1.686 ^{b-d}
High CP+ probiotic ³	15 d	1.652 ^{b-d}	2.617 ^{d-f}	1.437 ^{e-g}	1.131 ^{a,b}	1.262 ^a	1.429 ^a	1.664 ^{c,d}
	29 d	1.750 ^{a,b}	3.231 ^a	1.875 ^a	0.870 ^{c-e}	1.122 ^{a-c}	1.109 ^{a,b}	1.908 ^{a,b}
	35 d	1.332 ^f	2.402 ^{e,f}	1.422 ^{f,g}	0.554 ^f	0.713 ^f	0.448 ^d	1.578 ^d
High CP+ saponin ⁴	15 d	1.747 ^{a,b}	2.732 ^{b-d}	1.310 ^g	1.002 ^{a-d}	1.185 ^{a,b}	1.281 ^{a,b}	1.833 ^{b-d}
	29 d	1.694 ^{b,c}	3.028 ^{a,b}	1.645 ^{b-d}	0.867 ^{c-e}	1.007 ^{b-e}	1.037 ^{b,c}	1.800 ^{b-d}
	35 d	1.667 ^{b-d}	2.747 ^{b-d}	1.618 ^{b-e}	0.703 ^{e,f}	0.933 ^{d,e}	0.731 ^{c,d}	1.859 ^{b,c}
<i>P</i> -value								
Diet	0.001	0.005	0.880	0.482	0.506	0.350	0.076	
Age	0.201	0.001	0.001	0.001	0.001	0.001	0.651	
Diet × age	0.003	0.001	0.019	0.017	0.030	0.047	0.017	

^{a-g}within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$).

¹concentrations were measured in a flux hood placed on meat chicken litter and flushed with 500 ml/min N₂.

²zinc bacitracin, ALBAC 150 (Zoetis).

³a combination of three *Bacillus subtilis* strains, ENVIVA PRO (Dupont Animal Nutrition).

⁴a blend of *Yucca schidigera* and *Quillaja saponaria*, NUTRAFITO PLUS (Desert King International).

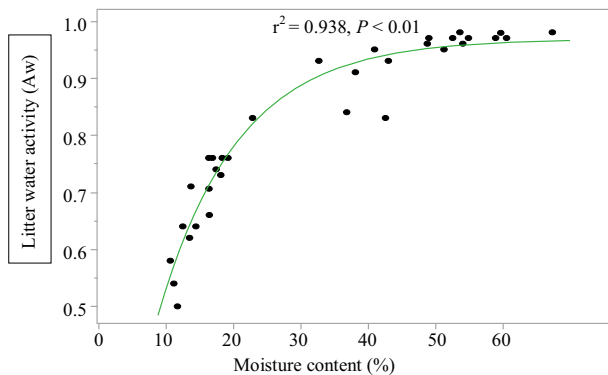
odorant (Sharma et al., 2015). In the same study, diets high in canola meal or soybean meal produced a high concentration of total elemental sulfur at the grower stage. Thus, meat chicken diets with different protein sources can affect the concentration of specific odorants. Similarly, manipulating dietary protein levels may alter the odorant concentrations in litter. Feeding low protein diets supplemented with a range of crystalline amino acids to provide required amino acids without excesses would be expected to reduce the excreted substrates for microbial fermentation to produce odorous metabolites.

In this study, reduction of dietary CP and simultaneous addition of synthetic amino acids lowered the litter headspace concentration of dimethyl amine, trimethyl amine, ammonia, H₂S, and phenol. Studies in pigs have shown reduced methyl sulfide, carbon disulfide, ethanethiol, phenol, 4-ethyl phenol, indole, and 3-methyl indole concentrations in manure by lowering CP in diet

(Le et al., 2007). Similarly, ammonia emissions have been reduced by feeding meat chickens with low CP diets supplemented with crystalline amino acids (Ferguson et al., 1998; Gates, 2000). Although lower emissions of ammonia may not necessarily correlate with the reduced odor emission rates (McGahan et al., 2002; Le et al., 2009), the simultaneous reduction of some odorants along with ammonia by feeding a low CP diet may reduce the overall odor intensity and offensiveness. Interestingly, the concentrations of 2,3-butanedione, 3-hydroxy-2-butanone (acetoin), propionic acid, and hexane were higher in litter from the group fed the low CP diet compared to all other treatments on d 35 but not on days 15 and 29. Also, the group fed the low CP diet as well as the high CP diet with saponin produced higher levels of 3-methyl-1-butanol and ethyl mercaptan in litter at d 35 compared to other groups. These findings suggest that a low CP diet can reduce the production of some odorous metabolites in litter but may

Table 6. Pearson correlation coefficient between litter moisture content and odorants.

Items	Litter moisture (r)	P-value
Litter moisture	1.0	–
Sulfur compounds	0.534	0.001
Methyl mercaptan (methanethiol)	0.453	0.006
Ethyl mercaptan (ethanethiol)	0.108	0.531
Hydrogen sulfide	0.482	0.003
Dimethyl sulfide	0.621	0.001
Dimethyl disulfide	0.316	0.061
Dimethyl trisulfide	0.230	0.178
Amines	0.384	0.021
Methyl amine	–0.309	0.086
Dimethyl amine	0.287	0.089
Trimethyl amine	0.526	0.001
Ammonia	0.532	0.001
Phenolic compounds	0.376	0.024
Phenol	0.409	0.013
m-cresol+p-cresol	0.296	0.079
Indolic compounds	0.441	0.007
Indole	0.503	0.002
Skatole (3-methylindole)	0.344	0.041
Alcohols	0.271	0.111
1-butanol+2-butanol	0.286	0.104
3-methyl-1-butanol	0.180	0.292
Aldehydes	–0.012	0.943
3-methylbutanal	–0.012	0.943
Ketones	0.210	0.218
2,3-butanedione	0.188	0.274
3-hydroxy-2-butanone (acetoin)	–0.018	0.918
2-butanone	0.319	0.058
Short chain fatty acids	–0.249	0.184
Acetic acid	0.001	0.995
Propionic acid	–0.330	0.071
Butanoic acid	–0.318	0.072

**Figure 4.** Relationship between litter moisture content and water activity (A_w).**Table 7.** Effects of low protein and high protein diets on litter pH and moisture content.

Diet	Litter pH	Litter moisture, %
High CP	7.5	38.3 ^a
Low CP	6.9	31.8 ^b
P-value	0.05	<0.01
SE	0.13	1.31

increase the production of some other odorants on d 35. The increase in some of the odorants on d 35 in the low CP group may be due to increased excretion and subsequently fermentation of carbohydrate-rich substrates relative to protein in litter. Birds fed the high CP diet, on the other hand, may have excreted more protein

and amino acids relative to carbohydrates resulting into a higher amount of protein/AA fermentation products like phenol, hydrogen sulfide, and ammonia.

Litter moisture content and litter water activity may also influence microbial growth and odor emissions. Increased litter moisture content favoured the growth of *Atopostipes* and *Bacillus* species in meat chicken litter (Wadud et al., 2012) and these bacteria positively correlated with the levels of phenol, indole, iso-butyric acid and iso-valeric acid in pigs manure (Cho et al., 2015). In this study, birds fed the high CP diet had 6 percentage points higher litter moisture than those fed the low CP diet. High litter moisture was positively correlated to higher concentrations of trimethyl amine, hydrogen sulfide, ammonia and phenol. Thus, higher concentrations of these odorants from the birds fed the high CP diet may also be related to high litter moisture content. Further, there was an exponential relationship between litter moisture content and A_w at d 29. A_w increased with litter moisture up to a level of 1.0 when the moisture level was nearly 50%. This finding was similar to the findings by Himathongkham et al. (1999). Increased litter water activity enhances the growth of micro-organisms (Payne et al., 2007), resulting in increased anaerobic degradation of litter. Thus, the difference in the flux of odorants from litter housing birds fed high and low protein diets may be related to the difference in substrates available in litter, changes in litter moisture content, litter water activity, and microbial ecosystem in litter.

Bacillus subtilis is a spore-forming bacterium that can be used as a probiotic in meat chicken diets (Dersjant-Li et al., 2014). In this study, birds fed the high CP diet with a probiotic based on three strains of *B. subtilis* produced a lower concentration of H_2S in litter than those fed the high CP diet. Our results agreed with the findings of Zhang et al. (2013) that there were lower concentrations of ammonia and H_2S in excreta of meat chickens fed diets supplemented with *B. subtilis*. Addition of *B. subtilis* also showed improved nutrient retention, decreased cecal *Clostridium* and *Coliform* counts (Sen et al., 2012), reduced ileal, cecal and excreta *Salmonella* populations, increased *Lactobacillus* count in the ileum, caecum, and excreta and reduced *E. coli* counts in the excreta of meat chickens (Jeong and Kim, 2014; Park and Kim, 2014). Thus, improved nutrient utilization and reduced counts of H_2S producing bacterial species such as *Salmonella* and *E. coli* in the gastrointestinal tract and excreta may have resulted in lower emissions of H_2S from litter in the probiotic fed group.

Saponins are natural detergents or surfactants found in a wide variety of plants. The major commercial saponin-containing products are those derived from *Yucca schidigera* and *Quillaja saponaria* (Cheeke, 2009). The group fed the high CP diet with saponin blend produced a lower concentration of trimethyl amine and phenol in litter compared to those fed the high CP diet alone. It has been reported that *Yucca*

extract reduced fecal odors in dogs and cats and altered the chemical array of fecal volatiles (Lowe et al., 1997; Lowe and Kershaw, 1997). The researchers mentioned that some components of *Yucca* extracts may directly bind odorants. They also noted that the addition of *Yucca* extracts to dilute aqueous solutions of dimethyl sulfide, dimethyl disulfide, indole, and skatole reduced the degree of offensiveness. Saponin is also reported to inhibit microbial fermentation of protein (Cheeke, 2000). All these properties of saponin may have resulted in a decreased concentration of some odorants in this study.

In conclusion, the results of this experiment showed that a low protein diet balanced with supplemental amino acids, a probiotic of three *Bacillus subtilis* strain and a blend of *Yucca* and *Quillaja* saponins were effective in reducing key odorants from meat chickens. However, none of the additives tested reduced the concentrations of odorants in litter below that produced from the low protein diet. Further research is warranted to investigate strategies to reduce odor emissions from birds fed low protein diets, which will have further commercial application in the future. There was a significant correlation between litter moisture content, litter water activity and odorant concentration in litter. A high litter moisture increased water activity and favored the emissions of sulfur containing odorants, trimethyl amine, phenol, indole, and skatole over others. These results may have implications in controlling odor emissions from meat chicken facilities through dietary manipulation and effective control of litter moisture and water activity.

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