MANAGEMENT AND PRODUCTION

Necrotic enteritis challenge and high dietary sodium level affect odorant composition or emission from broilers

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ABSTRACT Necrotic enteritis (NE) challenge and high dietary sodium (from sodium chloride) level on odor flux from broiler litter was investigated using 160 day-old Ross 308 male chicks randomly assigned to 4 dietary treatments with 4 replicates of 10 birds each. A 2 × 2 factorial arrangement of treatments was employed. Factors were: presence or absence of NE challenge and normal (1.6 g/kg) or high (4.0 g/kg) dietary sodium (Na) level. On d 20, odorants were collected from litter headspace with a flux hood and measured using selected ion flow tube mass spectrometry (SIFT-MS). On d 33, while challenge did not lead to higher mortality, it reduced feed intake by 5.48% ($P < 0.05$) and body weight gain by 9.02% ($P < 0.01$) and worsened FCR by 5 points ($P < 0.01$), indicating subclinical necrotic enteritis occurred in challenged birds. Challenge increased ($P < 0.01$) litter moisture and litter headspace concentrations of dimethyl sulfide ($P < 0.05$), propyl mercaptan ($P < 0.05$), total butanols ($P < 0.05$), acetoin ($P < 0.01$), skatole ($P = 0.05$), butyric acid ($P < 0.05$), and methyl amine ($P < 0.05$) and tended to increase concentrations of ethyl mercaptan ($P = 0.07$), carbon disulfide ($P = 0.09$), indole ($P = 0.10$), and formic acid ($P = 0.10$) compared to the unchallenged group. The birds fed a high Na diet produced higher litter moisture ($P < 0.01$) and higher litter headspace concentration of sulfur compounds and phenol ($P < 0.01$) compared to those fed a normal Na diet. In the birds fed a high Na diet, challenge increased the litter flux of some additional odorants, which included 2,3-butanedione ($P < 0.05$), acetic acid ($P < 0.01$), propionic acid ($P < 0.01$), isobutyric acid ($P < 0.01$), isovaleric acid ($P < 0.01$), pentanoic acid ($P < 0.05$), 2-butanone ($P < 0.05$), and 3-methyl-1-butanol ($P < 0.05$). These findings suggest that both a high Na diet and sub-clinical NE increase the odor nuisance potential of broiler farms.

Key words: *Clostridium perfringens*, diet, necrotic enteritis, odor, wet litter

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INTRODUCTION

Necrotic enteritis (**NE**) affected flocks have poor enteric health and nutrient digestibility leading to decreased performance and increased excretion of nutrients (Barekatain et al., 2013; Hofacre et al., 2003). Sub-clinical forms of NE can lead to sticky droppings and dark and moist litter (Kalshusdal and Hofshagen, 1992). Evidence suggests that there is an association between diarrhea, wet litter, and NE (Williams, 2005), but it is unclear whether the occurrence of NE leads to wet litter or vice-versa (Hermans and Morgan, 2007). Acute forms of NE may lead to diarrhea (Helmboldt and Bryant, 1971), but this is not always the case (Nairn and Bamford, 1967). If NE affected birds produce sticky droppings, diarrhea, and wet litter, then NE may exacerbate odor emissions. However, this has not been investigated, to the best of our knowledge.

Wet litter in commercial poultry farms is an increasing problem around the world, and litter quality has come under great scrutiny after new standards were set by animal welfare organizations in Australia (RSPCA, 2013). According to RSPCA (2013) broiler chicken standard, "litter must be maintained in a dry and friable condition." Litter is also considered to be the primary source of odor because the majority of odorants are released during the decomposition of organic matter (Hobbs et al., 2004; Mackie et al., 1998). Litter with high moisture content has been shown to instigate the emission of highly odorous sulfur compounds (Sharma et al., 2017a). Increasing dietary levels of sodium (**Na**) has been shown to increase water intake and litter moisture (Francesch and Brufau, 2004). Water-to-feed-intake ratio, litter moisture, and emission of odorants that produce a noxious smell from broiler chicken houses may be heightened in birds that are challenged with NE or fed diets with high levels

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

	Normal Na diet			High Na diet		
Ingredients, %	Starter	Grower	Finisher	Starter	Grower	Finisher
Wheat	55.8	60.4	64.9	54.6	59.2	65.2
Soybean meal	25.1	19.2	14.2	25.3	19.4	15.8
Canola meal	8.0	10.0	10.0	8.0	10.0	7.3
Meat meal	5.9	5.0	4.6	5.9	5.0	4.7
Canola oil	3.6	3.9	4.9	4.0	4.3	4.9
Limestone	0.31	0.32	0.33	0.31	0.32	0.33
Salt	0.11	0.12	0.12	0.72	0.73	0.73
Na bicarbonate	0.150	0.150	0.150	0.150	0.150	0.150
D,L-methionine	0.309	0.252	0.220	0.311	0.250	0.237
L-Lysine HCl	0.260	0.246	0.248	0.258	0.240	0.250
L-Threonine	0.181	0.146	0.128	0.181	0.150	0.134
Vitamin-mineral premix[1]	0.200	0.200	0.200	0.200	0.200	0.200
Choline Cl, 70%	0.070	0.070	0.060	0.070	0.070	0.060
Calculated nutrients						
ME, MJ/kg	12.55	12.76	13.18	12.55	12.76	13.18
Crude protein	24.5	22.5	20.5	24.5	22.5	20.3
Crude fibre	2.83	2.89	2.83	2.81	2.87	2.69
Standardized ileal digestible (SID)						
Lysine	1.28	1.15	1.03	1.28	1.15	1.03
M+C	0.95	0.87	0.80	0.95	0.87	0.80
Arginine	1.43	1.27	1.12	1.43	1.27	1.13
Isoleucine	0.92	0.84	0.75	0.92	0.83	0.76
Threonine	0.86	0.77	0.69	0.86	0.77	0.69
Valine	1.05	0.97	0.87	1.05	0.96	0.87
Ca	0.96	0.86	0.80	0.96	0.86	0.80
Total P	0.79	0.74	0.70	0.79	0.74	0.69
Av. P	0.48	0.43	0.40	0.48	0.43	0.40
Na	0.16	0.16	0.16	0.40	0.40	0.40
K	0.94	0.85	0.76	0.94	0.85	0.76
Cl	0.20	0.20	0.20	0.57	0.57	0.57
Analyzed nutrients						
Dry matter	90.7	91.2	91.1	91.3	91.4	91.2
Crude protein	25.6	23.7	21.2	25.4	23.5	20.9
Na	0.15	0.16	0.15	0.42	0.40	0.40

[1]Vitamin-Mineral concentrate supplied per kilogram of diet: retinol, 12000 IU; cholecalciferol, 5000 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; cyanocobalamine, 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.

of Na. Understanding the emissions of odorants from NE affected flocks in wet litter conditions provides opportunity to target combating these specific odorants through the use of in-feed or litter additives. This study was conducted to investigate the effect of sub-clinical NE and high dietary Na level on odorants emission from broiler litter.

MATERIALS AND METHODS

Animal Ethics

All the experimental procedures were approved by the Animal Ethics Committee at the University of New England, Australia (Authority No.: AEC16-019).

Bird Husbandry, Experimental Design, and Diets

A total of 160 day-old Ross 308 male broiler chicks was assigned to 4 dietary treatments, each with 4 replicates of 10 birds/pen (pen size: 1.2 m × 0.65 m up to d 10 and 0.84 m × 0.60 m thereafter) with fresh pine shavings (Hysorb wood shavings, ECW, Carole Park, Qdl., Australia) as bedding material. A litter collection tray measuring 0.46 m × 0.29 m × 0.065 m was placed in each pen away from the feeder and drinker before litter was spread over the pens covering the tray. A 2 × 2 factorial arrangement of treatments was employed in a completely randomized design to study the effect of NE challenge (no, yes), dietary Na level (1.6 g/kg, 4.0 g/kg), and their interaction on litter headspace concentration of odorants. The high Na diet contained a high level of salt. The diets contained wheat, soybean, canola, and meat meals as basal ingredients and were formulated to meet the 2014 Ross 308 nutrient specifications, except for the high Na diet, which contained 2.5 times more Na level than the normal diet. Feed was provided in three phases: starter (0 to 10 d), grower (10 to 24 d), and finisher (24 to 33 d). The composition of the experimental diets and their calculated and analyzed nutrients are presented in Table 1. Feed was mixed and cold pelleted at 65 °C at the University of New England, Australia. All diets were fed in crumble form to 10 d and as 3 mm pellets thereafter until finishing the 33-day study period. Feed and water were provided ad libitum

throughout the study. The lighting program was provided according to the Ross 308 breed management manual (Aviagen, 2014).

Necrotic Enteritis Challenge

NE challenge was based on a previously conducted procedure (Rodgers et al., 2015; Wu et al., 2010). The challenged birds were given a one-off dose of 1 mL *per os Eimeria* (***E***) species (*E. acervulina*, 5,000 oocytes; *E. maxima*, 5,000 oocytes; *E. brunetti*, 2,500 oocytes) in PBS on d 9, and the same dose *per os* of sterile PBS was administered to the birds in the non-challenge group. On d 14, each bird in the challenge group was subjected to 2 mL *per os* the culture of *Clostridium perfringens* (***Cp***) type A strain EHE-NE18 (CSIRO, Geelong, Vic. AU) containing approximately 10^7cfu/mL in thioglycollate (Thermo-Fisher Scientific Oxoid Ltd, Basingstoke, UK) broth supplemented with peptone and starch. The non-challenge group received the same dose of sterilized broth. An aseptic technique was applied in the growth of broth culture and the inoculation processes to prevent the chance of contaminations.

Performance Measurements

Birds and leftover feeds were weighed in all pens on d 16 and 33 to measure feed intake (**FI**), body weight gain (**BWG**), and feed conversion ratio (**FCR**, corrected for mortality) during these periods. Water intake (**WI**) and FI were measured from d 13 to 20 to calculate WI:FI during this period. On d 16, 2 birds from each pen were randomly selected and euthanized by cervical dislocation to investigate the intestinal lesions caused by NE challenge.

Flux hood and litter collection trays

The flux hood used in this experiment was similar to the one used to measure odor flux from litter by Sharma et al. (2017a). 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 (±1°C) with continuous air ventilation.

SIFT-MS Measurement of Odorants

Immediately before measurements, the aluminium foil was removed and the sample litter tray was covered with the fabricated flux hood. Emissions of odorants from sample litter headspace were measured on d 20 using selected ion flow tube mass spectrometry (**SIFT-MS**, Voice 200^{TM} SYFT technologies, Christchurch, New Zealand) as per the method described by Sharma et al. (2017a). In short, the flux hood was purged with ultra-high purity N_2 gas at 500 mL/min until the gases under it reached the equilibrium concentration. The other end of the flux hood was connected to the SIFT-MS, which drew the gas sample at 14 mL/min. Measurements were taken at 2 different points in the tray and averaged to get a single value. Prior to each analysis, the SIFT-MS was calibrated using the method described in a previous study (Sharma et al., 2016a). The odorants were measured using selected ion method (**SIM**) scan mode of SIFT-MS. The odorants with their odor characters and odor threshold values are reported in Sharma et al. (2017b).

Litter Moisture and Litter pH

Litter samples were collected from each pen on d 20 to measure the moisture content. From d 25 to d 30, several litter samples were collected from the pens and analyzed for pH and moisture contents. Litter pH was determined by mixing litter and de-ionised water in a ratio of 1:5 and measuring the pH with a pH meter (EcoScan 5/6 pH meter, Eutech Instrument Pte Ltd, Singapore).

Chemical and Gross Energy Analysis

Dry matter content of the diets and litter were determined by subjecting samples to forced air at 105 °C for 48 h until the weight was constant. Nitrogen content of the diets was determined on approximately a 0.25 g sample with a combustion analyzer (Leco model FP-2000N analyzer, Leco Corp., St. Joseph, MI) using EDTA as a calibration standard. Crude protein was calculated by multiplying percentage N by a correction factor (6.25). Mineral contents in the feed were analyzed using an 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 a calibration standard.

Statistical Analysis

Data were analyzed following a 2 × 2 factorial arrangement using JMP statistical software version 8 (SAS Institute Inc, Cary, NC) to test the main effects of diet, NE challenge, and the interaction between them. Odorant concentrations were not normally distributed and thus were transformed to a base 10 logarithm before analysis. Data were subjected to 2-way ANOVA, and means were separated by Tukey's HSD test at a probability level of 0.05. The relationship between litter moisture and pH was investigated by linear regression analysis using JMP software.

Table 2. Effect of *Cp* challenge and high dietary sodium level on performance, water-to-feed-intake ratio, and litter moisture content of broilers.

Treatments	FI, g	BWG, g 0 to 16 d	FCR	FI, g	BWG, g 16 to 33 d	FCR	FI, g	BWG, g 0 to 33 d	FCR	WI:FI 13 to 20 d	Litter moisture, % d 20
Diet											
Normal Na[1]	737	605	1.238[a]	2544[a]	1786	1.424	3281	2391	1.373	2.24	32
High Na[2]	739	619	1.203[b]	2408[b]	1709	1.409	3147	2328	1.353	2.81	51
SEM	12.3	9.3	0.01	38.3	22.5	0.01	47.5	28.1	0.01	0.06	0.75
CP challenge											
No	799[a]	706	1.131[b]	2506	1766	1.419	3304[a]	2471[a]	1.337[b]	2.43	35
Yes	678[b]	518	1.310[a]	2446	1730	1.414	3123[b]	2248[b]	1.389[a]	2.62	47
SEM	12.3	9.3	0.01	38.3	22.5	0.01	47.5	28.1	0.01	0.06	0.75
Treatments											
Normal Na+ No challenge	814	716[a]	1.138	2535	1765[a,b]	1.437	3349	2481	1.350	2.23[c]	23[d]
Normal Na + *Cp* challenge	661	494[b]	1.339	2552	1808[a]	1.411	3212	2301	1.396	2.25[c]	40[c]
High Na + No challenge	783	696[a]	1.125	2476	1766[a,b]	1.402	3259	2462	1.324	2.62[b]	48[b]
High Na + *Cp* challenge	695	542[b]	1.282	2339	1652[b]	1.416	3034	2195	1.383	2.99[a]	54[a]
SEM	17.4	13.1	0.02	54.2	31.8	0.01	67.1	39.6	0.01	0.06	1.07
P-value											
Diet	0.92	0.29	0.05	<0.05	<0.05	0.31	0.07	0.14	0.15	<0.001	<0.01
Cp challenge	<0.01	<0.01	<0.01	0.29	0.28	0.69	0.02	<0.01	<0.01	<0.05	<0.01
Diet × *Cp* challenge	0.08	<0.05	0.20	0.18	<0.05	0.18	0.52	0.29	0.60	<0.05	<0.01

[a–d]Within each treatment factor, means in the same column with a different superscript differ significantly ($P \leq 0.05$).
[1]Na- 1.6 g/kg.
[2]Na- 4.0 g/kg.

RESULTS

Feed Analysis

The calculated and analyzed nutrient contents of the diets are presented in Table 1. The analyzed Na contents in the diets were similar to the calculated values.

Growth Performance, Water-to-feed-intake Ratio, and Litter Moisture

The overall mortality during the entire study period was less than 3%, and there was no diet or challenge related mortality ($P > 0.05$, data not shown). The effects of NE challenge and high dietary Na level on performance of birds are shown in Table 2. On d 16, the birds in the challenged group had 15.14% lower FI ($P < 0.01$) and 18 points poorer FCR ($P < 0.01$) than those in the unchallenged group. The birds fed the high Na diet had 4 points better FCR ($P = 0.05$) than those fed the normal Na diet. There was diet × challenge effect on BWG. NE challenge reduced BWG by 31% in the birds fed the normal Na diet but by only 22% in those fed the high Na diet ($P < 0.05$). During the period of 0 to 33 d, NE challenge reduced FI by 5.48% ($P < 0.05$) and BWG by 9.02% ($P < 0.01$) and worsened FCR by 5 points ($P < 0.01$). There was diet × challenge effect on WI:FI of birds during the 13 to 20 d period. During this period, NE challenge had no effect on WI:FI in the birds fed the normal Na diet but increased it by 14% ($P < 0.05$) in the birds fed the high Na diet. Birds fed the high Na diet had higher WI:FI compared to those fed the normal Na diet ($P < 0.05$) irrespective of presence or absence of the NE challenge. Necrotic enteritis challenge increased ($P < 0.01$) litter moisture irrespective of dietary Na level with that of challenged being 1.7 times that of unchallenged (from 23 to 40%) in the group fed the normal Na diet, and that of challenged being only 1.1 times that of unchallenged (from 48 to 54%) in those fed the high Na diet. Similarly, the birds fed the high Na diet had higher litter moisture than those fed the normal Na diet irrespective of presence or absence of the NE challenge ($P < 0.01$) with high Na diet giving 2.1 times the moisture of normal Na diet in unchallenged birds but only 1.4 times in challenged birds.

Litter Headspace Concentration of Odorants

The odorants under the flux hood in the litter headspace reached equilibrium concentration after 7 to 8 scans in 15 to 20 minutes.

Odorants Belonging to the Group of Sulfur and Phenolic Compounds The effect of diet and NE challenge on concentration of odorants belonging to the group of sulfur and phenolic compounds is presented in Table 3. No interactions between Na level in diet and necrotic enteritis challenge were observed ($P > 0.05$). The birds fed the high Na diet produced higher concentrations of dimethyl sulfide ($P < 0.01$), dimethyl disulfide ($P < 0.01$), hydrogen sulfide ($P < 0.05$), ethyl mercaptan ($P < 0.01$), propyl mercaptan ($P < 0.05$), and phenol ($P < 0.01$) and tended to produce higher concentrations of dimethyl trisulfide ($P = 0.08$) and methyl mercaptan ($P = 0.10$) in litter headspace compared to those fed the normal Na diet. NE challenged birds produced higher concentrations of dimethyl sulfide ($P < 0.05$) and propyl mercaptan ($P < 0.05$) and tended to produce higher concentrations of ethyl mercaptan

Table 3. Main effect of diet or *Cp* challenge on the litter headspace concentration of odorants belonging to the group of sulfur compounds, phenols, and cresols on d 20 ($\log_{10}\mu gm^{-3}$).[1]

	Odorants									
Treatments	Dimethyl sulfide	Dimethyl disulfide	Dimethyl trisulfide	Hydrogen Sulfide	Methyl mercaptan	Ethyl mercaptan	Propyl mercaptan	Carbon disulfide	Phenol	Total cresols[2]
Diet										
Normal Na[3]	1.15[b]	0.50[b]	0.73	0.78[b]	0.94	1.23[b]	0.97[b]	1.27	0.58[b]	0.87
High Na[4]	1.75[a]	1.38[a]	0.86	1.38[a]	1.30	1.77[a]	1.14[a]	1.46	1.44[a]	0.93
SEM	0.07	0.16	0.05	0.13	0.14	0.07	0.05	0.08	0.15	0.04
Cp challenge										
No	1.32[b]	1.04	0.80	1.20	1.08	1.40	0.94[b]	1.26	1.10	0.90
Yes	1.57[a]	0.85	0.79	0.96	1.15	1.61	1.17[a]	1.47	0.91	0.90
SEM	0.07	0.16	0.05	0.13	0.14	0.07	0.05	0.08	0.15	0.04
P-value										
Diet	<0.01	<0.01	0.08	<0.05	0.10	<0.01	<0.05	0.13	<0.01	0.27
Cp challenge	<0.05	0.42	0.92	0.23	0.76	0.07	<0.05	0.09	0.41	0.99
Diet × *Cp* challenge	0.83	0.55	0.72	0.10	0.66	0.35	0.10	0.08	0.54	0.24

[a,b]Within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$).
[1]Concentrations were measured in litter headspace using a flux hood placed on meat chicken litter and flushed with 500 mL/min N_2.
[2]m-cresol+p-cresol.
[3]1.6 g/kg Na.
[4]4.0 g/kg Na.

Table 4. Main effect of diet or *Cp* challenge on the litter headspace concentration of odorants belonging to the group of alcohols, aldehydes, ketones, amines, and carboxylic acids on d 20 ($\log_{10}\ \mu gm^{-3}$).[1]

		Odorants						
Treatments	Total butanol[2]	Acetoin	Indole	Skatole	3-methyl butanal	Formic acid	Butanoic acid	Methyl amine
Diet								
Normal Na[3]	1.85	2.01	0.34	0.34	1.49[a]	2.95	3.12	1.66
High Na[4]	1.94	1.93	0.55	0.60	1.23[b]	2.91	3.34	1.66
SEM	0.08	0.11	0.08	0.08	0.06	0.09	0.14	0.01
Cp challenge								
No	1.74[b]	1.73[b]	0.34	0.33[b]	1.30	2.81	2.98[b]	1.64[b]
Yes	2.05[a]	2.21[a]	0.56	0.61[a]	1.43	3.06	3.48[a]	1.68[a]
SEM	0.08	0.11	0.08	0.08	0.06	0.09	0.14	0.01
P-value								
Diet	0.40	0.65	0.10	0.06	<0.05	0.77	0.30	0.76
Cp challenge	<0.05	<0.01	0.10	0.05	0.16	0.10	<0.05	<0.05
Diet × *Cp* challenge	0.13	0.06	0.33	0.58	0.29	0.26	0.14	0.56

[a,b]Within each treatment factor, means in the same column with a different superscript differ significantly ($P \leq 0.05$).
[1]Concentrations were measured in litter headspace using a flux hood placed on meat chicken litter and flushed with 500 mL/min N_2.
[2]1-butanol+2-butanol.
[3]1.6 g/kg Na.
[4]4.0 g/kg Na.

($P = 0.07$) and carbon disulfide ($P = 0.09$) in the litter headspace compared to the unchallenged birds.

Odorants Belonging to the Group of Alcohols, Aldehydes, Ketones, Amines, and Carboxylic Acids As shown in Table 4, the birds fed the high Na diet produced a lower concentration of 3-methyl butanal ($P < 0.05$) and tended to produce higher concentrations of skatole ($P = 0.06$) and indole ($P = 0.10$) in the litter headspace compared to those fed the normal Na diet. NE challenged birds produced higher concentrations of total butanols ($P < 0.05$), acetoin ($P < 0.01$), skatole ($P = 0.05$), butyric acid ($P < 0.05$), and methyl amine ($P < 0.05$) and tended to produce higher concentrations of indole ($P = 0.10$) and formic acid ($P = 0.10$) in the litter headspace compared to the unchallenged group.

Significant interactions between diet and NE challenge were observed in the concentrations of odorants belonging to the group of alcohols, aldehydes, ketones, amines, and carboxylic acids (Table 5). In the birds fed the high Na diet, NE challenge increased the litter headspace concentrations of 2,3-butanedione ($P < 0.05$), acetic acid ($P < 0.01$), propionic acid ($P < 0.01$), isobutyric acid ($P < 0.01$), isovaleric acid ($P < 0.01$), pentanoic acid ($P < 0.05$), 2-butanone ($P < 0.05$), and 3-methyl-1-butanol ($P < 0.05$) and decreased the concentrations of dimethyl amine ($P < 0.05$) and trimethyl amine ($P < 0.05$), but NE challenge had no effect on these odorants in the birds fed the normal Na diet. Similarly, the birds fed the high Na diet produced lower litter headspace concentrations of acetic acid ($P < 0.01$), propionic acid ($P < 0.01$), isobutyric acid ($P < 0.01$), isovaleric acid ($P < 0.01$), and pentanoic acid ($P < 0.05$) and higher concentrations of dimethyl amine ($P < 0.05$) and trimethyl amine ($P < 0.05$) compared to those fed the normal Na diet in unchallenged birds

Table 5. Interaction effect of diet and *Cp* challenge on the litter headspace concentration of odorants belonging to the group of alcohols, aldehydes, ketones, amines, and carboxylic acids on d 20 ($\log_{10}\mu gm^{-3}$).[1]

	Odorants									
Treatments	2,3-butanedione	Acetic acid	Propionic acid	Isobutyric acid	Isovaleric acid	Pentanoic acid	Dimethyl amine	Trimethyl amine	2-butanone	3-methyl-1-butanol
Diet										
Normal Na[2]	1.57	2.77	1.98	2.13	1.62	1.85	1.06	1.44	2.16	1.61
High Na[3]	1.57	2.01	1.50	1.68	1.35	1.48	1.19	2.19	2.83	2.16
SEM	0.08	0.10	0.07	0.06	0.06	0.05	0.02	0.09	0.16	0.08
No	1.36	2.00	1.44	1.63	1.29	1.51	1.20	2.05	2.11	1.73
Yes	1.78	2.79	2.04	2.18	1.68	1.82	1.05	1.58	2.88	2.04
SEM	0.08	0.10	0.07	0.06	0.06	0.05	0.02	0.09	0.16	0.08
Treatments										
Normal Na+ No challenge	$1.52^{a,b}$	2.68^{a}	1.97^{a}	2.07^{a}	1.59^{a}	1.78^{a}	1.10^{b}	1.47^{b}	2.07^{b}	1.60^{b}
Normal Na + *Cp* challenge	$1.62^{a,b}$	2.85^{a}	1.98^{a}	2.20^{a}	1.65^{a}	1.91^{a}	1.02^{b}	1.41^{b}	2.26^{b}	1.62^{b}
High Na + No challenge	1.19^{b}	1.31^{b}	0.91^{b}	1.20^{b}	0.99^{b}	1.23^{b}	1.30^{a}	2.63^{a}	2.15^{b}	1.87^{b}
High Na+ *Cp* challenge	1.94^{a}	2.72^{a}	2.10^{a}	2.16^{a}	1.70^{a}	1.74^{a}	1.08^{b}	1.75^{b}	3.50^{a}	2.46^{a}
SEM	0.11	0.14	0.10	0.08	0.08	0.07	0.02	0.13	0.23	0.12
P-value										
Diet	0.98	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	<0.05	<0.01
Cp challenge	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05
Diet × *Cp* challenge	<0.05	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05	<0.05	<0.05

[a,b]Within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$).
[1]Concentrations were measured in litter headspace using a flux hood placed on meat chicken litter and flushed with 500 ml/min N_2.
[2]1.6 g/kg Na.
[3]4.0 g/kg Na.

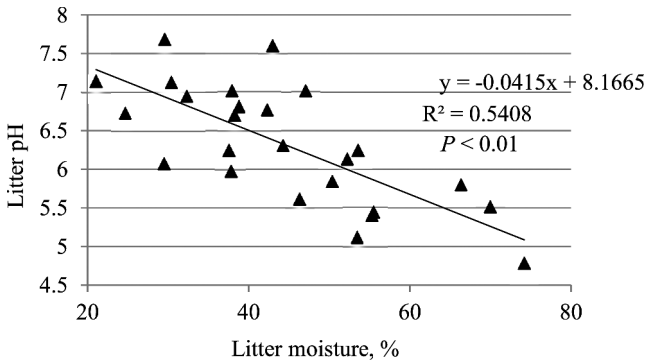

Figure 1. Relationship between litter moisture content and litter pH.

but not in challenged birds. Moreover, the birds fed the high Na diet produced higher litter headspace concentrations of 2-butanone ($P < 0.05$) and 3-methyl-1-1butanol ($P < 0.05$) only in challenged birds but not in unchallenged birds.

Litter Moisture Content and pH

There was a negative linear relationship between litter moisture and litter pH ($r^2 = 0.54$, $P < 0.01$), as shown in Figure 1. As litter moisture increased, litter pH decreased and vice-versa.

DISCUSSION

Recent studies suggest that odorants emission from broiler production may be reduced by dietary manipulation (Sharma et al., 2015; 2017a). However, the understanding of the odorants that are emitted from wet litter or diseased flock has not been shown, while this is important to provide an opportunity for nutritionists to target reduction of specific odorants by using feed or litter additives in odor abatement studies. This study aimed to investigate the effect of NE challenge and high dietary Na level on litter moisture and litter headspace concentration of odorants in broiler houses.

The challenge model used in this study was successful at inducing sub-clinical NE in broilers. The lower FI, lower BWG, and poorer FCR in the challenged group as compared to the unchallenged group are in agreement with previous findings on sub-clinical NE affected broilers (Kalshusdal and Hofshagen, 1992; Lovland and Kaldhusdal, 2001). The performance losses may be associated with chronic intestinal mucosal damage caused by *Cp* resulting in poor nutrient digestion and absorption, and higher excretion of nutrients (Van Immerseel et al., 2004; Timbermont et al., 2011). The high water-to-feed-intake ratio (WI:FI) in NE affected broilers observed in this study and the possible higher excretion of undigested nutrients may have resulted in increased litter moisture in the NE affected group. A high WI:FI has been correlated with a poor litter quality in a recent study (Sharma et al., 2016b). A high WI:FI in NE affected birds has also been reported previously (van der Sluis, 2000), which supports our findings.

Cp may produce a wide range of odorous metabolites in the culture media, the concentrations of which vary according to the media type and *Cp* concentration in the media (Sharma et al., 2017c). This suggests that odor produced from *Cp* infected chickens may differ with change in diet composition or *Cp* concentration in the excreta. The principal odorants produced in this in-vivo experiment were similar to the in-vitro experimental findings of Sharma et al. (2017c). In the current study, sub-clinical NE resulted in higher

litter flux of odorants belonging to the group of sulfur compounds (dimethyl sulfide, propyl mercaptan, and ethyl mercaptan), alcohols (total butanols), ketones (acetoin), carboxylic acids (butyric acid), and amines (methyl amine). In addition, sub-clinical NE increased the litter flux of additional odorants in the group fed the high Na diet but not in those fed the normal Na diet. These included 2,3-butanedione, acetic acid, propionic acid, isobutyric acid, isovaleric acid, pentanoic acid, 2-butanone, and 3-methyl-1-butanol. These odorants are carbohydrate and protein fermentation products (Mackie et al., 1998; Wadud, 2011) and were produced at concentrations higher than minimum odor detection threshold (ODT) (Sharma et al., 2017b). These findings suggest that sub-clinical NE affected broilers may further increase odor emissions if the disease comes together with wet litter conditions. This may be due to high litter water activity ($\mathbf{A_w}$) associated with wet litter conditions. Data collected by Carr et al. (1995), van der Hoeven-Hangoor et al. (2014), and Sharma et al. (2017a) showed that A_w in litter reached 0.98 to 1.00 when litter moisture content reached 38 to 55%. It has been reported that *Clostridium spp.* grow well at high litter A_w of 0.90 to 0.97 (Dunlop et al., 2016). Thus, when litter is wet, *Clostridium spp.* may grow rapidly, resulting in higher production of odorous metabolites.

Most of the sulfur compounds were produced at higher levels in the litter headspace of the pens housing birds fed the high Na diet with greater litter moisture compared with those fed the normal Na diet, possibly due to the anaerobic biodegradation of litter in wet litter conditions (McGahan et al., 2002; Hobbs et al., 2004). Sulfur compounds have very low ODT (Sharma et al., 2017b), and a higher emission of these odorants may highly affect the surrounding environment when the ventilation carries these odorants downwind the broiler sheds. Most of these sulfur compounds were positively correlated with litter moisture, as illustrated in a previous study (Sharma et al., 2017a). Litter moisture was negatively correlated with litter pH in this study, which implies that a low litter pH may favor the emissions of sulfur-containing odorants. Thus, feeding a high Na diet resulted in wet litter causing lower litter pH and higher litter flux of sulfur-containing odorants.

In conclusion, both a high Na diet (wet litter) and sub-clinical NE may increase the odor nuisance potential of broiler farms. Therefore, control of litter conditions of sheds and prevention of enteric diseases such as NE in broilers may alleviate the possible odor problems around the chicken sheds.

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