Original Research Article

Effect of oat hulls as a free choice feeding on broiler performance, short chain fatty acids and microflora under a mild necrotic enteritis challenge

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A B S T R A C T

Structure of fibre has been reported to enhance performance, intestinal function and modify the composition and quantity of the microbial population in the chicken gastrointestinal tract. It is hypothesised that insoluble fibre in oat hulls may improve gut health and reduce intestinal *Clostridium perfringens* number. This research assessed the effect of free choice oat hulls (OH) on performance and gut microbiota in broilers during a mild (subclinical) necrotic enteritis (NE) challenge. A total of 240 day-old male Ross 308 broiler chickens were assigned to 24 cages in a 2×2 factorial arrangement of treatments. Factors were challenge − or +; and OH − or +. On d 16, challenged broilers had lower weight gain and feed intake ($P < 0.05$) compared with unchallenged broilers. On d 16, broilers given OH had lower feed intake ($P < 0.05$) and tended to have lower ($P = 0.062$) feed conversion ratio (FCR) compared with those without access to OH. Broiler performance, however, was not affected by OH nor by challenge on d 24 and 35. The broilers given OH had heavier gizzards ($P < 0.05$) compared with those without OH at d 35 but not at d 13 or 16. Increased numbers of *C. perfringens* ($P < 0.001$) and reduced numbers ($P < 0.05$) of *Lactobacillus* and *Salmonellae* were observed in the caecal contents of challenged broilers on d 16. Challenged broilers had a lower concentration of caecal acetic acid ($P < 0.01$) compared with unchallenged broilers at d 16. The broilers given OH had lower concentrations of caecal acetic acid ($P < 0.05$), propionic acid ($P < 0.05$), and valeric acid ($P < 0.01$) compared with those without access to OH. An OH by challenge interaction on succinic acid concentration was observed on d 16 ($P < 0.05$). Oat hulls elevated the caecal succinic acid concentration only in the unchallenged broilers. This study indicated a positive role of OH through improved gizzard function and increased succinic acid in the gut but its role in controlling NE was not conclusive.

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1. Introduction

Necrotic enteritis (NE) is a widespread and economically important enteric disease in broiler flocks (Van der Sluis, 2000). The financial cost of NE has been estimated to be US $6 billion per year to the world's poultry industry (Wade and Keyburn, 2015). In broiler flocks, the mild (subclinical) form of NE, which is not manifested by clear signs or symptoms (Skinner et al., 2010), is more common than the clinical form (Kaldhusdal, 2000), which has visible signs of the disease and leads to high mortality. The subclinical NE mostly results in higher feed conversion ratio and lower gain, which cannot be regained via compensatory growth in the modern broiler, thus leading to massive economic losses (Kaldhusdal and Hofshagen, 1992; Kaldhusdal et al., 1999; Engström et al., 2003). In addition, the disease causes wet litter (Williams, 2005) and possible contamination of poultry products for human consumption (Timbermont et al., 2011). Necrotic enteritis is known to change the

composition and quantity of the gastrointestinal flora (Wu et al., 2014; Stanley et al., 2014). It was reported that counts of *Lactobacillus* decreased following NE challenge (Dahiya et al., 2005; Feng et al., 2010). The causative agent of NE is *Clostridium perfringens*, a ubiquitous bacterium found in soil, dust, feed, animal feces, used poultry litter, and the intestines of normally healthy animals (Ewing and Cole, 1994; Wages and Opengart, 2003). Although *C. perfringens* is the primary causative agent of subclinical NE, contributory factors alter the intestinal microbial balance to favor the proliferation of *C. perfringens* allowing them to colonise the upper intestines. Predisposing factors include diet composition, management related stress, and presence of other intestinal diseases such as coccidiosis (*Eimeria maxima and E. acervulina*) (Shane et al., 1985; Kaldhusdal and Løvland, 2000; McDevitt et al., 2006; Wu et al., 2014). Traditionally, NE has been controlled by in-feed antibiotics (Williams, 2005). However, consumer pressure and legislation to ban the use of in-feed antibiotics in the poultry industry in the EU has increased the incidence of enteric disorders including NE in broilers (Caly et al., 2015). The strategies to control NE without the reliance on antibiotics have focused on improving gut health using a range of feed additives, such as probiotics, prebiotics, bacteriophages, enzymes and phytobiotics (Caly et al., 2015; M'Sadeq et al., 2015). It is speculated that structural fibres play an important role in enhancing performance and intestinal health by selectively stimulating the growth and/or activity of beneficial bacteria in gut. Insoluble fibre, a key component of structural fibres, certainly improves intestinal barrier function and host immunity by reducing pathogen load (e.g., Clostridia), and enhancing short chain fatty acid production (Glenn and Roberfroid, 1995; Amerah et al., 2009; Choct, 2009; Slavin, 2013; Jiménez-Moreno et al., 2016; Kheravii et al., 2016b). It has also been reported that feeding broilers insoluble fibre improves gizzard function and stimulates mucosal layer, and increases gut motility, thereby reducing *C. perfringens* adhering to the mucosal surface in the distal part of the gastrointestinal tract (Kalmendal et al., 2011). Wu et al. (2011) and Kheravii et al. (2017) reported that ingestion of litter by broilers enhances gizzard development. Furthermore, a well-developed gizzard increases digesta retention time in the proximal part of the gastrointestinal tract and enhances secretion of HCl in the proventriculus, thereby resulting in reduced gizzard pH (Kimiaeitalab et al., 2016). This, in turn, has an antimicrobial impact on pathogenic bacteria entering the distal part of digestive tract (Engberg et al., 2002). Jiménez Moreno et al. (2011) reported that the inclusion of oat hulls, which contain 847 g/kg lignified insoluble fibre (Hetland and Svihus, 2001), in broiler diets improved gizzard function and reduced the counts of caecal *C. perfringens*. The present research assessed the response of broilers under subclinical NE challenge to free choice oat hulls (OH) on performance, gizzard development and caecal microflora.

2. Material and methods

2.1. Design and husbandry

A total of 240 day-old male Ross 308 chicks were obtained on the day of hatch from the Baiada Hatchery in Tamworth, NSW, Australia. On arrival, chicks were randomly allocated to 24 multi-tiered brooder cages measuring (600 cm × 420 cm × 23 cm) with 6 replicates per treatment and 10 broilers per replicate. The cages were physically partitioned according to the challenge treatments (12 cage per partition). A 2 × 2 factorial arrangement of treatments was employed with the factors being: challenge - without (−) or with (+); and free choice OH without (−) or with (+). Each cage was equipped with 2 nipple drinkers and a trough feeder. Broilers had *ad libitum* access to water and feed. The feeders were divided into 2 portions: the first portion had 4 access holes to

the feed and the second portion either had 2 holes to access the free choice OH (OH+) or blocked holes (OH). The lighting, relative humidity and temperature followed the Ross 308 strain (Aviagen, 2014) management guidelines.

2.2. Diets and oat hulls

All broilers were fed the same diets in 3 phases: starter (placement to d 10), grower (d 11 to 24), and finisher (d 25 to 35). The diets were formulated with wheat, sorghum, soybean meal, meat meal, and canola meal according to the Ross 308 nutrient specifications (Table 1). The diets were thoroughly mixed and pelleted at 65 °C. The diets were also assayed for crude protein, fat, and fibre, ash, NDF, ADF, moisture content, insoluble non-starch polysaccharides (NSP), soluble NSP and free sugars (Table 1). The OH was obtained locally from a commercial supplier (Grazag Company, Armidale, NSW, Australia). The composition of OH was determined ("as is" basis) for total NSP following the method developed by Englyst et al. (1994). The OH contained 535 g/kg insoluble NSP and soluble NSP was not present at detectable level.

2.3. Animal ethics

This experiment was approved by the Animal Ethic Committee of the University of New England (Approval No. AEC14-068). All broiler management procedures including health care, husbandry and use of laboratory animals fulfilled the requirements of the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). This experiment was conducted in September 2014.

2.4. Broiler performance

On d 16, 24 and 35, broilers and leftover feed were weighed, and mortality was recorded daily. Feed conversion ratio (FCR) was adjusted for mortality. Live broiler feed intake was calculated as FCR × weight gain during the measured periods.

2.5. Lesion scoring

On d 16, 2 broilers were randomly selected from each pen and euthanised by cervical dislocation. Duodenum, jejunum and ileum were excised and scored following the lesion scoring system reported by Prescott et al. (1978).

2.6. Analysis of short chain fatty acids (SCFA)

The caecal SCFA were analysed according to method described by Jensen et al. (1995) with minor modifications. Frozen caecal samples were defrosted and homogenised. Approximately 1 g of homogenised caecal sample from 2 broilers at d 16 was suspended in 1 mL of internal standard (0.01 mol/L 2-ethylbutyric acid), thoroughly mixed and centrifuged at $2,050 \times g$ for 15 min at 5 °C. Then, 2.5 mL of diethyl ether and 0.5 mL of concentrated HCl (36%) were added to 1 mL of the supernatant and thoroughly mixed by using a vortex mixer. Meanwhile, using the same method, a blank and an internal standard solution were prepared by replacing 1 mL of the supernatant with the same amount of water and standard acid mixture respectively. The mixture was centrifuged at $2,050 \times g$ at 5 °C for 15 min. An aliquot of 400 μL of the supernatant was transferred to a gas chromatograph vial (2 mL) and mixed with 40 μL of N-tert-butyldimethlsilyl-N-methyltrifluoroacetamide (MTBSTFA) and incubated at 80 °C for 20 min. The mixture was left at room temperature for at least 48 h. The caecal SCFA were

Table 1
Ingredient composition, and calculated and analysed nutrients of the experimental diets.

Item	Starter	Grower	Finisher
Ingredients (DM basis), g/kg			
Sorghum	250	285	306
Wheat	319	333	330
Soycomil K SPC	–	20.0	–
SBM	247	107	85.2
Canola meal solvent	70.3	127	141
Meat meal	40.0	60.0	65.0
Canola oil	40.0	45.0	55.8
Limestone	11.03	4.87	3.95
Dicalcium phosphate 18P/21Ca	8.42	4.37	1.92
Xylanase powder (500 g/t)	0.500	0.500	0.500
NaCl	1.076	0.665	0.584
Na bicarb	2.00	2.00	2.00
UNE Vit[1] (0.5 kg/Mt inclusion)	0.500	0.500	0.500
UNE TM[2] (0.75 kg/Mt inclusion)	0.750	0.750	0.750
Choline Cl 60%	0.824	0.902	0.838
L-lysine HCl 78.4	3.84	3.69	2.59
DL-methionine	3.52	2.62	2.08
L-threonine	1.96	1.50	1.19
Calculated nutrients (DM basis), g/kg			
ME, kcal/kg	3,025	3,130	3,178
Crude protein	230	216	202
Crude fat	63.6	73.6	82.9
Crude fibre	27.0	28.8	28.9
Digestible arginine	13.1	11.4	10.2
Digestible lysine	12.9	11.5	9.70
Digestible methionine	6.50	5.53	4.85
Digestible Met + Cys	9.40	8.40	7.60
Digestible tryptophan	2.39	1.93	1.73
Digestible isoleucine	8.60	7.52	6.79
Digestible threonine	8.30	7.30	6.50
Digestible valine	9.83	8.86	8.16
NDF	110	122	127
ADF	45.28	51.29	53.74
Soluble NSP	8.09	8.60	8.52
Insoluble NSP	61.6	63.0	61.1
Calcium	10.5	9.00	8.50
Available P	4.50	4.50	4.20
Sodium	1.60	1.60	1.60
Chloride	2.18	2.00	1.73
Choline, mg/kg	1,600	1,500	1,400
Linoleic (18:2)	19.2	20.5	21.5
Analysed nutrients composition ("as is" basis), g/kg			
Crude protein	237	224	215
Crude fat	62.0	74.2	82.5
Crude fibre	29.9	34.6	34.9
Ash	60.7	54.6	47.9
NDF	105	109	142
ADF	44.9	56.0	57.0
Total insoluble NSP	70.4	70.4	73.6
Total soluble NSP	7.44	8.10	8.35
Free sugar	33.0	29.3	25.8

SPC = soy protein concentrate; SBM = soybean meal; NDF = neutral detergent fiber; ADF = acid detergent fiber; NSP = non-starch polysaccharides.

[1] Vitamin 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.

[2] Trace mineral premix supplied per kilogram of diet: 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.

measured using a Varian CP3400 CX gas Chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA).

2.7. Gizzard measurements

On d 13, 16, and 35, empty gizzards and gizzard contents were weighed and recorded along with broiler weight using a digital scale. Gizzard contents of 2 broilers were collected and homogenised to measure pH by using a calibrated pH meter (EcoScan pH 6). The relative gizzard weight was calculated as mass per unit of live body weight (g/100 g of live body weight).

2.8. Caecal content collection

On d 16, the caecal contents from 2 broilers per pen were pooled and mixed in specimen containers and stored at −20 °C until SCFA quantification. During collection of the samples, approximately 2 g of caecal digesta from the homogenised caecal content in specimen containers were transferred into a 2-mL Eppendorf cap lock tube, snap-frozen in liquid N$_2$, and stored at −20 °C until DNA extraction for bacterial quantification.

2.9. Extraction of caecal DNA

Caecal DNA was extracted following the protocol of ISOLATE II Plant DNA Kit (Bioline, NSW, Australia) with slightly modification. Approximately 200 mg of freshly defrosted caecal content were placed in a 2-mL Eppendorf tube contained 300 mg of glass beads. An aliquot of 450 μL Lysis Buffer PA1 was added to the samples and thoroughly mixed with a vortex mixer. The samples were transferred to a block bead mill (Retsch GmbH & Co, Haan, Germany) to disrupt at a frequency of 30/s for 5 min prior to being heated at 95 °C for 5 min. The digesta was lysed and homogenised after adding 200 μL and then 100 μL of Extraction Buffer with vortexmixing following each addition. An aliquot of 10 μL of RNase was added to 600 μL of the lysate in a 1.5-mL microcentrifuge tube in order to remove RNA. The solution was incubated at 65 °C for 10 min. The incubated mixture was centrifuged for 1 min at 11,000 × g to pellet potential impurities. An aliquot of 450 μL of Binding Buffer was used to capture DNA by vortexing thoroughly and then centrifuging for 1 min at 11,000 × g. Then 400 and 700 μL of Wash Buffer PAW1 and PAW2 respectively were added at independent steps to purify DNA, centrifuged for 1 min at 11,000 × g to remove the wash buffer and to dry the silica membrane completely. An aliquot of 50 μL of Elution Buffer was used to elute DNA into a 1.5-mL Eppendorf tube.

2.10. Quantification of caecal bacteria

The quantification of caecal bacterial groups, *Bifidobacterium* spp., *Lactobacillus* spp., *Bacillus* spp., *Ruminococcus* spp., *Bacteroides* spp., *Salmonella* spp., *C. perfringens* and Enterobacteriaceae were achieved by using the methods of Wise and Siragusa (2007). The extracted caecal DNA was diluted 20 times in autoclaved Milli-Q water. The Rotorgene 6000 real-time PCR machine (Corbett, Sydney, Australia) was employed for qPCR assay of the desired bacteria from the extracted cecal DNA. The PCR was performed in duplicate for each sample in 10 μL of reaction. For PCR reactions, a SYBR Green containing Mix (SensiMix SYBR No-Rox, Bioline, Sydney, Australia) was applied for all groups of bacteria except *C. perfringens* for which the SensiFAST Probe SYBR No-ROX (Bioline, Sydney, Australia) was used. The reaction in a volume of 10 μL contained 5 μL of 2 × SensiMix, 300 mmol/L of each primer and 2 μL of DNA template. However, for *C. perfringens*, the reaction in a volume of 10 μL contained 5 μL of 2 × SensiFAST Probe, 400 mmol/L of each primer, 500 mmol/L of probe and 2 μL of DNA template. The below specific 16S rRNA primers and/or probe were used for quantification of different groups of bacteria: GCG TCC GCT GTG GGC and CTT CTC CGG CAT GGT GTT G for *Bifidobacterium* spp. (Requena et al., 2002); CAC CGC TAC ACA TGG AG and AGC AGT AGG GAA TCT TCC A for *Lactobacillus* spp. (Wise and Siragusa, 2007); GCA ACG AGC GCA ACC CTT GA and TCA TCC CCA CCT TCC TCC GGT for *Bacillus* spp.

(Zhang et al., 2015); GGC GGC YTR CTG GGC TTT and CCA GGT GGA TWA CTT ATT GTG TTA A for *Ruminococcus* spp. (Ramirez-Farias et al., 2009); GAG AGG AAG GTC CCC CAC and CGC TAC TTG GCT GGT TCA G for *Bacteroides* spp. (Layton et al., 2006); CGT TTC CTG CGG TAC TGT TAA TT and AGA CGG CTG GTA CTG ATC GAT AA for *Salmonella* spp.; CAT TGA CGT TAC CCG CAG AAG AAG C and CTC TAC GAG ACT CAA GCT TGC for the Enterobacteriaceae; CGG YCC AGA CTC CTA CGG G and TTA CCG CGG CTG CTG GCA C for the total bacteria; and CGC ATA ACG TTG AAA GAT GG and CCT TGG TAG GCC GTT ACC C; TaqMan probe: 5′-FAM-TCA TCA TTC AAC CAA AGG AGC AAT CC-TAMRA-3′ for the *C. perfringens*.

The PCR was performed according to the following cycles: DNA templates were denatured at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 30 s and annealing 63 °C for 45 s and extension at 78 °C for 45 s for all the primers except *Salmonella* spp. and *C. perfringens* primers which the annealing/extension was conducted at 56/72 and 55/70 °C, respectively. A threshold cycle averaged from the duplicate samples was used for data analysis. Serial dilutions of linearised plasmid DNA (pCR 4-TOPO Vector, Life Technologies, Carlsbad, USA) inserted with respective bacterial amplicons were used to construct a standard curve. The concentrations of the plasmid DNA were measured using NanoDrop ND-8000 (Thermo Fisher Scientific, Waltham, USA) prior to the serial dilutions. The number of target DNA copies was calculated from the mass of DNA taking into account the size of the amplicon insert in the plasmid. Bacteria numbers were expressed as $\log_{10}$ (genomic DNA copy number)/g digesta.

2.11. NE challenge

The NE challenge was performed according to Wu et al. (2014) with modifications. *Eimeria acervulina* (batch E1-4/14-042), *E. brunetti* (batch E9-7/14-012A) and *E. maxima* (batch E2-6/14-043) were obtained from Bioproperties Pty. Ltd. (Glenorie, NSW, Australia). *C. perfringens* type A strain EHE-NE18 (CSIRO Livestock Industries, Geelong, Australia) was incubated overnight at 39 °C in 100 mL of sterile thioglycollate broth (USP alternative; Oxoid) followed by subsequent overnight incubation of 1 mL of the previous culture in 100 mL of cooked meat medium (Oxoid), and then in 700 mL of thioglycollate broth (USP alternative; Oxoid) containing starch (10 g/L) and pancreatic digest of casein (5 g/L) to obtain the challenge inoculum. On d 9, broilers in the challenge room were inoculated with 5,000 sporulated oocysts each of *E. maxima* and *E. acervulina* and 2,500 sporulated oocysts of *E. brunetti* in 1 mL of

1% (wt/vol) sterile saline. Unchallenged broilers received 1 mL of 1% (wt/vol) sterile saline as a sham treatment. On d 14 and 15, the challenge group were inoculated *per os* with 2 mL of *C. perfringens* suspension (10×10^7 cfu/mL).

2.12. Statistical analysis

All data were analysed using the General Linear Models (GLM) procedure of SPSS statistics version 22 (IBM, Armonk, New York, US) for the main effect of challenge and free choice OH and interactions. Short chain fatty acids data were transformed as ($\log_{10} + 1$) to achieve normal distribution of the data. Differences between mean values were determined using the Tukey HSD test at the level of $P < 0.05$.

3. Results

3.1. Broiler performance

Performance results are presented in Table 2. On d 16, challenged broilers had lower weight gain and feed intake ($P < 0.05$) compared with unchallenged broilers. The FCR in challenged broilers tended to be higher ($P = 0.052$) compared with unchallenged broilers. On d 16, broilers given OH had lower feed intake ($P < 0.05$) compared with those without access to OH. Broilers with access to OH tended ($P = 0.062$) to have lower FCR compared with those without access to OH. However, on d 24 and 35, the challenge and free choice OH had no effect on weight gain, feed intake, FCR and livability. No challenge × free choice OH interaction was observed for any performance parameters during all the trial period ($P > 0.05$).

3.2. Relative gizzard weight, gizzard contents and pH

As shown in Table 3, at 13 d, i.e., 4 days following *Eimeria* inoculation, the relative gizzard weight, gizzard contents and pH were not affected by *Eimeria* inoculation. However, on d 16, broilers challenged with NE exhibited decreased gizzard contents compared with unchallenged broilers, whereas the relative gizzard weight and pH were not affected by challenge. On d 13 and 16, the free choice OH had no effect on the relative gizzard weight, gizzard contents and pH. However, the broilers given OH had heavier ($P < 0.05$) gizzards compared with those without access to OH at d 35. No significant differences were observed on gizzard contents

Table 2
Impact of free choice oat hulls (OH) and necrotic enteritis challenge on performance in broilers at d 16, 24, and 35.

Treatments		FCR			Weight gain, g/bird			Feed intake, g/bird			Livability, %		
		d 0 to 16	d 0 to 24	d 0 to 35	d 0 to 16	d 0 to 24	d 0 to 35	d 0 to 16	d 0 to 24	d 0 to 35	d 0 to 16	d 0 to 24	d 0 to 35
Challenge	OH												
No	No	1.179	1.276	1.444	623	1,321	2,241	736	1,686	3,236	97	97	93
No	Yes	1.154	1.258	1.417	602	1,276	2,273	695	1,605	3,218	100	100	100
Yes	No	1.194	1.258	1.414	577	1,300	2,323	688	1,633	3,284	98	98	97
Yes	Yes	1.180	1.264	1.401	562	1,312	2,307	663	1,650	3,231	100	100	98
Main effect													
Challenge													
No		1.167	1.267	1.430	612	1,299	2,257	715[a]	1,646	3,227	98	98	97
Yes		1.187	1.261	1.408	569	1,306	2,315	675[b]	1,642	3,257	99	99	98
OH													
No		1.187	1.267	1.429	600	1,311	2,282	712[a]	1,659	3,260	98	98	95
Yes		1.167	1.261	1.409	582	1,294	2,290	679[b]	1,628	3,224	100	100	99
P-value													
Challenge		0.052	0.610	0.082	0.008	0.776	0.258	0.013	0.887	0.614	0.542	0.542	0.701
OH		0.062	0.661	0.114	0.241	0.519	0.875	0.037	0.287	0.555	0.078	0.078	0.066
Challenge × OH		0.607	0.353	0.579	0.844	0.275	0.636	0.599	0.103	0.763	0.542	0.542	0.257

[a,] [b]Means sharing the same superscripts are not significantly different from each other at $P < 0.05$.

Table 3
Impact of free choice oat hulls (OH) and necrotic enteritis challenge on the relative gizzard weight, gizzard pH and contents in broilers at d 13, 16, and 35.

Treatments		Relative gizzard weight, g/100 g BW			Gizzard content, g/100 g BW			Gizzard pH		
		d 13	d 16	d 35	d 13	d 16	d 35	d 13	d 16	d 35
Challenge	OH									
No	No	2.45	2.11	1.03	12.91	17.38	14.78	2.89	2.97	3.17
No	Yes	2.44	2.02	1.16	12.01	15.65	20.32	2.89	2.88	2.89
Yes	No	2.41	2.11	1.08	11.67	13.60	18.19	2.71	2.98	3.33
Yes	Yes	2.48	2.10	1.21	11.62	14.57	19.37	2.77	3.06	3.24
Main effect										
Challenge										
No		2.45	2.07	1.09	12.46	16.51[b]	17.55	2.89	2.92	3.03[b]
Yes		2.44	2.11	1.14	11.64	14.09[a]	18.78	2.74	3.02	3.28[a]
OH										
No		2.43	2.11	1.05[b]	12.29	15.49	16.49	2.80	2.97	3.25
Yes		2.46	2.06	1.18[a]	11.81	15.11	19.85	2.83	2.97	3.06
P-value										
Challenge		0.963	0.615	0.343	0.316	0.028	0.737	0.077	0.468	0.048
OH		0.751	0.498	0.017	0.551	0.714	0.362	0.695	0.984	0.140
Challenge × OH		0.656	0.586	0.931	0.599	0.202	0.553	0.725	0.517	0.463

[a, b]Means sharing the same superscripts are not significantly different from each other at $P < 0.05$.

and pH between broilers with and without access to OH at d 35. On the other hand, challenged broilers had lower gizzard content at d 16, and higher gizzard pH ($P < 0.05$) at d 35 compared with those unchallenged broilers. However, no differences were observed in relative gizzard weight and gizzard content between challenged and unchallenged broilers at d 35 ($P > 0.05$). No challenge × free choice OH interaction was observed for relative gizzard weight, gizzard contents or pH throughout study ($P > 0.05$).

3.3. Bacterial quantification

On d 16, the challenge led to significant changes in the counts of caecal microflora (Table 4). Increased numbers of *C. perfringens* ($P < 0.001$) and Enterobacteriaceae ($P < 0.05$) and reduced numbers of *Lactobacillus* ($P < 0.05$) and *Salmonellae* ($P < 0.05$) were observed in the caecal contents of challenged broilers. However, no changes were observed in caecal *Bifidobacteria*, *Bacillus*, *Ruminococcus*, *Bacteroides* or total bacteria as a result of challenge ($P > 0.05$). The OH had no effect on caecal bacterial counts on d 16. An OH by challenge interaction was observed for Enterobacteriaceae group counts ($P < 0.001$). In the unchallenged broilers, those without OH had lower counts of Enterobacteriaceae compared with those accessed to OH but no effect of OH was observed in challenged broilers. Also, the challenged broilers

had higher counts of Enterobacteriaceae than unchallenged broilers without accessed to OH.

3.4. NE lesions

On d 13, which was between *Eimeria* and *C. perfringens* challenges, there were no significant differences ($P > 0.05$) of lesion scores between treatments for the duodenal, jejunal and ileal samples. On d 16, no significant differences ($P > 0.05$) of lesion scores were detected between treatments for all parts of small intestine (data not shown).

3.5. Caecal SCFA

On d 16, the caecal concentrations of SCFA were significantly affected by challenge and OH (Table 5). The challenged broilers had lower concentrations of caecal acetic acid ($P < 0.01$) and succinic acid ($P < 0.05$) compared with unchallenged broilers, while challenge had no effect on the concentrations of propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid and lactic acid ($P > 0.05$). The OH had no effect on the concentrations of isobutyric acid, butyric acid, and isovaleric acid. However, the broilers given OH had lower concentrations of acetic acid ($P < 0.05$), propionic acid ($P < 0.05$), and valeric acid ($P < 0.01$) and a higher

Table 4
Impact of free choice oat hulls (OH) and necrotic enteritis challenge on caecal microflora ($\log_{10}$ cfu) in broilers at d 16.

Treatments		*Lactobacillus*	*Bifidobacteria*	*Bacillus*	*Ruminococcus*	*Bacteroides*	Enterobacteriaceae	*C. perfringens*	*Salmonella*	Total bacteria
Challenge	OH									
No	No	8.40	8.46	8.67	9.39	4.52	6.54[b]	0.00	6.97	10.00
No	Yes	8.54	8.71	8.96	9.30	4.62	7.19[a]	0.00	6.92	9.99
Yes	No	8.26	8.70	8.74	9.43	4.56	7.39[a]	9.58	6.77	10.06
Yes	Yes	8.15	8.58	9.03	9.29	4.98	6.98[ab]	9.49	6.75	10.03
Main effect										
Challenge										
No		8.47[a]	8.59	8.81	9.34	4.57	6.86[b]	0.00[b]	6.94[a]	9.99
Yes		8.21[b]	8.64	8.88	9.36	4.77	7.18[a]	9.54[a]	6.76[b]	10.05
OH										
No		8.33	8.58	8.71	9.41	4.54	6.96	4.79	6.87	10.03
Yes		8.34	8.64	8.99	9.30	4.80	7.09	4.75	6.84	10.01
P-value										
Challenge		0.035	0.666	0.764	0.765	0.185	0.023	<0.001	0.026	0.282
OH		0.913	0.601	0.214	0.132	0.087	0.327	0.853	0.658	0.649
Challenge × OH		0.304	0.133	0.988	0.723	0.290	0.001	0.853	0.861	0.859

[a, b]Means sharing the same superscripts are not significantly different from each other at $P < 0.05$.

Table 5
Impact of free choice oat hulls (OH) and necrotic enteritis challenge on caecal SCFA (μmol/g digesta) in broilers at d 16.

Treatments		Acetic acid	Propionic acid	Isobutyric acid	Butyric acid	Isovaleric acid	Valeric acid	Lactic acid	Succinic acid
Challenge	OH								
No	No	111.6	4.13	0.449	15.65	0.094	1.64	0.331	8.07[b]
No	Yes	78.80	2.66	0.257	10.92	0.024	1.03	5.530	18.84[a]
Yes	No	68.72	3.83	0.300	11.92	0.072	1.28	0.823	7.42[b]
Yes	Yes	60.51	2.66	0.283	10.99	0.073	0.937	2.110	6.74[b]
Main effect									
Challenge									
No		95.22[a]	3.39	0.353	13.29	0.059	1.34	2.93	13.45[a]
Yes		64.61[b]	3.24	0.292	11.46	0.072	1.11	1.46	7.08[b]
OH									
No		90.18[a]	3.98[a]	0.374	13.79	0.083	1.46[a]	0.58	7.74[b]
Yes		69.66[b]	2.66[b]	0.270	10.96	0.048	0.983[b]	3.82	12.79[a]
P-value									
Challenge		0.003	0.808	0.316	0.381	0.579	0.174	0.404	0.015
OH		0.031	0.043	0.095	0.183	0.152	0.008	0.075	0.047
Challenge × OH		0.178	0.811	0.156	0.364	0.138	0.404	0.269	0.026

[a], [b]Means sharing the same superscripts are not significantly different from each other at $P < 0.05$.

concentration of succinic acid compared with those without access to OH. Furthermore, the broilers with access to OH tended to have a higher concentration of lactic acid ($P = 0.075$) compared with those without access to OH. Interestingly, there was no significant challenge × free choice OH interaction observed for all SCFA except for succinic acid concentration ($P < 0.05$). Succinic acid concentration in the caeca was reduced by challenge of broilers only when the broilers had access to OH. On the other hand, OH access elevated succinic acid concentration only in the unchallenged broilers.

4. Discussion

Necrotic enteritis imposes a significant economic burden on the global broiler industry as it affects broiler welfare and increases the risk of contamination of broiler for human consumption (Timbermont et al., 2011). The broilers in this study were inoculated with *Eimeria* spp. and *C. perfringens* to produce a subclinical form of NE experimentally in order to investigate the possible role of free choice OH on performance, gut development and health in challenged broilers.

Subclinical NE was successfully induced after inoculation with *Eimeria* spp. and *C. perfringens* as shown by the depression of weight gain and feed intake of broilers on d 16. A number of studies have shown impaired broiler performance when birds are challenged with subclinical NE (Cooper, 2007; Timbermont et al., 2011; Saleem, 2013). Most of studies also have shown improved broiler performance when broilers are fed fibre supplementation in their diets at different ages (Hetland et al., 2003; González-Alvarado et al., 2010; Jiménez-Moreno et al., 2009, 2010, 2013a, 2016; Mateos et al., 2012; Kheravii et al., 2016b). The improvement in growth performance due to the addition of moderate amounts of structural fibre in the diet was more pronounced in young broilers (Jiménez-Moreno et al., 2009, 2010, 2013a, 2016). Hetland and Svihus (2001) reported increased feed intake in broilers fed OH. In the current study, broilers with free choice access to OH consumed less feed and tended to have lower FCR compared with those without access to OH.

It has been speculated that insoluble fibre might increase the release of cholecystokinin (Svihus et al., 2004), which acts through the vagus nerve to stimulate pancreatic enzyme secretion and gastro-duodenal reflux (Duke, 1992; Li and Owyang, 1993). This, in turn, may assist digestion and absorption of nutrients in the small intestine and hence result in performance improvement.

Several studies have shown that feeding broilers with insoluble fibre, such as OH, increases gizzard size at different ages (Jiménez-

Moreno et al., 2013b; Kimiaeitalab et al., 2016). Indeed, feeds need to be ground to certain particle size before leaving the gizzard. Jiménez-Moreno et al. (2013b) stated that the relative weight of the gizzard and its dry matter content were increased, and gizzard pH was reduced with OH inclusion (2.5% or 5%) at all ages (d 6, 12, and 18). This is different from the findings of this study where younger broilers (up to d 16) might not have eaten enough OH to increase the relative gizzard weight and reduce the pH, as it is possible that young broilers prefer to consume less OH which is provided as free choice. However, the relative gizzard weight of the broilers at d 35 increased with access to OH. A large and well-developed gizzard is able to grind feed particles more thoroughly (Amerah et al., 2007), to elevate pancreatic enzyme secretion through increased release of cholecystokinin (Svihus, 2011), to increase proteolysis by pepsin, trypsin and other endogenous proteases in the small intestine, and to improve gastrointestinal tract motility (Ferket, 2000; Gonzalez-Alvarado et al., 2008) and to improve nutrient digestibility (Amerah, 2008). Thus, gut development and health can be enhanced (Choct, 2009). This study has shown that infection with a pathogenic bacterium *C. perfringens* strain led to the development of necrotic enteritis, which altered the populations of some specific organisms harboured in the caeca, such as *Lactobacillus* spp. Feng et al. (2010) and Dahiya et al. (2005) noted that counts of *Lactobacillus* decreased following *C. perfringens* challenge. Stanley et al. (2012) suggested the artificial boosting of *C. perfringens* caused an imbalance in the numbers of Lactobacillales orders. The disruption of the gut flora resulted from *C. perfringens* infection could also disrupt the ability of *Lactobacillus* spp. to colonise. In the poultry industry, *Lactobacillus* species are often regarded as probiotics due to their health promoting properties (Klaenhammer et al., 2008) that are characterised by reduced pathogens in the gut, improved immune function, and increased chicken performance (Salim et al., 2013; Zhang et al., 2012). It is possible that a notable reduction in these beneficial probiotic bacteria may predispose chickens to the onset of the other bacterial diseases.

The structural components of feed that impart beneficial effects on the gizzard consist largely of fibre, which is the sum of NSP and lignin. In addition to the direct effects that fibre has on the gizzard, certain components of fibre bring about gut health benefits via the production of prebiotics *in situ*. The effect of OH was not notable on the number of the selected bacteria examined in this study. However, SCFA as the products from the fermentation of dietary fibre by the anaerobic bacteria were clearly influenced by free choice OH. This may indicate the OH as the substrate significantly influenced the number of some groups of bacteria. For example, the broilers with access to free choice OH had a higher amount of caecal

succinic acid compared with those without access to free choice OH. It has been reported that succinic acid improves performance in broilers possibly due to its bactericidal properties (Broz et al., 2009).

Succinic acid has been shown along with other SCFA to have immunomodulatory effects (Lawhon et al., 2002; Cavaglieri et al., 2003; Maslowski et al., 2009). It has been reported that succinate or succinic acid increased pro-inflammatory cytokines such as interleukin-8 and interleukin-1β (Graham et al., 2013; Tannahill et al., 2013), which play an important role in cell signaling as inflammatory reaction develops (Brat et al., 2005; Tannahill et al., 2013). In this study, the amount of caecal succinic acid in challenged broilers with access to OH was lower than those unchallenged with access to OH. It is possible that challenged broilers with access to OH might have used the succinic acid for immunological purposes. Hence, offering broilers with dietary fibre may not only affect overall broiler performance, but may also contribute to immune function and health.

5. Conclusion

In conclusion, free choice OH was beneficial in improving broiler performance at least at younger ages. The benefit of OH may be due to altered SCFA types and concentrations as a result of mediated changes in the gut microbiota. In addition, offering broilers with OH resulted in a well-developed gizzard that has been considered as the pacemaker organ for digestion and health in poultry.

Acknowledgements

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