Poultry Science

Comparison of necrotic enteritis effects on growth performance and intestinal health in two different meat-type chicken strains Athens Canadian Random Bred and Cobb 500

Doyun Goo, Hanseo Ko, Milan Kumar Sharma, Venkata Sesha Reddy Choppa, Deependra Paneru, Hanyi Shi, and Woo Kyun Kim[1]

Department of Poultry Science, University of Georgia, Athens, GA 30602, USA

ABSTRACT Chickens have undergone genetic improvements in the past few decades to maximize growth efficiency. However, necrotic enteritis (**NE**), an enteric disease primarily caused by *C. perfringens*, remains a significant problem in poultry production. A study investigated the differences in intestinal health between the nonselected meat-type chicken Athens Canadian Random Bred (**ACRB**) and the modern meat-type Cobb 500 broilers (**Cobb**) when challenged with experimental NE. The study utilized a 2 × 3 factorial arrangement, consisting of two main effects of chicken strain and NE challenge model (nonchallenged control, NC; NE challenge with 2,500/12,500 *Eimeria maxima* oocysts + 1 × 10^9 *C. perfringens*, NE2.5/NE12.5). A total of 432 fourteen-day-old male ACRB and Cobb were used until 22 d (8 d postinoculation with *E. maxima* on d 14, dpi), and the chickens were euthanized on 6 and 8 dpi for the analysis. All data were statistically analyzed using a two-way ANOVA, and Student's *t*-test or Tukey's HSD test was applied when $P < 0.05$. The NE12.5 group showed significant decreases in growth performance and relative growth performance from d 14 to 20, regardless of chicken strain ($P < 0.01$). The ACRB group exhibited significant decreases in relative body weight and relative body weight gain compared to the Cobb group from d 14 to 22 ($P < 0.01$). On 6 and 8 dpi, both NE challenge groups showed significant decreases in intestinal villus height to crypt depth ratio, jejunal goblet cell count, and jejunal *MUC2* and *LEAP2* expression ($P < 0.01$). Additionally, the NE12.5 group had significantly higher intestinal NE lesion score, intestinal permeability, fecal *E. maxima* oocyst count, intestinal *C. perfringens* count, and jejunal *IFNγ* and *CCL4* expression compared to the NC group ($P < 0.05$). In conclusion, NE negatively impacts growth performance and intestinal health in broilers, parameters regardless of the strain.

Key words: Athens Canadian Random Bred, *Clostridium perfringens*, Cobb 500, intestinal health, necrotic enteritis

2024 Poultry Science 103:103599
https://doi.org/10.1016/j.psj.2024.103599

INTRODUCTION

The Athens Canadian Random Bred (**ACRB**) is a white-plumaged meat-type control strain that originated from the Ottawa Meat Control Strain, and it has been maintained solely in Athens (University of Georgia) since 1958 (Hess, 1962; Collins et al., 2014). The ACRB can be considered the oldest pedigreed commercial broiler chicken strain in existence today, as the Ottawa Meat Control Strain began to nonpedigreed in 1974 (Collins et al., 2016). The ACRB has been used as a control broiler strain in many poultry experiments, particularly in the field of genetics, to observe long-term changes in commercial broilers (Collins et al., 2016). The Cobb 500 chicken (**Cobb**) is one of the most commonly raised modern meat-type broilers, selected based on its high-meat yielding, rapid growth rate, and feed efficiency (Su et al., 2020). The Cobb is a fast-growing chicken that has been genetically improved to optimize the yield of breast muscles. Compared to the nonselected slow-growing chickens, ACRB, the Cobb exhibits significant differences in growth performance and meat yield; at 42 d (6 wk), the Cobb shows an increased BW of about 4.6 times (0.59 vs. 2.73 kg), an improved feed conversion ratio (**FCR**) of about 2.9 times (5.19 vs. 1.76 g/g), and an increased breast meat yield of 9.8 times (59.7 vs. 586.6 g) (Collins et al., 2014).

Necrotic enteritis (**NE**) is a bacterial disease in poultry that affects the intestinal health and growth performance (Goo et al., 2023b). It is mainly caused by a bacterium called *Clostridium perfringens*, which is Gram-positive. NE is developed due to various predisposing factors such as

Received January 15, 2024.
Accepted February 23, 2024.

[1]Corresponding author: wkkim@uga.edu

Eimeria infection, heat, viral infection, or changes in the intestinal environment (Moore, 2016). It is estimated that NE causes an annual loss of $6 billion USD worldwide and is considered a major enteric disease in poultry with a significant economic impact on the poultry industry (Wade and Keyburn, 2015). Necrotic enteritis coinfection, especially with *Eimeria maxima*, which is a common parasite in chickens, can be a crucial predisposing factor for NE. *E. maxima* directly damages the intestinal epithelium during its asexual reproduction, leading to NE (Paiva and McElroy, 2014; Goo et al., 2023b). Chickens affected by NE typically exhibit decreased growth performance, poor feed efficiency, diarrhea, intestinal (jejunal) lesions, and mortality. Subclinical NE, which refers to decreased growth without mortality, causes the greatest economic loss in poultry production (Shojadoost et al., 2012).

For over half a century, modern broilers have been bred through genetic improvement for rapid growth and muscle deposition, resulting in high growth efficiency and stable profits for poultry production (Schmidt et al., 2009; Zuidhof et al., 2014). However, along with the benefits of rapid growth, Cobb also face negative issues such as immune malfunction and skeletal abnormalities (Rath et al., 2000; Cheema et al., 2003). Such problems are directly correlated to high mortality, lameness, and skin lesions, resulting in economic losses in broiler production (Hartcher and Lum, 2020). Along with this, NE infection is also an important external factor of economic loss in the broiler production under antibiotics-free conditions and has yet to find a complete solution. To gain a better understanding of how NE affects different chicken strains, we conducted a study using a NE coinfection model (*E. maxima* + *C. perfringens*) on two different strains of chickens, the nonselected ACRB and the modern-type Cobb. There have been limited experiments comparing *Eimeria* challenge or NE challenge in ACRB and Cobb, and there is a lack of research on how genetic selection and improvement in broilers have enhanced the intestinal health and immunity of modern-type broilers.

Our hypothesis was that there would be differences in various intestinal health parameters and intestinal immunity between the genetically nonselected ACRB and the modern-type Cobb under NE challenge. Therefore, the aim of this study was to investigate the effects of experimental NE on growth performance, intestinal health, and intestinal immunity in ACRB and Cobb.

MATERIALS AND METHODS

Chickens and Experimental Design

The current study was conducted at the Poultry Research Center (**PRC**), University of Georgia and approved by the Institutional Animal Care and Use Committee (A2021 12-012). Athens Canadian Random Bred eggs were collected from artificially inseminated ACRB breeding stocks at University of Georgia. Fertile ACRB eggs were stored at 14°C and 70% **RH** prior to incubation. For the egg incubation, ACRB eggs were moved to the PRC hatchery NMC 2,000 incubator (NatureForm Incubator Co., Jacksonville, FL) and incubated at 37.8°C and 53% RH for 18 d. After 18 d of incubation, all eggs were transferred to an NMC 2000 hatcher (NatureForm Incubator Co., Jacksonville, FL) and incubated at 36.9°C and 65% RH until hatching. On d 0, a total of 576 male ACRB and Cobb were raised in nipple-installed battery cages and raised until 14 d of age. On d 14, a total of 432 ACRB and Cobb were re-allocated with an average BW. The average BW of ACRB and Cobb on d 14 was 113.1 ± 0.46 g and 436.4 ± 0.99 g, respectively. The treatments in the current experiment consisted of 6 treatments (2 × 3 factorial design) with 6 replicates of 12 chickens per cage. The two main effects were chicken strains (ACRB vs. Cobb) and NE models (nonchallenge and two different NE challenge groups). The treatments in this study were as follows: (1) ACRB, nonchallenged (**NC**); (2) Cobb, NC; (3) ACRB, challenged with 2,500 *E. maxima* with *C. perfringens* (NE2.5); (4) Cobb, NE2.5. (5) ACRB, challenged with 12,500 *E. maxima* with *C. perfringens* (NE12.5); and (6) Cobb, NE12.5. The 2-phase corn-soybean meal-based mash diets (Table 1) were formulated and fed ad libitum. The experiment was conducted until d 22. On d 14, 20, and 22, BW and feed intake (**FI**) were measured. Body weight gain (**BWG**) and FCR were calculated using BW and FI. In addition, the relative BW (**RBW**), relative BWG (**RBWG**), relative FI (**RFI**), and relative FCR (**RFCR**) were calculated by setting the nonchallenged group of each strain to 100%. To determine the effect of NE, chickens were euthanized, and samples were collected on d 20 (6 dpi) and 22 (8 dpi).

Necrotic Enteritis Model and Jejunal Lesion Score

The NE model in the current study was used with *E. maxima* and *C. perfringens*, followed by previous experiment conducted by Goo et al. (2023b). In brief, all chickens in the NE challenge group were given 1 mL of *E. maxima* (either 2,500 or 12,500 oocysts) orally, followed by the inoculation of NE B-like toxin (**NetB**) positive *C. perfringens* strain Del-1 at a concentration of 1×10^9 of *E. maxima* on 4 d postinoculation (**dpi**). For the nonchallenged groups, chickens were given **PBS** instead of the pathogens. To increase the pathogenicity of *C. perfringens* in the intestine, the basal diet (containing 21% CP) was switched to a high CP diet (containing 24% CP) on d 18 (4 dpi) until the end of the experiment on d 22 (Goo et al., 2023b). On d 20 and 22 (6 and 8 dpi), three chickens per cage were euthanized by cervical dislocation, and 20 to 30 cm jejunum (the segment between the end of the duodenal loop and Meckel's diverticulum) were collected to measure NE lesion scores. The jejunal NE lesion score, measured on a scale of 0 to 3, was determined by 2 independent observers, following the methods outlined in previous studies (Lee et al., 2011; Shojadoost et al., 2012). Examples of jejunal NE lesions for each group in this experiment is presented in Figure 1.

Table 1. Diet composition of the current study (as-fed basis, %).

Ingredients, %	d 0−18	d 19−22
Corn, grain	59.85	53.86
Soybean meal, 48%	31.52	39.54
Soybean oil	2.20	3.69
Dicalcium phosphate	1.94	1.24
Sand	1.89	-
Limestone	1.27	0.99
Salt	0.35	0.35
DL-Met	0.34	0.15
L-Lys	0.31	-
Thr	0.15	-
Mineral premix[1]	0.08	0.08
Vitamin premix[2]	0.10	0.10
Total	100.0	100.0
Calculated value		
ME (kcal/kg)	3,000	3,100
Crude protein, %	21.10	24.00
Total Ca, %	0.99	0.76
Available P, %	0.50	0.38
dLys[3]	1.22	1.19
dMet	0.64	0.49
dTSAA	0.91	0.80
dThr	0.83	0.81
dArg	1.28	1.52
dVal	0.98	1.15
dTrp	0.23	0.28

[1]Mineral premix provided the following per kg of diet: Mn, 100.5 mg; Zn, 80.3 mg; Ca, 24 mg; Mg, 20.1 mg; Fe, 19.7 mg; Cu, 3 mg; I, 0.75 mg; Se, 0.30 mg.

[2]Vitamin premix provided the following per kg of diet: vitamin A, 3,527 IU; vitamin D_3, 1,400 IU; vitamin E, 19.4 IU; niacin, 20.28 mg; D-pantothenic acid, 5.47 mg; riboflavin, 3.53 mg; vitamin B_6, 1.46 mg; menadione, 1.10 mg; thiamin, 0.97 mg; folic acid, 0.57 mg; biotin, 0.08 mg; vitamin B_{12}, 0.01 mg.

[3]digestible.

Intestinal Permeability

Intestinal permeability was measured on 2 sampling d (6 and 8 dpi) using blood fluorescein isothiocyanate-dextran (**FITC-d**, Molecular weight 4,000; Sigma-Aldrich, Canada) levels, following a previously reported experiment (Teng et al., 2020) with minor modifications. Briefly, 2 mg/mL FITC-d solution was prepared with PBS under dark conditions and orally administered to one chicken per cage. Two h after the administration of FITC-d solution, the chickens were euthanized by cervical dislocation, and blood samples were directly collected via heart puncture. The collected blood samples were stored in a dark room for 2 h and then centrifuged at 2,000 × *g* for 10 min to obtain the supernatant. A standard curve was prepared using 6 nonexperimental control chickens (three chickens per strain) to calculate the blood FITC-d levels. Serum samples (100 μL each) were transferred to 96 dark flat-bottom plates, and fluorescence was measured at optical density (**OD**) 485/525 nm using a microplate reader (Spectra Max 5 microplate reader, Molecular Devices, Sunnyvale, CA).

Fecal Water Content and E. maxima Oocysts Count

To analyze the water content and count the number of *E. maxima* oocysts in fecal samples, approximately 150 g of fresh feces were collected on each sampling day (6 and 8 dpi). The analysis of fecal water content and fecal *E. maxima* oocyst count followed a previous experiment (Goo et al., 2023b) with some slight modifications. The day before collecting the feces (5 and 7 dpi), all feces were removed, and clean metal trays were placed under each cage. On each sampling day, the feces were collected into 2 separate sample bags. In order to measure the fecal water content, the weight of the feces was recorded before drying. The fecal samples were then dried for 5 d in a 70°C drying oven, and the weight of the dried feces was recorded to calculate the fecal water content. To measure the fecal *E. maxima* oocyst count, 5 g of gently mixed fresh feces was mixed with 35 mL of tap water in a 50-mL centrifuge tube. Then, 1 mL of the mixed feces solution was combined with 10 mL of a saturated salt solution. Approximately 600 μL of the mixed solution was then added to a McMaster counting chamber (Vetlab Supply, Palmetto Bay, FL) to count the *E. maxima* oocysts in 6 columns. The total fecal *E. maxima* oocyst count was expressed on a $\log_{10}$ scale.

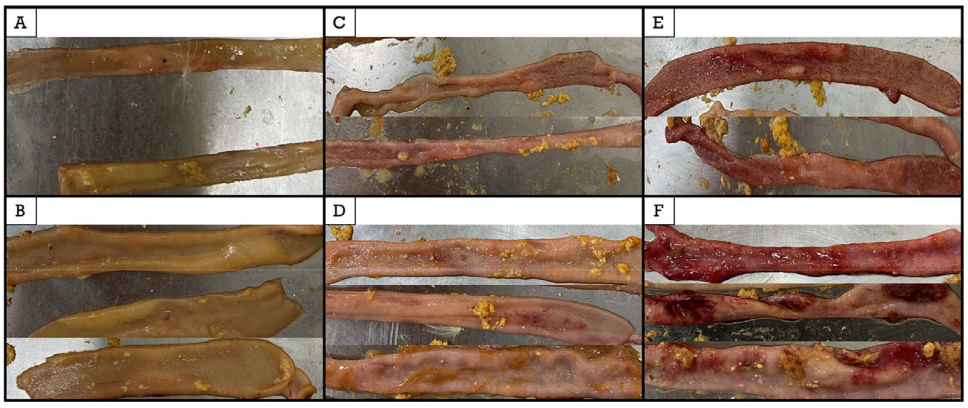

Figure 1. Necrotic enteritis effects on jejunal necrotic enteritis lesion score in two different meat-type chicken strains ACRB and Cobb 500 on d 20 (6 dpi). The necrotic enteritis lesion was scored by 2 independent observers with the scale from 0 (clean intestine with no lesion) to 3 (obvious necrotic enteritis lesion with blood). (A) Nonchallenged control of ACRB. (B) Nonchallenged control of Cobb 500. (C) ACRB with inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18. (D) Cobb 500 with inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18. (E) ACRB with inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18. (F) Cobb 500 with inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18.

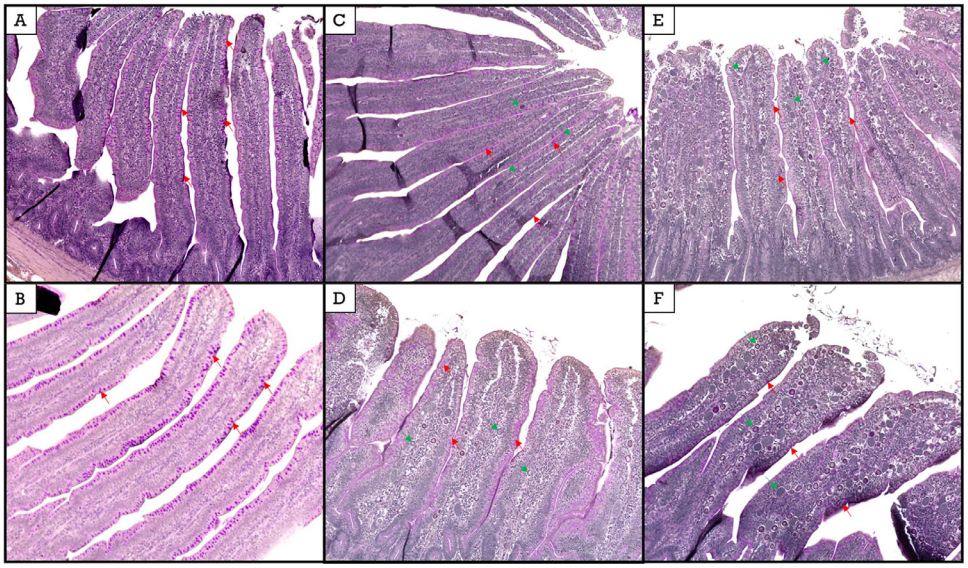

Figure 2. Necrotic enteritis effects on jejunal goblet cells in two different meat-type chicken strains ACRB and Cobb 500 on d 20 (6 dpi). The jejunal section was stained with Period acid-Schiff (PAS) and counterstained with hematoxylin, and image captured by Keyence microscope. The red arrows point to jejunal goblet cells, and the green dotted arrow point to *E. maxima*. (A) Nonchallenged control of ACRB. (B) Nonchallenged control of Cobb 500. (C) ACRB with inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18. (D) Cobb 500 with inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18. (E) ACRB with inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18. (F) Cobb 500 with inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18.

Intestinal C. perfringens *Colony Count*

Intestinal *C. perfringens* colony counting was conducted on two sampling days (6 and 8 dpi), following a previously reported study (Goo et al., 2023b) with slight modifications. In brief, one chicken per cage was euthanized by cervical dislocation, and approximately 20 g of intestinal contents (from the middle of the jejunum to the end of the ileum) were collected using filter bags (WhilePak, Nasco, Fort Atkinson, WI). Next, 10 mL of 0.1% buffered peptone water (BPW; Himedia, Mumbai, India) was added to each filter bag and homogenized for 60 sec using a homogenizer (Masticator Silver Panoramic, Neutec Group Inc., Farmingdale, NY). The homogenized intestinal content was then transferred into a sterile dilution tube and diluted to 10^{-8} by a serial dilution. Subsequently, 100 μL of the diluted intestinal contents in each dilution tube (10^{-4}, 10^{-6}, and 10^{-8}) were dispensed onto Tryptose Sulfite Cycloserine and Shahadi Ferguson Perfringens (TSC/SFP; Oxoid Ltd., Hampshire, UK) agar plates and gently spread. The TSC/SFP agar plates were then anaerobically incubated (AnaeroPack, Thermo Scientific, MA) at 37℃ for up to 48 h. After the incubation, the colonies were counted and recorded.

Intestinal Morphology and Goblet Cell Count

To assess the morphology of the jejunum and ileum, as well as the number of goblet cells, one chicken per cage were euthanized on each sampling day (6 and 8 dpi), using cervical dislocation. The jejunum and ileum were collected and then directly fixed in a 10% formalin solution. The fixed intestine sections were stained with Periodic acid-Schiff (**PAS**) and counterstained with hematoxylin, following the methodology described in a previous experiment (Liu et al., 2022). The intestinal sections were then visualized using a microscope (BZ-X810, Keyence, Osaka, Japan) and measured the villus height to crypt depth ratio (**VH:CD**) at 4X magnification. Additionally, the goblet cells were counted within each villus at 10X magnification. Examples of stained jejunal sections and goblet cells within the villi for each group in the current study were shown in Figure 2.

Jejunal qRT-PCR Analysis

To measure gene expression in the jejunum, one chicken per cage was euthanized by cervical dislocation on each sampling day (6 and 8 dpi). Approximately 5 cm of jejunal section was collected from each chicken. The jejunal samples were immediately placed in liquid nitrogen and stored at −80°C for further analysis. For RNA extraction, approximately 100 mg of jejunal sample was mixed with 1 mL of QIAzol lysis reagent (Qiagen, Valencia, CA) and homogenized using a bead beater (Biospec Products, Bartlesville, OK) for 90 sec. RNA was then extracted following the manufacturer's protocol, and the quantity and purity were checked using a NanoDrop 2,000 spectrophotometer (Thermo Scientific, Waltham, MA). Next, cDNA was synthesized using a high-capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA). Then, 20 μL of synthesized cDNA samples were diluted with 180 μL of HyPure Molecular Biology Grade Water (Cytiva, HyClone Laboratories, South Logan, UT) and stored at −20°C for further analysis. Quantitative real-time reverse transcriptase polymerase chain reaction (**qRT-PCR**) analysis was performed using a Step One thermocycler (Applied Biosystems, Foster City, CA) and SYBR

Table 2. Primers for RT-PCR in the current study.

Primers[1]	Primer sequences	Product size, bp	Exons	Accession number / Ensembl gene ID
Tight junction proteins				
CLDN2	F: TTGGAGGCTTCATCCTCTGT	126	2 / 2	NM_001277622.1
	R: ACTCACTCTTGGGCTTCTGC			ENSGALG00010015115
JAM2	F: AAGGATTCTGGGACCTACCG	143	4 / 5	NM_001397141.1
	R: GTTCCCGTCATTGCAGAGTT			ENSGALG00010010838
OCLN	F: GCAGCACCTACCTCAACCA	114	2 / 3	NM_205128.1
	R: AGAAGCAGATGAGGCAGAGC			ENSGALG00010028579
ZO2	F: GAAAGCAGACCCTGCTCAAC	141	7 / 8	NM_001396726.1
	R: TGGATGAATGCAAATCCAGA			ENSGALG00010016887
Mucins				
MUC2	F: AGTGCTCCTGCAGACTCCAT	123	8 / 9	XM_046942297.1
	R: ATGTGCCTGGACAGGGTAAA			ENSGALG00010023823
MUC13	F: GGGACGCTGTATGTTCTTCA	112	1 / 2	XM_040703557.2
	R: TCCTGGTTGTACTGCTGCAA			ENSGALG00010020087
Inflammatory cytokines				
IL1β	F: CCTTCACCCTCAGCTTTCAC	138	1 / 2	NM_204524.2
	R: CCCTCCCATCCTTACCTTCT			ENSGALG00010018460
IL2	F: TGCAGTGTTACCTGGGAGAA	149	5 / 6	NM_204153.2
	R: CTTGCATTCACTTCCGGTGT			ENSGALG00010004232
IFNγ	F: GGCGTGAAGAAGGTGAAAGA	133	3 / 4	NM_205149.2
	R: TCCTTTTGAAACTCGGAGGA			ENSGALG00010011933
IL4	F: AACCTGCAGGGTCTCTTCCT	100	5 / 6	NM_001398461.1
	R: TTGAAGTAGTGTTGCCTGCTG			ENSGALG00010016327
IL6	F: GCTACAGCACAAAGCACCTG	112	3 / 4	NM_204628.2
	R: GACTTCAGATTGGCGAGGAG			ENSGALG00010001941
IL10	F: GCTGCGCTTCTACACAGATG	150	3 / 4	NM_001004414.4
	R: CTCCTCTTCTCGCAGGTGAA			ENSGALG00010027138
CCL4	F: CGGGAAGATGAAGCTCTCTG	113	2 / 3	NM_204720.3
	R: TGTAAGTGGTGCAGCAGGTC			ENSGALG00000034478
CXCL8	F: ATGTGAAGCTGACGCCAAG	131	2 / 3	NM_205498.2
	R: GGCCATAAGTGCCTTTACGA			ENSGALG00010005131
TLR/NFκB signaling pathway-related proteins				
NFκB1	F: TCACCAGGAGGACAACACAA	145	21 / 22	NM_001396395.1
	R: TTTGCGGAAGGAGGTCTCTA			ENSGALG00010005476
Myd88	F: AGGATGGTGGTCGTCATTTC	125	4 / 5	NM_001030962.5
	R: GTCTTGCACTTGACCGGAAT			ENSGALG00010026550
TLR2	F: ACATGTGTGAATGGCCTGAA	110	3 / 3	NM_001161650.3
	R: AGCACTAACGTCCAGCACTTC			ENSGALG00010014865
TLR4	F: ACTCTTGGGGTGCTGCTG	110	1 / 2	NM_001030693.2
	R: TGTCCTGTGCATCTGAAAGC			ENSGALG00010028914
Host defense peptides				
AvBD3	F: TCGTGAAGACCTGCTCCAG	137	5 / 6	XM_046938413.1
	R: AGCTCCCAACACGACAGAAT			ENSGALG00010007870
AvBD9	F: GCTGACACCTTAGCATGCAG	113	2 / 2	NM_001001611.3
	R: CATTTGCAGCATTTCAGCTT			ENSGALG00010011980
CATHL3	F: ACAGCTGCGAGTTCAAGGAG	100	2 / 3	NM_001311177.2
	R: GAGTCCACGCAGGTGACAT			ENSGALG00010025935
LEAP2	F: TTATTCTTCTCGCTGCTGCTC	125	1 / 2	NM_001001606.2
	R: GAGGCTCCAACAGGTCTCAG			ENSGALG00010013704
Reference genes				
GAPDH	F: CCTCTCTGGCAAAGTCCAAG	126	3 / 4	NM_204305.2
	R: CCGTTCTCAGCCTTGACAGT			ENSGALG00010022038

[1]CLDN2, claudin 2; JAM2, junctional adhesion molecule 2; OCLN, occludin; ZO2, zonula occludens 2; MUC, mucin; IL, interleukin; IFNγ, interferon gamma; CCL4, C-C motif chemokine ligand 4; CXCL8, C-X-C motif chemokine ligand 8; NFκB1, nuclear factor kappa B subunit 1; Myd88, myeloid differentiation primary response protein 88; TLR, toll like receptor; AvBD; avian beta-defensin; CATHL3, cathelicidin 3; LEAP2, liver enriched antimicrobial peptide 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Green Master Mix. The final volume of the qRT-PCR mixture was 10 μL, containing 5.0 μL of SYBR Green Master Mix, 2.5 μL of cDNA, 0.375 μL of forward primer, 0.375 μL of reverse primer, and 1.75 μL of nuclease-free water. The thermal cycles for all reactions were as follows: polymerase activation and DNA denaturation for 5 min at 95°C, followed by 38 cycles of denaturation at 95°C for 15 s, and annealing/extension at 60°C for 1 min. After amplification, a melting curve analysis was performed by collecting fluorescence data. Glyceraldehyde-3-phosphate dehydrogenase (***GAPDH***) was used as the reference gene for the jejunum tissue samples. The relative fold changes in gene expression levels were determined using the $2^{-\Delta\Delta Ct}$ method, comparing them to the group of nonchallenged ACRB. The primer sequences used in the current study are shown in Table 2.

Statistical Analysis

All statistical analyses were conducted using RStudio software (R Version 4.2.2, RStudio PBC, Boston, MA), and figures were created using GraphPad Prism software (GraphPad Prism 5.0, GraphPad Software Inc., San Diego, CA). All data were analyzed using a 2 × 3

Table 3. Necrotic enteritis effects on body weight in two different meat-type chicken strains ACRB and Cobb 500 from d 14 to 22 (0−8 dpi).

Item		Body weight, g			Relative body weight[1], %		
		d 14 (0 dpi[2])	d 20 (6 dpi)	d 22 (8 dpi)	d 14 (0 dpi)	d 20 (6 dpi)	d 22 (8 dpi)
Strain	*NE model*[3]						
ACRB	NC	113.8	172.0[d]	191.0[c]	100.0	100.0	100.0
	NE2.5	112.8	161.8[de]	172.8[c]	99.3	94.1	90.6
	NE12.5	112.5	153.7[e]	162.5[c]	98.9	89.3	85.0
Cobb 500	NC	436.3	807.5[a]	954.6[a]	100.0	100.0	100.0
	NE2.5	435.5	788.2[b]	934.5[a]	99.9	97.6	97.9
	NE12.5	437.5	748.7[c]	870.2[b]	100.3	92.7	91.2
SEM (n = 6)		1.39	4.21	11.77	0.58	0.95	1.92
Main effect							
Strain							
ACRB		113.1[b]	162.3[b]	175.4[b]	99.4	94.5[b]	91.9[b]
Cobb 500		436.4[a]	781.4[a]	919.8[a]	100.1	96.8[a]	96.4[a]
SEM (n = 18)		0.80	2.43	6.80	0.33	0.55	1.11
NE model							
NC		275.1	489.8[a]	572.8[a]	100.0	100.0[a]	100.0[a]
NE2.5		274.2	475.0[b]	553.6[a]	99.6	95.9[b]	94.2[b]
NE12.5		275.0	451.2[c]	516.3[b]	99.6	91.0[c]	88.1[c]
SEM (n = 12)		0.98	2.98	8.32	0.41	0.67	1.36
P-value							
Strain		<0.001	<0.001	<0.001	0.168	<0.01	<0.01
NE model		0.767	<0.001	<0.001	0.685	<0.001	<0.001
Interaction		0.606	<0.001	<0.05	0.461	0.134	0.139

[a-e]Means in the same column with different superscripts are statistically different ($P < 0.05$).
[1]Each NC group was set to 100% relative body weight.
[2]Days postinoculation of *E. maxima* on d 14.
[3]NE model: NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18.

factorial arrangement of treatments with a 2-way ANOVA. In the current study, the interaction between the 2 main effects was tested first, following by testing the main effects themselves (strain and NE model) were tested. Differences among the NE models (3 groups, n = 12) were determined using Tukey's honestly significant differences (HSD) test, while differences between strains (2 groups, n = 18) were determined using Student's *t*-test if the *P*-value from the ANOVA was less than 0.05 ($P < 0.05$). The SEM was also presented in tables and figures.

RESULTS

Growth Performance

The results of NE challenge on BW and RBW data from d 14 to 22 (0−8 dpi) are shown in Table 3. Interactions between the strain and the NE model were observed in BW on d 20 and 22, but no interactions were observed in RBW. On d 20, there were significant differences between NE challenge groups in the Cobb group, whereas no differences were observed in the ACRB group ($P < 0.001$). On d 22, the Cobb group still showed significant differences in the NE12.5 group compared to the NC group, whereas the ACRB group showed no significant differences in any of the groups ($P < 0.05$). Throughout the entire experimental period (0 to 8 dpi), the Cobb group had significantly higher BW compared to the ACRB group ($P < 0.001$). In the NE challenge model, the NE12.5 group significantly decreased BW compared to the NC and NE2.5 groups on both 6 and 8 dpi ($P < 0.001$). However, there was no significant difference in BW between the NE2.5 and NC groups at 8 dpi. Regarding RBW on 6 and 8 dpi, the ACRB group showed a greater decrease in BW than the Cobb group ($P < 0.01$). The RBW between the NE model groups showed that both NE challenge groups significantly decreased RBW compared to the NC group, and the NE12.5 group significantly decreased RBW compared to the NE2.5 group on both 6 and 8 dpi ($P < 0.001$). The results of BWG and RBWG data during the acute NE infection periods (d 14−20 and d 14−22) are shown in Table 4. Interactions between the strain and the NE model were observed in BWG from d 14 to 20 and d 14 to 22 BWG, and RBWG from d 14 to 20. In terms of BWG, there no significant differences among the ACRB groups, whereas the Cobb group showed significant differences between the NC and NE12.5 challenge groups from d 14 to 20 ($P < 0.001$) and d 14 to 22 ($P < 0.05$). For RBWG form d 14 to 20, all NE challenged ACRB groups showed significantly decreased RBWG compared to the NC ACRB group, whereas the NE2.5 Cobb group showed no difference compared to the NC Cobb group ($P < 0.05$). Throughout all experimental periods, the ACRB groups showed significantly lower BWG and RBWG compared to the Cobb group ($P < 0.001$). There was a significant decrease in BWG in the NE12.5 group compared to the NC group in both d 14 to 20 and d 14 to 22, whereas there was no difference between the NE2.5 and NC groups in d 14 to 22 ($P < 0.001$). All NE challenged groups had a significant reduction in RBWG compared to the NC group ($P < 0.001$). No interaction between the strain and the NE

Table 4. Necrotic enteritis effects on body weight gain in two different meat-type chicken strains ACRB and Cobb 500 from d 14 to 22 (0−8 dpi).

Item		Body weight gain, g		Relative body weight gain[1], %	
		d 14−20 (0−6 dpi[2])	d 14−22 (0−8 dpi)	d 14−20 (0−6 dpi)	d 14−22 (0−8 dpi)
Strain	*NE model*[3]				
ACRB	NC	58.2[d]	77.2[c]	100.0[a]	100.0
	NE2.5	48.9[d]	60.0[c]	84.0[b]	77.8
	NE12.5	41.0[d]	49.9[c]	70.5[c]	64.6
Cobb 500	NC	371.4[a]	518.4[a]	100.0[a]	100.0
	NE2.5	352.5[b]	498.9[a]	94.9[a]	96.2
	NE12.5	311.0[c]	432.8[b]	83.8[b]	83.5
SEM (n = 6)		4.11	11.60	2.43	4.22
Main effect					
Strain					
ACRB		49.4[b]	62.4[b]	84.8[b]	80.8[b]
Cobb 500		345.0[a]	483.4[a]	92.9[a]	93.2[a]
SEM (n = 18)		2.37	6.70	1.40	2.44
NE model					
NC		214.8[a]	297.8[a]	100.0[a]	100.0[a]
NE2.5		200.7[b]	279.5[a]	89.5[b]	87.0[b]
NE12.5		176.0[c]	241.3[b]	77.1[c]	74.1[c]
SEM (n = 12)		2.91	8.21	1.72	2.98
P-value					
Strain		<0.001	<0.001	<0.001	<0.001
NE model		<0.001	<0.001	<0.001	<0.001
Interaction		<0.001	<0.05	<0.05	0.052

[a-c]Means in the same column with different superscripts are statistically different ($P < 0.05$).
[1]Each NC group was set to 100% relative body weight gain.
[2]Days postinoculation of *E. maxima* on d 14.
[3]NE model: NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18.

model was observed for FI, RFI, FCR, and RFCR (Table 5 and 6). The ACRB group had significantly lower FI compared to the Cobb group on both periods ($P < 0.001$). The ACRB group also had significantly lower RFI from d 14 to 22 compared to the Cobb group ($P < 0.05$), but not from d 14 to 20. From d 14 to 20, the NE12.5 group significantly decreased FI ($P < 0.001$) and RFI ($P < 0.01$) compared to the NC group, whereas there was no difference between the NE2.5 and NE12.5 groups. The Cobb group exhibited significantly lower FCR compared to the ACRB group throughout the entire experimental period ($P < 0.001$), but there were no differences in RFCR. In the NE challenge model groups, the NE12.5 group significantly increased FCR and RFCR compared to the NC group, whereas there were no significant differences between the NE2.5 and NC groups.

Jejunal NE Lesion Score and Intestinal Permeability

The results of the jejunal NE lesion score and intestinal permeability are presented in Table 7. NE lesion scoring examples are shown in Figure 1. Regarding the jejunal NE lesion score, no interaction was found between strain and the NE model. There was no significant difference in NE lesion score between the ACRB and Cobb groups. On d 20 (6 dpi), both NE challenge groups (NE2.5 and NE12.5) showed a significant increase in NE lesion score compared to the NC group ($P < 0.001$). However, on d 22 (8 dpi), there was no difference between the NE2.5 and NC groups ($P < 0.05$). An interaction between the strain and the NE model was observed in intestinal permeability. On d 20, both NE challenge ACRB groups significantly increased serum FITC-d levels compared to the NC ACRB group, whereas there was no significant difference in FITC-d levels in any of the Cobb groups ($P < 0.01$). Throughout the entire experimental period, the ACRB group showed a significant increase in FITC-d levels compared to the Cobb group ($P < 0.001$). On d 20 and 22 (6 and 8 dpi), the NE12.5 group significantly increased FITC-d levels compared to the NC group. The NE2.5 group showed a significant increase in FITC-d levels compared to the NC group on d 20 ($P < 0.001$), whereas there was no significant difference on d 22 ($P < 0.01$).

Fecal Water Content, Fecal E. maxima Oocyst Count, and Intestinal C. perfringens Colony Count

The data for fecal water content, fecal *E. maxima* oocyst count, and intestinal *C. perfringens* colony count are presented in Table 8. There was no interaction observed between the strain and the NE model for fecal water content and intestinal *C. perfringens* colony count. On d 20 and 22 (6 and 8 dpi), the Cobb group exhibited significantly higher fecal water content compared to the ACRB group ($P < 0.001$). There was no significant difference in fecal water content among the NE challenge groups. An interaction between strain and the NE model was observed for fecal *E. maxima* oocyst

Table 5. Necrotic enteritis effects on feed intake in two different meat-type chicken strains ACRB and Cobb 500 from d 14 to 22 (0−8 dpi).

Item		Feed intake, g		Relative feed intake[1], %	
		d 14−20 (0−6 dpi[2])	d 14−22 (0−8 dpi)	d 14−20 (0−6 dpi)	d 14−22 (0−8 dpi)
Strain	*NE model*[3]				
ACRB	NC	185.6	275.7	100.0	100.0
	NE2.5	174.5	258.9	94.0	93.9
	NE12.5	156.9	240.4	84.5	87.2
Cobb 500	NC	567.5	813.9	100.0	100.0
	NE2.5	557.7	826.0	98.3	101.5
	NE12.5	531.2	789.8	93.6	97.1
SEM (n = 6)		7.71	17.16	2.67	3.45
Main effect					
Strain					
ACRB		173.3[b]	258.3[b]	92.9	93.7[b]
Cobb 500		552.1[a]	809.9[a]	97.3	99.5[a]
SEM (n = 18)		4.45	9.91	1.54	1.99
NE model					
NC		376.6[a]	544.8	100.0[a]	100.0
NE2.5		366.1[a]	542.5	96.2[a]	97.7
NE12.5		344.0[b]	515.1	89.1[b]	92.1
SEM (n = 12)		5.46	12.13	1.89	2.44
P-value					
Strain		<0.001	<0.001	0.052	<0.05
NE model		<0.001	0.175	<0.01	0.080
Interaction		0.826	0.710	0.252	0.342

[a, b]Means in the same column with different superscripts are statistically different ($P < 0.05$).
[1]Each NC group was set to 100% relative feed intake.
[2]Days postinoculation of *E. maxima* on d 14.
[3]NE model: NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18.

count on d 22. Both NE challenge groups in each chicken strain showed a significant increase in *E. maxima* oocyst count compared to the NC groups. The ACRB NE2.5 and NE12.5 groups exhibited a significantly higher fecal *E. maxima* oocyst count compared to the Cobb NE2.5 and NE12.5 groups, respectively ($P < 0.05$). On d 22 (8 dpi), the ACRB group had a significantly higher fecal *E. maxima* oocyst count compared to the Cobb group

Table 6. Necrotic enteritis effects on feed conversion ratio in two different meat-type chicken strains ACRB and Cobb 500 from d 14 to 22 (0−8 dpi).

Item		Feed conversion ratio, g/g		Relative feed conversion ratio[1], %	
		d 14−20 (0−6 dpi[2])	d 14−22 (0−8 dpi)	d 14−20 (0−6 dpi)	d 14−22 (0−8 dpi)
Strain	*NE model*[3]				
ACRB	NC	3.22	3.63	100.0	100.0
	NE2.5	3.58	4.35	111.4	119.7
	NE12.5	3.83	4.95	119.1	136.5
Cobb 500	NC	1.53	1.57	100.0	100.0
	NE2.5	1.58	1.66	103.6	105.4
	NE12.5	1.71	1.86	112.1	118.2
SEM (n = 6)		0.115	0.206	4.05	6.92
Main effect					
Strain					
ACRB		3.54[a]	4.31[a]	110.2	118.7
Cobb 500		1.61[b]	1.69[b]	105.2	107.9
SEM (n = 18)		0.067	0.119	2.34	4.00
NE model					
NC		2.37[b]	2.60[b]	100.0[b]	100.0[b]
NE2.5		2.58[ab]	3.00[ab]	107.5[ab]	112.6[ab]
NE12.5		2.77[a]	3.41[a]	115.6[a]	127.3[a]
SEM (n = 12)		0.082	0.146	2.87	4.89
P-value					
Strain		<0.001	<0.001	0.146	0.065
NE model		<0.01	<0.01	<0.01	<0.01
Interaction		0.174	0.055	0.575	0.392

[a,b]Means in the same row with different superscripts are statistically different ($P < 0.05$).
[1]Each NC group was set to 100% relative feed conversion ratio.
[2]Days postinoculation of *E. maxima* on d 14.
[3]NE model: NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18.

Table 7. Necrotic enteritis effects on necrotic enteritis lesion score and intestinal permeability in two different meat-type chicken strains ACRB and Cobb 500 on d 20 and 22 (6 and 8 dpi).

Item		NE lesion score, 0−3 scale		FITC-dextran level, ng/mL	
		d 20 (6 dpi[1])	d 22 (8 dpi)	d 20 (6 dpi)	d 22 (8 dpi)
Strain	*NE model*[2]				
ACRB	NC	0	0	125[b]	58
	NE2.5	0.83	0.50	926[a]	138
	NE12.5	1.83	0.67	906[a]	228
Cobb 500	NC	0	0.17	48[b]	31
	NE2.5	0.75	0.50	138[b]	61
	NE12.5	2.08	0.83	254[b]	68
SEM (n = 6)		0.266	0.236	113.5	31.3
Main effect					
Strain					
ACRB		0.89	0.39	652[a]	141[a]
Cobb 500		0.94	0.50	145[b]	53[b]
SEM (n = 18)		0.154	0.136	65.5	16.4
NE model					
NC		0[c]	0.08[b]	87[b]	45[b]
NE2.5		0.79[b]	0.50[ab]	530[a]	100[ab]
NE12.5		1.96[a]	0.75[a]	580[a]	148[a]
SEM (n = 12)		0.188	0.167	80.3	20.2
P-value					
Strain		0.800	0.586	<0.001	<0.001
NE model		<0.001	<0.05	<0.001	<0.01
Interaction		0.810	0.920	<0.01	0.053

[a-c]Means in the same row with different superscripts are statistically different ($P < 0.05$).
[1]Days postinoculation of *E. maxima* on d 14.
[2]NE model: NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18.

($P < 0.001$), while there was no difference in fecal *E. maxima* oocyst count on d 20. On d 20 and 22 (6 and 8 dpi), both NE challenge groups showed a significant increase in fecal *E. maxima* oocyst count compared to the NC group, and at both same sampling points, the NE12.5 group showed a significantly higher *E. maxima* oocyst count compared to the NE2.5 group ($P < 0.001$). The inherent intestinal *C. perfringens* contents of the NC groups (ACRB and Cobb groups) were $\log_{10}{}^{6.18}$ and $\log_{10}{}^{6.62}$ on d 20 and 22, respectively. There were no

Table 8. Necrotic enteritis effects on fecal water content, fecal oocyst count, and intestinal *C. perfringens* colony count in two different meat-type chicken strains ACRB and Cobb 500 on d 20 and 22 (6 and 8 dpi).

Item		Fecal water content, %		*E. maxima* oocyst, $\log_{10}$/g of feces		*C. perfringens* colony count, $\log_{10}$ cfu/g of intestinal contents	
		d 20 (6 dpi[1])	d 22 (8 dpi)	d 20 (6 dpi)	d 22 (8 dpi)	d 20 (6 dpi)	d 22 (8 dpi)
Strain	*NE model*[2]						
ACRB	NC	39.4	22.5	0	0[d]	6.13	6.42
	NE2.5	35.5	29.4	2.92	3.81[ab]	6.98	7.72
	NE12.5	38.3	30.5	3.96	4.24[a]	7.59	7.73
Cobb 500	NC	75.6	72.9	0	0[d]	6.23	6.81
	NE2.5	74.7	72.0	3.19	3.24[c]	7.28	7.56
	NE12.5	70.0	70.0	3.52	3.65[bc]	7.53	8.04
SEM (n = 6)		1.77	2.56	0.154	0.111	0.181	0.221
Main effect							
Strain							
ACRB		37.7[b]	27.5[b]	2.29	2.68[a]	6.90	7.29
Cobb 500		73.4[a]	71.6[a]	2.23	2.30[b]	7.01	7.47
SEM (n = 18)		0.96	1.39	0.088	0.064	0.105	0.127
NE model							
NC		57.5	47.7	0[c]	0[c]	6.18[b]	6.61[b]
NE2.5		55.1	50.7	3.05[b]	3.52[b]	7.13[a]	7.64[a]
NE12.5		54.2	50.2	3.74[a]	3.94[a]	7.56[a]	7.88[a]
SEM (n = 12)		1.20	1.83	0.109	0.078	0.128	0.156
P-value							
Strain		<0.001	<0.001	0.645	<0.001	0.443	0.324
NE model		0.458	0.414	<0.001	<0.001	<0.001	<0.001
Interaction		0.090	0.071	0.079	<0.05	0.611	0.412

[a-d]Means in the same row with different superscripts are statistically different ($P < 0.05$).
[1]Days postinoculation of *E. maxima* on d 14.
[2]NE model: NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18.

Table 9. Necrotic enteritis effects on intestinal morphology and goblet cell count in two different meat-type chicken strains ACRB and Cobb 500 on d 20 and 22 (6 and 8 dpi).

Item		Villus height to crypt depth ratio				Goblet cell count, cell count/villus			
		Jejunum		Ileum		Jejunum		Ileum	
		d 20 (6 dpi[1])	d 22 (8 dpi)	d 20 (6 dpi)	d 22 (8 dpi)	d 20 (6 dpi)	d 22 (8 dpi)	d 20 (6 dpi)	d 22 (8 dpi)
Strain	*NE model*[2]								
ACRB	NC	6.15	6.54	5.40[ab]	5.30[b]	123.3	141.0	91.0	96.7
	NE2.5	3.45	3.69	2.50[c]	3.34[c]	86.3	100.7	61.3	83.3
	NE12.5	3.09	2.68	2.74[c]	2.61[c]	92.3	101.7	62.0	65.0
Cobb 500	NC	5.51	6.35	5.73[a]	6.58[a]	185.0	216.3	139.0	139.0
	NE2.5	4.14	3.96	4.45[b]	3.17[c]	164.7	161.7	112.7	120.3
	NE12.5	3.00	3.03	2.92[c]	2.70[c]	133.3	139.0	87.3	105.0
SEM (n = 6)		0.256	0.416	0.289	0.271	9.50	8.25	7.60	6.58
Main effect									
Strain									
ACRB		4.23	4.30	3.54[b]	3.75	100.7[b]	114.4[b]	71.4[b]	81.7[b]
Cobb 500		4.22	4.45	4.37[a]	4.15	161.0[a]	172.3[a]	113.0[a]	121.4[a]
SEM (n = 18)		0.148	0.240	0.167	0.157	5.48	4.76	4.39	3.80
NE model									
NC		5.83[a]	6.45[a]	5.57[a]	5.94[a]	154.2[a]	178.7[a]	115.0[a]	117.8[a]
NE2.5		3.80[b]	3.82[b]	3.47[b]	3.26[b]	125.5[b]	131.2[b]	87.0[b]	101.8[a]
NE12.5		3.05[c]	2.85[b]	2.83[b]	2.66[b]	112.8[b]	120.3[b]	74.7[b]	85.0[b]
SEM (n = 12)		0.181	0.294	0.205	0.192	6.72	5.83	5.38	4.65
P-value									
Strain		0.941	0.674	<0.01	0.084	<0.001	<0.001	<0.001	<0.001
NE model		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction		0.051	0.792	<0.01	<0.05	0.162	0.083	0.194	0.921

[a-c]Means in the same row with different superscripts are statistically different ($P < 0.05$).

[1]Days postinoculation of *E. maxima* on d 14.

[2]NE model: NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18.

significant differences in intestinal *C. perfringens* colony count between the ACRB and Cobb groups on d 20 and 22. Both NE challenge groups showed a significant increase in intestinal *C. perfringens* colony count compared to the NC group; however, there was no significant difference between the NE2.5 and NE12.5 groups ($P < 0.001$).

Intestinal Morphology and Goblet Cell Count

Jejunal and Ileal VH:CD and goblet cell count of villus data are shown in Table 9. Examples of stained jejunal sections are presented in Figure 2. Interactions were observed between the strain and the NE model in the ileal VH:CD on d 20 and 22. On d 20 (6 dpi), the ileal VH:CD of both NE challenge groups significantly decreased compared to the NC group ($P < 0.01$). However, the NE2.5 group in Cobb showed a significantly higher ileal VH:CD compared to the NE12.5 group and had similar ileal VH:CD compared to the NC group in ACRB ($P < 0.01$). On d 22 (8 dpi), both NE challenged groups significantly decreased ileal VH:CD compared to the NC groups, whereas, in the NC groups, the Cobb had a significantly higher ileal VH:CD compared to the ACRB ($P < 0.05$). The ACRB group significantly decreased ileal VH:CD on d 20 compared to the Cobb group ($P < 0.01$). In both sampling points, both NE challenge groups significantly decreased jejunal and ileal VH:CD compared to the NC group, and the NE12.5 group significantly decreased jejunal VH:CD on d 20 compared to the NE2.5 group ($P < 0.001$). No interaction between the strain and the NE model was observed in the intestinal goblet cell count. In both sampling points, the Cobb group had a significantly higher intestinal goblet cell count compared to the ACRB group ($P < 0.001$). The NE12.5 group significantly decreased jejunal and ileal goblet cell counts (d 20 and 22) compared to the NC group ($P < 0.001$), whereas there was no significant difference in the ileal goblet cell count between the NE2.5 and NC groups on d 22.

Jejunal Gene Expression

The relative gene expression levels of tight junction proteins (**TJP**) and mucins in the jejunum are presented in Figures 3 and 4. No interaction between the strain and the NE model was observed in the jejunal gene expression of TJPs and mucins. On d 20 (6 dpi), there were no significant differences in the gene expression levels of junctional adhesion molecule 2 (***JAM2***) and mucin13 (***MUC13***) in the jejunum. However, the gene expression levels of claudin 2 (***CLDN2***; $P < 0.001$), occludin (***OCLN***; $P < 0.05$), and zonula occludens 2 (***ZO2***; $P < 0.05$) were significantly upregulated in the ACRB group compared to the Cobb group. Conversely, the gene expression levels of mucin 2 (***MUC2***; $P < 0.001$) was significantly downregulated in the ACRB group compared to the Cobb group on d 20 (6 dpi). On d 20 (6 dpi), the NE12.5 group significantly downregulated the gene expression levels of *ZO2* ($P < 0.05$) and *MUC2* ($P < 0.001$) in the jejunum compared to the NC group. On d 22 (8 dpi), there were no significant differences in the gene expression levels of *CLDN2*. The ACRB group significantly upregulated *JAM2* and *ZO2* gene expression

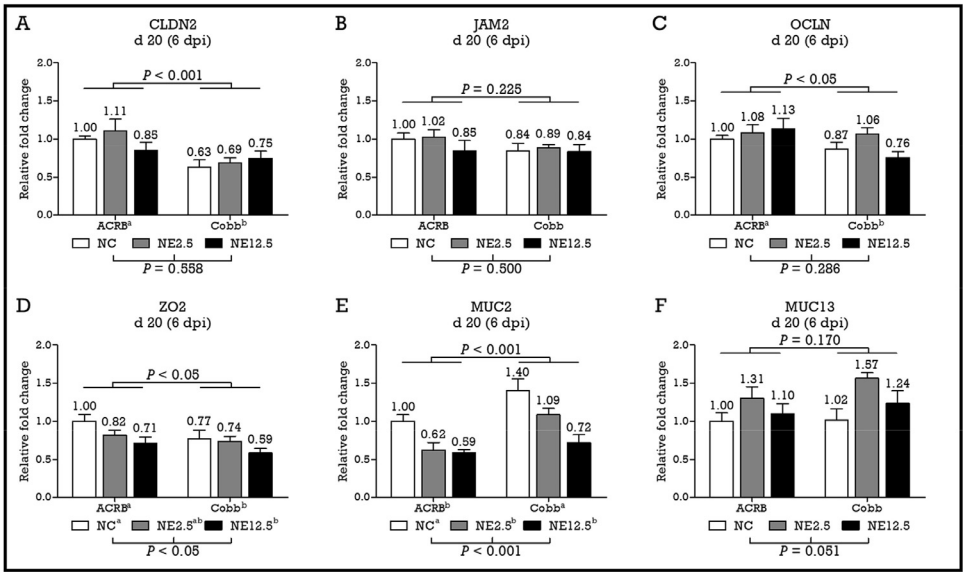

Figure 3. Necrotic enteritis effects on relative jejunal gene expression of tight junction proteins and mucins in two different meat-type chicken strains ACRB and Cobb 500 on d 20 (6 dpi). No interaction was observed. The different superscripts (a and b) of each group (ACRB vs. Cobb; NC vs. NE2.5 vs. NE12.5) represent a statistical difference ($P < 0.05$). Each bar indicates the standard error of the mean (n = 6). Abbreviations: dpi, days postinoculation of *E. maxima* on d 14; NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; ACRB, Athens Canadian Random Bred; Cobb, Cobb 500; CLDN2, claudin 2; JAM2, junctional adhesion molecule 2; OCLN, occludin; ZO2, zonula occludens 2; MUC, mucin. (A) The expression of CLDN2 on 6 dpi. (B) The expression of JAM2 on 6 dpi. (C) The expression of OCLN on 6 dpi. (D) The expression of ZO2 on 6 dpi. (E) The expression of MUC2 on 6 dpi. (F) The expression of MUC13 on 6 dpi.

levels ($P < 0.05$) and downregulated *MUC2* and *MUC13* gene expression levels ($P < 0.01$) compared to the Cobb group. Both NE challenge groups significantly downregulated *OCLN*, *MUC2*, and *MUC13* gene expression levels compared to the NC group ($P < 0.001$). The relative gene expression levels of inflammatory cytokines and chemokines in the jejunum are shown in Figures 5 and 6. There was an interaction between the strain and the NE model in the gene expression levels of interleukin (**IL**) 1 beta (***IL1β***) on d 20 (6 dpi). On d 20, the NE12.5 group in the Cobb significantly upregulated *IL1β* levels compared to all other groups ($P < 0.05$). On d 20 (6 dpi), no

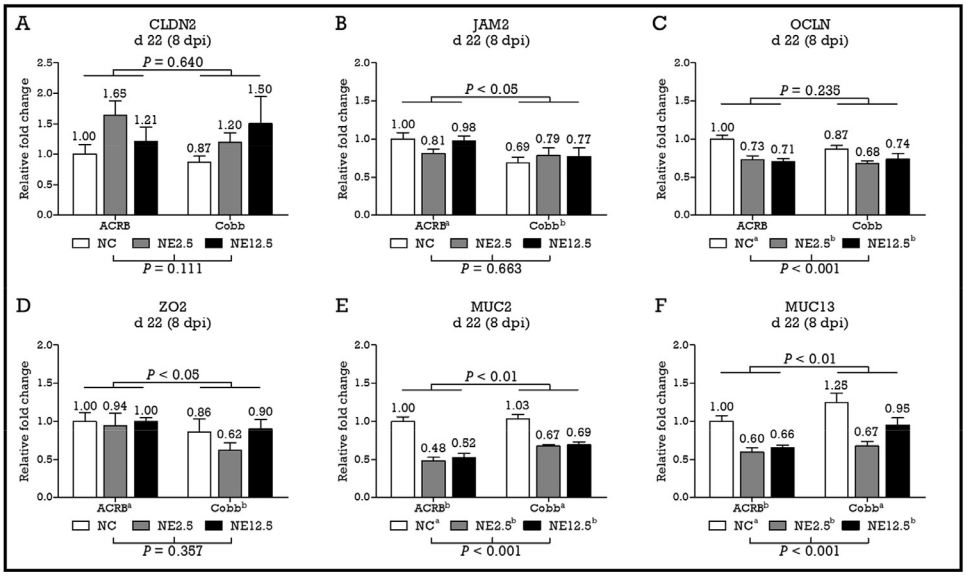

Figure 4. Necrotic enteritis effects on relative jejunal gene expression of tight junction proteins and mucins in two different meat-type chicken strains ACRB and Cobb 500 on d 22 (8 dpi). No interaction was observed. The different superscripts (a and b) of each group (ACRB vs. Cobb; NC vs. NE2.5 vs. NE12.5) represent a statistical difference ($P < 0.05$). Each bar indicates the standard error of the mean (n = 6). Abbreviations: dpi, days postinoculation of *E. maxima* on d 14; NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; ACRB, Athens Canadian Random Bred; Cobb, Cobb 500; CLDN2, claudin 2; JAM2, junctional adhesion molecule 2; OCLN, occludin; ZO2, zonula occludens 2; MUC, mucin. (A) The expression of CLDN2 on 8 dpi. (B) The expression of JAM2 on 8 dpi. (C) The expression of OCLN on 8 dpi. (D) The expression of ZO2 on 8 dpi. (E) The expression of MUC2 on 8 dpi. (F) The expression of MUC13 on 8 dpi.

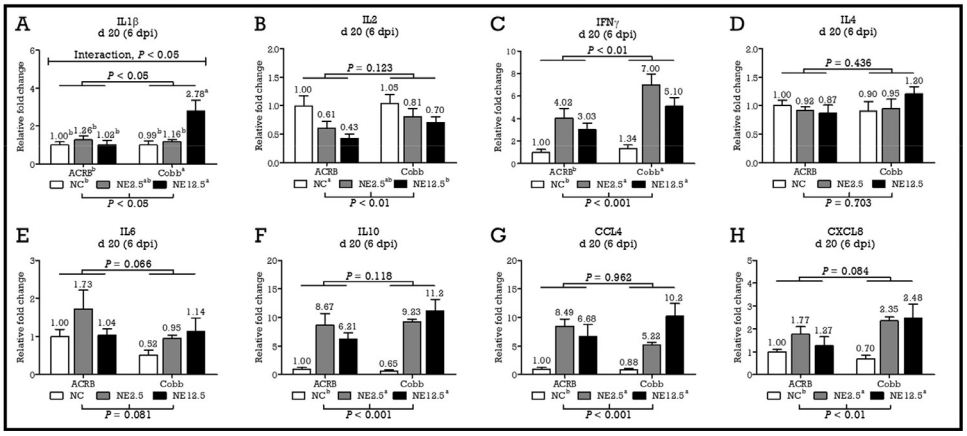

Figure 5. Necrotic enteritis effects on relative jejunal gene expression of inflammatory cytokines and chemokines in two different meat-type chicken strains ACRB and Cobb 500 on d 20 (6 dpi). The different superscripts (a and b) of each group (ACRB vs. Cobb; NC vs. NE2.5 vs. NE12.5) represent a statistical difference ($P < 0.05$). Each bar indicates the standard error of the mean (n = 6). Abbreviations: dpi, days postinoculation of *E. maxima* on d 14; NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; ACRB, Athens Canadian Random Bred; Cobb, Cobb 500; IL, interleukin; IFNγ, interferon gamma; CCL4, C-C motif chemokine ligand 4; CXCL8, C-X-C motif chemokine ligand 8. (A) The expression of IL1β on 6 dpi. (B) The expression of IL2 on 6 dpi. (C) The expression of IFNγ on 6 dpi. (D) The expression of IL4 on 6 dpi. (E) The expression of IL6 on 6 dpi. (F) The expression of IL10 on 6 dpi. (G) The expression of CCL4 on 6 dpi. (H) The expression of CXCL8 on 6 dpi.

significant differences in jejunal gene expression levels of *IL4* and *IL6* were observed. The Cobb group showed significant upregulation of *IL1β* ($P < 0.05$) and interferon gamma (***IFNγ***; $P < 0.01$) gene expression levels compared to the ACRB group on d 20 (6 dpi). The NE12.5 group significantly upregulated *IL1β* ($P < 0.05$), *IFNγ* ($P < 0.001$), *IL10* ($P < 0.001$), C-C motif chemokine ligand 4 (***CCL4***; $P < 0.001$), and C-X-C motif chemokine ligand 8 (***CXCL8***; $P < 0.01$) gene expression levels compared to the NC group. The NE12.5 group showed significant downregulation of *IL2* gene expression levels ($P < 0.01$) compared to the NC group on d 20 (6 dpi). On d 22 (8 dpi), there was no interaction observed between the chicken strain and the NE model in the jejunal gene expression levels of inflammatory cytokines and chemokines. On d 22 (8 dpi), there were no significant differences in jejunal gene expression levels of *IL2*, *IL4*, and *CXCL8*. On d 22 (8 dpi), the Cobb group had significant upregulation of *IFNγ* and *IL10* gene expression levels ($P < 0.05$) compared to the NC group, while downregulating *IL6* gene expression levels ($P < 0.05$). The NE12.5 group showed significant upregulation of *IFNγ* ($P < 0.01$) and *CCL4* ($P < 0.001$) gene expression levels compared to the NC group on d 22 (8 dpi). On d 22, the NE2.5 group significantly upregulated IL1β gene expression levels compared to the NC group ($P < 0.01$), but there was no significant difference between the NE2.5 and NE12.5 groups. The relative gene expression levels of

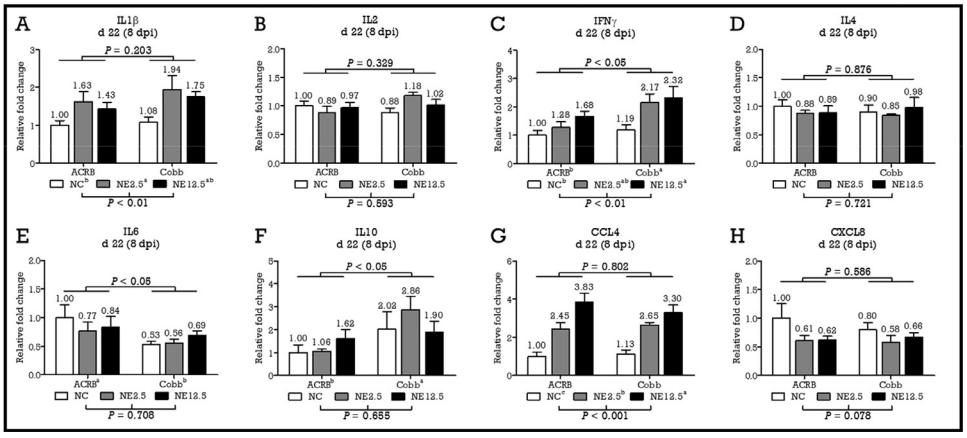

Figure 6. Necrotic enteritis effects on relative jejunal gene expression of inflammatory cytokines and chemokines in two different meat-type chicken strains ACRB and Cobb 500 on d 22 (8 dpi). No interaction was observed. The different superscripts (a, b, and c) of each group (ACRB vs. Cobb; NC vs. NE2.5 vs. NE12.5) represent a statistical difference ($P < 0.05$). Each bar indicates the standard error of the mean (n = 6). Abbreviations: dpi, days postinoculation of *E. maxima* on d 14; NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; ACRB, Athens Canadian Random Bred; Cobb, Cobb 500; IL, interleukin; IFNγ, interferon gamma; CCL4, C-C motif chemokine ligand 4; CXCL8, C-X-C motif chemokine ligand 8. (A) The expression of IL1β on 8 dpi. (B) The expression of IL2 on 8 dpi. (C) The expression of IFNγ on 8 dpi. (D) The expression of IL4 on 8 dpi. (E) The expression of IL6 on 8 dpi. (F) The expression of IL10 on 8 dpi. (G) The expression of CCL4 on 8 dpi. (H) The expression of CXCL8 on 8 dpi.

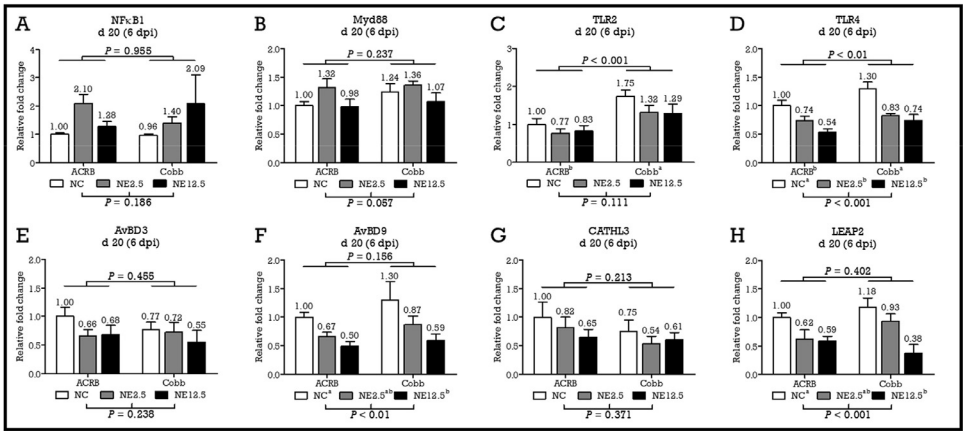

Figure 7. Necrotic enteritis effects on relative jejunal gene expression of TLR/NFκB signaling pathway-related proteins and host defense peptides in two different meat-type chicken strains ACRB and Cobb 500 on d 20 (6 dpi). No interaction was observed. The different superscripts (a and b) of each group (ACRB vs. Cobb; NC vs. NE2.5 vs. NE12.5) represent a statistical difference ($P < 0.05$). Each bar indicates the standard error of the mean (n = 6). Abbreviations: dpi, days postinoculation of *E. maxima* on d 14; NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; ACRB, Athens Canadian Random Bred; Cobb, Cobb 500; NFκB1, nuclear factor kappa B subunit 1; Myd88, myeloid differentiation primary response protein 88; TLR, toll like receptor; AvBD, avian beta-defensin; CATHL3, cathelicidin 3; LEAP2, liver enriched antimicrobial peptide 2. (A) The expression of NFκB1 on 6 dpi. (B) The expression of Myd88 on 6 dpi. (C) The expression of TLR2 on 6 dpi. (D) The expression of TLR4 on 6 dpi. (E) The expression of AvBD3 on 6 dpi. (F) The expression of AvBD9 on 6 dpi. (G) The expression of CATHL3 on 6 dpi. (H) The expression of LEAP2 on 6 dpi.

toll-like receptor/nuclear factor kappa B (**TLR**/**NFκB**) signaling pathway-related proteins and host defense peptides (**HDP**) in the jejunum are shown in Figures 7 and 8. There was no interaction between the strain and the NE model in terms of gene expression levels of TLR/NFκB signaling pathway-related proteins and HDPs in the jejunum. On d 20 (6 dpi), there were no significant differences in the gene expression levels of *NFκB1*, myeloid differentiation primary response protein 88 (***Myd88***), avian beta-defensin 3 (***AvBD3***), and cathelicidin 3 (***CATHL3***). The Cobb group had significant upregulation of *TLR2* ($P < 0.001$) and *TLR4* ($P < 0.01$) gene expression levels compared to the ACRB group on d 20 (6 dpi). The NE12.5 group exhibited significant downregulation of *TLR4* ($P < 0.001$), *AvBD9* ($P < 0.01$), and liver enriched antimicrobial peptide 2 (***LEAP2***; $P < 0.001$) gene expression levels compared to the NC group on d 20 (6 dpi). On d 22 (8 dpi), there were no significant differences in the gene expression levels of *Myd88*, *AvBD9*, and *CATHL3* in the jejunum. The Cobb group had higher *TLR2* ($P < 0.001$) and *TLR4* ($P < 0.01$) gene expression levels compared to the ACRB group, while exhibiting significant downregulation of *NFκB1* gene expression levels ($P < 0.01$) on d 22 (8 dpi). On d 22

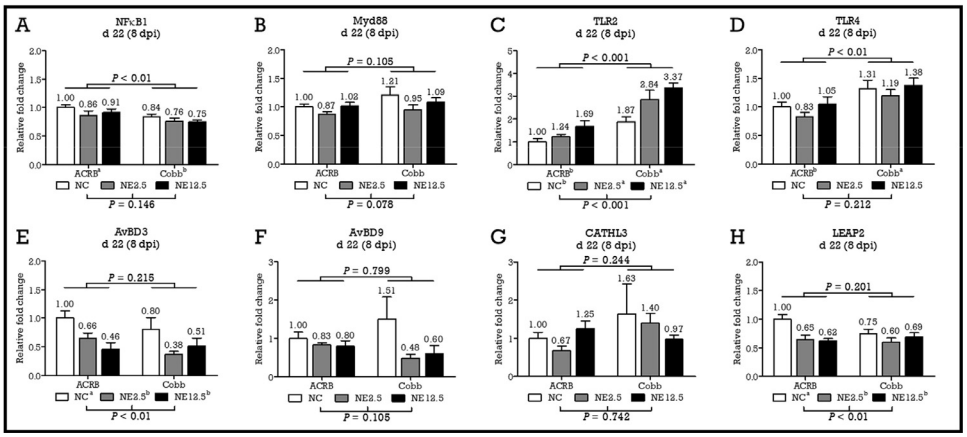

Figure 8. Necrotic enteritis effects on relative jejunal gene expression of TLR/NFκB signaling pathway-related proteins and host defense peptides in two different meat-type chicken strains ACRB and Cobb 500 on d 22 (8 dpi). No interaction was observed. The different superscripts (a and b) of each group (ACRB vs. Cobb; NC vs. NE2.5 vs. NE12.5) represent a statistical difference ($P < 0.05$). Each bar indicates the standard error of the mean (n = 6). Abbreviations: dpi, days postinoculation of *E. maxima* on d 14; NC, nonchallenged of both *E. maxima* and *C. perfringens*; NE2.5, inoculation of 2,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; NE12.5, inoculation of 12,500 *E. maxima* oocysts on d 14 and *C. perfringens* 1×10^9 on d 18; ACRB, Athens Canadian Random Bred; Cobb, Cobb 500; NFκB1, nuclear factor kappa B subunit 1; Myd88, myeloid differentiation primary response protein 88; TLR, toll like receptor; AvBD, avian beta-defensin; CATHL3, cathelicidin 3; LEAP2, liver enriched antimicrobial peptide 2. (A) The expression of NFκB1 on 8 dpi. (B) The expression of Myd88 on 8 dpi. (C) The expression of TLR2 on 8 dpi. (D) The expression of TLR4 on 8 dpi. (E) The expression of AvBD3 on 8 dpi. (F) The expression of AvBD9 on 8 dpi. (G) The expression of CATHL3 on 8 dpi. (H) The expression of LEAP2 on 8 dpi.

(8 dpi), both NE challenged groups significantly downregulated *AvBD3* and *LEAP2* gene expression levels ($P < 0.01$) compared to the NC group, while showing significant upregulation of *TLR2* gene expression levels ($P < 0.001$).

DISCUSSION

The purpose of the current study was to investigate the impact of NE challenge on the intestinal health of the old (genetically nonselected) meat-type ACRB and modern meat-type Cobb. For the NE challenge, we used the NetB^{+} strain of *C. perfringens* (Goo et al., 2023a). In addition, based on previous experimental data, the current study used two NE challenge models using *E. maxima* as a predisposing factor (Goo et al., 2023b). Both NE challenge models involved a coinfection of *E. maxima* and *C. perfringens* but differed in the oocyst dose of *E. maxima*. One model used 2,500 *E. maxima* oocysts (NE2.5 group), while the other used 12,500 *E. maxima* oocysts (NE12.5 group). Although our previous research indicated that 2,500 *E. maxima* oocysts may be insufficient to effectively induce NE, the ACRB used in this experiment had a significantly lower BW than Cobb on d 14 (average BW of ACRB and Cobb was 113.1 g and 436.4 g, respectively). Consequently, we anticipated a higher mortality rate in the ACRB group compared to Cobb under NE challenge. Thus, 2,500 *E. maxima* oocysts group was chosen as another NE challenge model in this study. However, contrary to our expectation, neither the ACRB nor the Cobb group experienced any mortality with both NE challenge doses from d 14 to 22 (0−8 dpi), consistent with the results of the previous study (Goo et al., 2023b). This suggests that the current study successfully generated experimental subclinical NE, without any deviations caused by minor or major mortality due to NE challenges. To gain more accurate understanding of the changes in intestinal health caused by NE challenge, we collected samples at 2 time points: 6 and 8 dpi (d 20 and 22). All growth performance data (BW, BWG, FI, and FCR) were converted into relative growth performance data (RBW, RBWG, RFI, and RFCR) and analyzed separately. Because there was a significant disparity in BW between ACRB and Cobb, relative data were used to express the percentage of change relative to the performance parameters of the nonchallenged group.

Relative body weight and RBWG decreased as the *E. maxima* level increased in each NE model. This is similar to the results of our previous study (Goo et al., 2023b). In particular, in the current experiment, the ACRB showed a greater reduction of RBW and RBWG under NE challenge than the Cobb. In addition, the Cobb did not show a significant decrease in the NE2.5 challenge group but instead showed an earlier recovery in BW and BWG on d 22 (8 dpi) compared to the ACRB. By comparing the decrease rate of RBWG in NE challenge groups, it was found that the RBWG of the Cobb from 0 to 8 dpi was either reduced or similar compared to the RBWG from 0 to 6 dpi. On the other hand, the RBWG of the ACRB showed a greater reduction from 0 to 8 dpi than from 0 to 6 dpi, indicating that the ACRB had more lasting acute NE effect and recovered more slowly from NE challenge than the Cobb did. A previous study reported that the ACRB showed a greater BW loss rate than the Cobb at d 21 (challenged on d 14) under *E. acervulina* challenge (Aggrey et al., 2019). However, few experiments have reported growth performance changes in the ACRB and Cobb under NE challenge conditions. The current study found that the ACRB shows greater BW loss under NE challenge than Cobb due to differences in their body mass and metabolism, but further research is needed to determine the exact difference. Regardless of the chicken strain, the NE2.5 group did not show any statistical differences in FI-related parameters (FI, RFI, FCR, and RFCR) compared to the NC group, which may be due to the possibility that the NE2.5 challenge model was insufficient to change FI. In addition, according to our intensive observations in this study, the ACRB was extremely active compared to the Cobb. Thus, the different behaviors of the two chicken strains may have also made a greater difference in energy efficiency, potentially affecting energy utilization against NE challenge.

The jejunal NE lesion score and serum intestinal permeability were measured on each sampling day (6 and 8 dpi) to directly determine the effect of NE infection. There was no difference in the NE lesion score between the chicken strains. The NE lesion score was the highest on 6 dpi and decreased on 8 dpi, indicating the recovery from the NE challenge started around 8 dpi. Intestinal permeability was also the highest on 6 dpi and decreased on 8 dpi. These results align with a previous study (Goo et al., 2023b) reporting that reduced effectiveness of NE infection might be due to the reproductive cycle of *E. maxima*, which acts as a predisposing factor for NE, having maximal effect between 4 to 6 dpi (Peek, 2010). This causes significant damage to the intestinal barrier, allowing *C. perfringens* to colonize and produce toxins between approximately 5 to 7 dpi (Van Immerseel et al., 2004, 2009), resulting in maximum NE lesions followed by gradual recovery. Intestinal permeability, which is evaluated by FITC-d levels in the serum, is a direct method of measuring intestinal barrier damage (Teng et al., 2020). FITC-d data were highly correlated to the NE lesion score in previous NE-related studies using *E. maxima* as a predisposing factor (Goo et al., 2023b, c; Shah et al., 2023), and the current study also showed similar results. However, when comparing the ACRB and Cobb, there was no statistical difference in the NE lesion score. The ACRB showed numerically lower NE lesion scores compared to the Cobb, but FITC-d levels were significantly higher in the ACRB throughout the entire period. The discrepancy between these 2 analyses (NE lesion score vs. intestinal permeability) in the 2 different stains may be due to the measurement methods being based on the Cobb in the current study, not the ACRB. In a previous study, very high FITC-d levels of about 580 to 780 ng/mL were observed in nonchallenged

chickens from d 2 to 7 using a similar method (Gilani et al., 2018). It is possible that the FITC-d levels used in the current experiment (2 mg/mL) may not be optimum for very small sizes of chickens such as ACRB. Additionally, applying the same criteria as Cobb to measure the NE lesion score in the ACRB may have led to different responses. Further studies are needed to determine the exact reason for these differences in the intestinal NE lesion scores in NE-challenged ACRB. Nevertheless, both analyses showed considerable similarity when comparing the NE challenge and nonchallenge, indicating that they are effective indicators for identifying NE symptoms at the earliest time. NE infection has been shown to deteriorate intestinal health regardless of the chicken strain, whether ACRB or Cobb.

Similar to a previous experiment (Goo et al., 2023b), both the fecal *E. maxima* oocyst count and intestinal *C. perfringens* colony count levels were increased in all NE challenge groups compared to the nonchallenge groups. However, while the fecal *E. maxima* level showed a significant difference according to the level of *E. maxima* inoculation, the *C. perfringens* colony count did not differ between the NE challenge groups. *C. perfringens* has optimal colonization conditions due to mucogenesis and serum leakage from the intestinal epithelium damage caused by *E. maxima* (Collier et al., 2008; Moore, 2016), resulting in a further increase in *C. perfringens* levels in the intestine (Park et al., 2008; Goo et al., 2023b). However, in the NE challenge model of the current study, only a numerical increase in the *C. perfringens* count was found in the NE12.5 group compared to the NE2.5 group, indicating that the difference between the two *E. maxima* inoculation levels did not produce a significant difference in the intestinal *C. perfringens* colonization. On d 22, the ACRB showed an increased fecal *E. maxima* oocyst count compared to the Cobb. While there was no significant difference in the fecal *E. maxima* oocyst count of the Cobb between the two sampling points (6 and 8 dpi), the fecal *E. maxima* oocyst count of the ACRB increased at 8 dpi compared to 6 dpi. This may suggest that the ACRB is recovering more slowly from *E. maxima* infection than the Cobb. There was no difference in fecal water content in the NE challenge; however, the Cobb showed significantly increased fecal water content compared to the ACRB. Diarrhea is one of the typical clinical signs of NE infection (Keyburn et al., 2010). In this case, nutrients in the intestine may not be properly absorbed and are discharged out of the body, resulting in a decrease in growth performance (Bilgili et al., 2010). However, in the current experiment, neither NE challenge models affected the fecal water content, so the impact of NE infection could not be determined. It is considered to exhibit subclinical NE signs with reduced growth performance without any clear clinical NE signs (Skinner et al., 2010). In the current study, the feces of the ACRB were very dry and in small amounts compared to the Cobb. Therefore, the difference in fecal water content between the two strains is thought to be due to the differences in feed and water intake or strain, regardless of NE challenge model, and a more detailed analysis is needed to determine the difference in fecal water content.

The intestinal VH:CD and goblet cell count were measured to investigate the impact of *E. maxima* and *C. perfringens* on intestinal morphology in the current study. Consistent with previous studies (Daneshmand et al., 2022; Goo et al., 2023b), the NE challenge model, using *E. maxima* as a predisposing factor, reduced VH:CD in both the jejunum and ileum. Specifically, the number of goblet cells in both the jejunum and ileum was reduced when challenged with 12,500 *E. maxima* and *C. perfringens* (NE12.5 group). The intestinal VH:CD and goblet cell count are important indicators of intestinal health, as they play a role in nutrient digestion, absorption, and mucin synthesis and secretion (Celi et al., 2017). In the current experiment, the NE challenge decreased intestinal VH:CD, which was attributed to diminished villus height and increased crypt depth. This indicates a decrease in intestinal surface area for nutrient absorption and an increase in cell turnovers due to epithelial cell damage (Paiva et al., 2014). In addition, the decrease in intestinal goblet cell numbers, caused by *Eimeria*-induced intestinal damage, leads to a reduction in mucin secretion. Since mucin primarily serves as a protective barrier for epithelial cells against the external environment, a reduction in mucin secretion increases the host's vulnerability to bacterial infection (Golder et al., 2011; Tan et al., 2014). The deterioration of intestinal morphology due to NE challenge in the current study is believed to be closely associated with the overall decrease in growth performance, regardless of chicken strain. In addition, the decrease in the number of goblet cells per villus in the jejunum and ileum of the ACRB compared to the Cobb may be influenced by differences in villi size, however, further studies are needed to uncover the exact reason.

Several types of TJPs and mucin genes were tested in the current study to determine the impact of NE challenge on their expression in the two chicken strains. These proteins constitute the intestinal barriers and play an important role in protecting the intestinal epithelium from external pathogenic bacteria (Forder et al., 2012; Awad et al., 2017). In the current study, NE challenge resulted in decreased expression of *ZO2* and *MUC2* gene on 6 dpi, and decreased expression of *OCLN*, *MUC2*, and *MUC13* genes on 8 dpi. Similar results have been reported in previous studies, where the expression of *ZO2* (Goo et al., 2023b) and *OCLN* (Song et al., 2017; Pham et al., 2020; Gharib-Naseri et al., 2021) was downregulated under NE challenge. The ZO group (*ZO1*, *ZO2*, and *ZO3*) and *OCLN*, which are the main components of TJPs, are closely associated with each other to form key barrier components and regulate epithelial permeability (Furuse et al., 1994; Awad et al., 2017). Therefore, the downregulation of TJPs gene expression indicates that the use of *E. maxima* and *C. perfringens* as NE challenge models in the current experiment negatively affected intestinal barrier function. The downregulation of *MUC2* and *MUC13* gene expression levels under NE challenge is closely related to the

decreased number of goblet cells because mucin is primarily secreted from goblet cells. Mucin plays a major role in effectively protecting the intestinal epithelium from bacterial infections, maintaining immune homeostasis, and preserving the integrity of the intestinal mucosal barrier (McGuckin et al., 2011). Mucin 2 is a secretory mucin that plays a crucial role in the formation of the mucus layer, while *MUC13* is a cell surface mucin that can contribute to intermediate cell signaling (Johansson et al., 2008; Maher et al., 2011). As mentioned before, the decrease in goblet cells due to NE challenge may lead to a decrease in mucin protein secretion, making the intestinal epithelium more vulnerable to bacterial infections and increasing inflammatory responses (Wei et al., 2012). When the ACRB and Cobb were compared, a consistent pattern was observed in all TJPs and mucin proteins. In the ACRB, all TJPs (*CLDN2*, *JAM2*, *OCLN*, and *ZO2*) showed upregulated expression levels compared to the Cobb, while mucin proteins (*MUC2* and *MUC13*) showed downregulated expression. This unexpected result suggests that, despite genetic improvement over a long period of time, the intestinal barrier function of broilers may not have significantly improved. However, the exact reason for this remains unclear.

E. maxima and *C. perfringens* challenge have been reported to activate cytokine and chemokine release and promote the migration of inflammatory cells that regulate the host immune system through innate and adaptive immune responses (Calefi et al., 2019; Park et al., 2022). In the current study, we investigated how the NE challenge model affects the secretion of various cytokines and chemokines in two different chicken strains. As a result, the NE challenge upregulated the expression of *IL1β* (6 and 8 dpi), *IFNγ* (6 and 8 dpi), *IL10* (6 dpi), *CCL4* (6 and 8 dpi), and *CXCL8* (6 dpi), while downregulating the expression of *IL2* (6 dpi). These findings are consistent with previous NE challenge models using *E. maxima* and *C. perfringens* (Goo et al., 2023b). Interleukin 1 beta is an important pro-inflammatory cytokine that mediates the innate immune response and is primarily produced by activated macrophages (Lee et al., 2015). Interferon gamma, which is produced by T cells and natural killer (**NK**) cells, plays a crucial role in modulating immune responses, promoting inflammation, and activating macrophages (Stark et al., 1998; Tau and Rothman, 1999). In the NE challenge, we observed a comprehensive inflammatory response in the small intestine, with increased levels of *IL1β* and *IFNγ* compared to the nonchallenged controls at both 6 and 8 dpi. Although the increase in pro-inflammatory cytokine levels in the NE challenge group was lower at 8 dpi than at 6 dpi, it still showed a significant difference compared to the nonchallenged group. These results suggest that the immune response induced by the NE challenge model in the current study lasted up to 8 dpi, potentially negatively impacting growth performance due to the increased energy expenditure on immune responses instead of growth and thermoregulation (Lochmiller and Deerenberg, 2000). According to Goo et al. (2023b), there is a contradictory relationship between the expression levels of *IL2* and *IL10* under NE challenge conditions, which was also observed in our experiment. Interleukin 2 is a T helper 1 (**Th_1**) cell cytokine and a growth factor for T cells and NK cells, whereas *IL10* is produced by various immune cells, including leukocytes, macrophages, dendritic cells, and granulocytes (Kany et al., 2019; Mizui, 2019). While most cytokines and chemokines were upregulated under NE challenge conditions, the downregulation of *IL2* expression level may be attributed to the immunosuppressive role of *IL10* (Groux and Cottrez, 2003). In our study, the expression level of *IL10* in the NE challenged group increased up to 17 times compared to the nonchallenged group. High expression level of *IL10* likely contributed to the inhibition of Th_1 cell function and the synthesis of proinflammatory cytokines, which in conclusion to the reduction of *IL2* expression levels (Rothwell et al., 2004; Bancherau et al., 2012). The effect of NE challenge on two chemokines has also been investigated. C-C motif chemokine ligand 4 is a chemoattractant for various immune cells and is produced by epithelial cells, neutrophils, and T cells (Lu et al., 2020). C-X-C motif chemokine ligand 8 is also a major chemoattractant and neutrophil activator (Banks et al., 2003). We observed a similar increasing pattern for all tested chemokines as observed for proinflammatory cytokines such as *IL1β* and *IFNγ* under NE challenge conditions, regardless of chicken strains, indicating an increased secretion of cytokines and chemokines in response to counteract NE infection. Intestinal epithelial cells play a crucial role in the interaction with adjacent immune cells and can protect the host from bacterial infection by secreting and regulating cytokines and chemokines (Mahapatro et al., 2021). Thus, the complex infection models, using *E. maxima* and *C. perfringens*, are primarily target the jejunum and may induce the production of more inflammatory cytokines and chemokines by intestinal epithelial cells to recruit neutrophils and T cells to the infection site (Rengaraj et al., 2016).

Cytokine secretion during an inflammatory response is regulated by complex endogenous cell signaling pathways. In this process, the innate immune response is typically initiated when pathogen recognition receptors, such as TLRs, react to pathogen-associated molecular patterns of bacteria or viruses (Takeuchi and Akira, 2010). The TLR/NFκB signaling pathway is activated by the recognition of pathogen-associated molecular patterns by TLRs, leading to the production of various inflammatory cytokines. This allows the host to effectively defend against the pathogen (Akira and Takeda, 2004). The TLRs trigger an inflammatory response through Myd88-dependent or independent signaling pathways that activate NFκB (Pasare and Medzhitov, 2004). However, in the current experiment, there were no differences observed in *NFκB1* and *Myd88* expression following NE challenge. Instead, NE challenge only resulted in a reduction of *TLR4* expression at 6 dpi, followed by an increase in *TLR2* expression at 8 dpi. These findings are likely due to the complex challenge model

used in the current study, which involved *E. maxima* and *C. perfringens*. Previous study by Tan et al. (2014) reported an increase in jejunal *TLR4* levels on 7 dpi following administration of an *Eimeria* spp. vaccine. On the other hand, Pham et al. (2020) reported a reduction in jejunal *TLR4* levels on 7 dpi in a NE challenge model using *E. maxima* and *E. necatrix*, with no difference in *TLR2* levels. Additionally, Zhou et al. (2023) reported that *TLR2* levels decreased on 7 dpi following *E. acervulina* challenge, but *TLR4* levels increased. These results suggest that the expression of inflammatory cytokines, chemokines, and TLR/NFκB signaling pathway-related proteins may vary depending on the reproductive cycle and status of *Eimeria* spp. used in the respective experiments (Zhou et al., 2023). Furthermore, it is important to note that *C. perfringens* toxin may also disrupt TLR-mediated inflammatory responses, leading to an excessive production of inflammatory cytokines. Ultimately, this disruption can negatively impact the host's immune system (Takehara et al., 2019), leading to unexpected consequences of TLR/NFκB signaling pathway-related proteins, as in the current study.

The TLRs and host defense peptides, particularly *LEAP2* and *CATHL3*, are highly relevant because they play crucial roles in the functioning of the innate immune system (Sumners et al., 2011). Additionally, TLRs may induce the production of host defense peptides, such as β-defensins and CATHLs when activated (Sumners et al., 2011; Fathima et al., 2022). Host defense peptides are known for their antimicrobial activity and immunomodulatory functions, and therefore, the current study aimed to investigate the impact of NE challenge on three representative classes of host defense peptides in avian species: β-defensins, CATHLs, and *LEAP2* (Cuperus et al., 2013). In the current study, NE challenge led to a downregulation in the expression of *AvBD3* (8 dpi), *AvBD9* (6 dpi), and *LEAP2* (6 and 8 dpi), with the exception of *CATHL3*. These findings are consistent with previous studies that observed a decrease in *AvBD3*, *AvBD9*, and *LEAP2* levels following NE challenge (Song et al., 2017; Goo et al., 2023b). Avian beta-defensin and *LEAP2* are typically expressed in the intestinal mucosa or epithelial cells, and they are responsible for protecting the host from pathogenic bacteria through the innate immune system (Townes et al., 2004; Cooper et al., 2019; Liu et al., 2019). Thus, the reduced expression of host defense peptides is believed to be a result of the complex NE challenge caused by *E. maxima* and *C. perfringens*, which may disrupt the innate immune system and make the host more susceptible to pathogenic bacterial infections, including *C. perfringens* (Tian et al., 2016). However, the current experiment does not provide clarification on the relationship between host defense peptides, inflammatory cytokines, and TLRs in the context of NE challenge conditions for both chicken strains.

The expression levels of several inflammatory cytokines under NE challenge were not consistent between the Cobb and ACRB. In the Cobb, the jejunal gene expression levels of *IL1β*, *IFNγ*, and *IL10* were upregulated compared to the ACRB, while *IL6* expression level was downregulated. Similar inconsistent results were also observed in the TLR/NFκB signaling pathway-related proteins. The Cobb showed upregulated *TLR2* and *TLR4* expression levels compared to the ACRB, but downregulated *NFκB1* expression levels. Additionally, there was no significant difference in the expression level of host defense peptides between the Cobb and ACRB. A previous study by Cheema et al. (2003) comparted the immune function of ACRB and modern-type chickens and reported that ACRB has a better humoral immune system and increased relative weights of lymphoid organs such as the bursa of Fabricius, spleen, and cecal tonsil compared to modern-type broilers. Aylward et al. (2022) further reported that modern-type chickens are more sensitive to immune stimulation, resulting in greater variations in key inflammatory cytokines compared to the ACRB. Thus, the ACRB responds more consistently to immune stimulation than modern-type chickens. Despite continuous genetic improvement and selection, it is difficult to determine that modern-type chickens have significantly advanced immune functions compared to the ACRB. However, these comparisons of immune functions have not specifically been made under NE challenge conditions. Additionally, differences may also occur between species within modern-type chickens (Cobb vs. Ross). Therefore, further studies are required to interpret the differences in immunity between the ACRB and Cobb.

In conclusion, the modern-type broiler strain Cobb has shown superior growth performance when compared to the old (genetically nonselected) ACRB strain. These variations are due to the substantial advancements made in broiler production efficiency through successful genetic selection in the industry over several decades. However, this study has confirmed that subclinical NE, which is known to impair performance and efficiency, has a negative impact on intestinal health and immune parameters, regardless of chicken strains. In other words, NE infection continues to be a significant factor in worsening intestinal health and reducing profitability, even in genetically improved modern-type chickens. Therefore, this experiment highlights the importance of addressing the issue of NE infection as a top priority in the broiler industry.

ACKNOWLEDGMENTS

This study was financed in part by a cooperative agreement 6040-32000-080-000D from United States Department of Agriculture-Agricultural Research Service.

DISCLOSURES

There is no conflict of interest.

REFERENCES

Aggrey, S. E., M. C. Milfort, A. L. Fuller, J. Yuan, and R. Rekaya. 2019. Effect of host genotype and Eimeria acervulina infection on the metabolome of meat-type chickens. PLoS One 14: e0223417.

Akira, S., and K. Takeda. 2004. Toll-like receptor signalling. Nat. Rev. Immunol. 4:499–511.
Awad, W. A., C. Hess, and M. Hess. 2017. Enteric pathogens and their toxin-induced disruption of the intestinal barrier through alteration of tight junctions in chickens. Toxins 9:60.
Aylward, B. A., C. N. Johnson, F. Perry, R. Whelan, C. Zhang, and R. J. Arsenault. 2022. Broiler chickens with 1950s genetics display a stable immune profile as measured by Kinome, mRNA expression, and metabolism when stimulated early in life with CpG. Poult. Sci. 101:101775.
Banchereau, J., V. Pascual, and A. O'garra. 2012. From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. Nat. Immunol. 13:925–931.
Banks, C., A. Bateman, R. Payne, P. Johnson, and N. Sheron. 2003. Chemokine expression in IBD. Mucosal chemokine expression is unselectively increased in both ulcerative colitis and Crohn's disease. J. Pathol. 199:28–35.
Bilgili, S. F., J. B. Hess, J. Donald, and B. Fancher. 2010. Practical considerations for reducing the risk of pododermatitis. Aviagen Brief 1:1–8.
Calefi, A. S., W. M. Quinteiro-Filho, A. de Siqueira, A. P. N. Lima, D. S. G. Cruz, N. Q. Hazarbassanov, F. A. Salvagni, A. Borsoi, C. D. O. M. S. Gomes, P. C. Maiorka, and A. J. P. Ferreira. 2019. Heat stress, *Eimeria spp.* and *C. perfringens* infections alone or in combination modify gut Th1/Th2 cytokine balance and avian necrotic enteritis pathogenesis. Vet. Immunol. Immunopathol. 210:28–37.
Celi, P., A. J. Cowieson, F. Fru-Nji, R. E. Steinert, A. M. Kluenter, and V. Verlhac. 2017. Gastrointestinal functionality in animal nutrition and health: new opportunities for sustainable animal production. Anim. Feed Sci. Tech. 234:88–100.
Cheema, M. A., M. A. Qureshi, and G. B. Havenstein. 2003. A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1519–1529.
Collier, C. T., C. L. Hofacre, A. M. Payne, D. B. Anderson, P. Kaiser, R. I. Mackie, and H. R. Gaskins. 2008. Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting *Clostridium perfringens* growth. Vet. Immunol. Immunopathol. 122:104–115.
Collins, K. E., B. H. Kiepper, C. W. Ritz, B. L. McLendon, and J. L. Wilson. 2014. Growth, livability, feed consumption, and carcass composition of the Athens Canadian Random Bred 1955 meat-type chicken versus the 2012 high-yielding Cobb 500 broiler. Poult. Sci. 93:2953–2962.
Collins, K. E., M. L. Marks, S. E. Aggrey, M. P. Lacy, and J. L. Wilson. 2016. History of the Athens Canadian random bred and the Athens random bred control populations. Poult. Sci. 95:997–1004.
Cooper, C. A., M. L. Tizard, T. Stanborough, S. C. Moore, P. S. Chandry, K. A. Jenkins, T. G. Wise, T. E. O'Neil, D. S. Layton, K. R. Morris, and R. J. Moore. 2019. Overexpressing ovotransferrin and avian β-defensin-3 improves antimicrobial capacity of chickens and poultry products. Transgenic Res 28:51–76.
Cuperus, T., M. Coorens, A. van Dijk, and H. P. Haagsman. 2013. Avian host defense peptides. Dev. Comp. Immunol. 41:352–369.
Daneshmand, A., H. Kermanshahi, J. Mohammed, M. H. Sekhavati, A. Javadmanesh, M. Ahmadian, M. Alizadeh, J. Razmyar, and R. R. Kulkarni. 2022. Intestinal changes and immune responses during *Clostridium perfringens*-induced necrotic enteritis in broiler chickens. Poult. Sci. 101:101652.
Fathima, S., W. G. A. Hakeem, R. Shanmugasundaram, and R. K. Selvaraj. 2022. Necrotic enteritis in broiler chickens: a review on the pathogen, pathogenesis, and prevention. Microorganisms 10:1958.
Forder, R. E. A., G. S. Nattrass, M. S. Geier, R. J. Hughes, and P. I. Hynd. 2012. Quantitative analyses of genes associated with mucin synthesis of broiler chickens with induced necrotic enteritis. Poult. Sci. 91:1335–1341.
Furuse, M., M. Itoh, T. Hirase, A. Nagafuchi, S. Yonemura, S. Tsukita, and S. Tsukita. 1994. Direct association of occludin with ZO-1 and its possible involvement in the localization of occludin at tight junctions. J. Cell Biol. 127:1617–1626.
Gharib-Naseri, K., S. Kheravii, C. Keerqin, R. A. Swick, M. Choct, and S. B. Wu. 2021. Differential expression of intestinal genes in necrotic enteritis challenged broiler chickens with 2 different *Clostridium perfringens* strains. Poult. Sci. 100:100886.
Gilani, S., G. S. Howarth, C. D. Tran, S. M. Kitessa, R. E. Forder, R. Barekatain, and R. J. Hughes. 2018. Effects of delayed feeding, sodium butyrate and glutamine on intestinal permeability in newly-hatched broiler chickens. J. Appl. Anim. Res. 46:973–976.
Golder, H. M., M. S. Geier, R. E. A. Forder, P. I. Hynd, and R. J. Hughes. 2011. Effects of necrotic enteritis challenge on intestinal micro-architecture and mucin profile. Br. Poult. Sci. 52:500–506.
Goo, D., I. Park, H. Nam, Y. Lee, J. Sawall, A. H. Smith, T. G. Rehberger, C. Li, and H. S. Lillehoj. 2023a. Collagen adhesin protein and necrotic enteritis B-like toxin as biomarkers for early diagnosis of necrotic enteritis in commercial broiler chickens. Poult. Sci. 102:102647.
Goo, D., J. Choi, H. Ko, V. S. R. Choppa, G. Liu, H. S. Lillehoj, and W. K. Kim. 2023b. Effects of *Eimeria maxima* infection doses on growth performance and gut health in dual-infection model of necrotic enteritis in broiler chickens. Front. Physiol. 14:1269398.
Goo, D., U. D. Gadde, W. K. Kim, C. G. Gay, E. W. Porta, S. W. Jones, S. Walker, and H. S. Lillehoj. 2023c. Hyperimmune egg yolk antibodies developed against Clostridium perfringens antigens protect against necrotic enteritis. Poult. Sci. 102:102841.
Groux, H., and F. Cottrez. 2003. The complex role of interleukin-10 in autoimmunity. J. Autoimmun. 20:281–285.
Hartcher, K. M., and H. K. Lum. 2020. Genetic selection of broilers and welfare consequences: a review. World Poult. Sci. J. 76:154–167.
Hess, C. W. 1962. Randombred populations of the southern regional poultry breeding project. World's Poult. Sci. J. 18:147–152.
Johansson, M. E., M. Phillipson, J. Petersson, A. Velcich, L. Holm, and G. C. Hansson. 2008. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc. Natl. Acad. Sci. 105:15064–15069.
Kany, S., J. T. Vollrath, and B. Relja. 2019. Cytokines in inflammatory disease. Int. J. Mol. Sci. 20:6008.
Keyburn, A. L., T. L. Bannam, R. J. Moore, and J. I. Rood. 2010. NetB, a pore-forming toxin from necrotic enteritis strains of Clostridium perfringens. Toxins 2:1913–1927.
Lee, K. W., D. K. Kim, H. S. Lillehoj, S. I. Jang, and S. H. Lee. 2015. Immune modulation by Bacillus subtilis-based direct-fed microbials in commercial broiler chickens. Anim. Feed Sci. Tech. 200:76–85.
Lee, K. W., H. S. Lillehoj, W. Jeong, H. Y. Jeoung, and D. J. An. 2011. Avian necrotic enteritis: experimental models, host immunity, pathogenesis, risk factors, and vaccine development. Poult. Sci. 90:1381–1390.
Liu, C., S. M. Radebe, H. Zhang, J. Jia, S. Xie, M. Shi, and Q. Yu. 2022. Effect of Bacillus coagulans on maintaining the integrity intestinal mucosal barrier in broilers. Veterinary Microbiol. 266:109357.
Liu, S. D., M. H. Song, W. Yun, J. H. Lee, H. B. Kim, and J. H. Cho. 2019. Effect of carvacrol essential oils on immune response and inflammation-related genes expression in broilers challenged by lipopolysaccharide. Poult. Sci. 98:2026–2033.
Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87–98.
Lu, M., W. H. Kim, H. S. Lillehoj, and C. Li. 2020. Development and characterization of novel mouse monoclonal antibodies against chicken chemokine CC motif ligand 4. Vet. Immunol. Immunopathol. 227:110091.
Mahapatro, M., L. Erkert, and C. Becker. 2021. Cytokine-mediated crosstalk between immune cells and epithelial cells in the gut. Cells 10:111.
Maher, D. M., B. K. Gupta, S. Nagata, M. Jaggi, and S. C. Chauhan. 2011. Mucin 13: structure, function, and potential roles in cancer pathogenesis. Mol. Cancer Res. 9:531–537.
McGuckin, M. A., S. K. Lindén, P. Sutton, and T. H. Florin. 2011. Mucin dynamics and enteric pathogens. Nat. Rev. Microbiol. 9:265–278.

Mizui, M. 2019. Natural and modified IL-2 for the treatment of cancer and autoimmune diseases. Clin. Immunol. 206:63–70.
Moore, R. J. 2016. Necrotic enteritis predisposing factors in broiler chickens. Avian Pathol. 45:275–281.
Paiva, D., and A. McElroy. 2014. Necrotic enteritis: applications for the poultry industry. J. Appl. Poult. Res. 23:557–566.
Paiva, D., C. Walk, and A. McElroy. 2014. Dietary calcium, phosphorus, and phytase effects on bird performance, intestinal morphology, mineral digestibility, and bone ash during a natural necrotic enteritis episode. Poult. Sci. 93:2752–2762.
Park, S. S., H. S. Lillehoj, P. C. Allen, D. W. Park, S. FitzCoy, D. A. Bautista, and E. P. Lillehoj. 2008. Immunopathology and cytokine responses in broiler chickens coinfected with Eimeria maxima and Clostridium perfringens with the use of an animal model of necrotic enteritis. Avian Dis. 52:14–22.
Park, I., S. Oh, H. Nam, P. Celi, and H. S. Lillehoj. 2022. Antimicrobial activity of sophorolipids against Eimeria maxima and Clostridium perfringens, and their effect on growth performance and gut health in necrotic enteritis. Poult. Sci. 101:101731.
Pasare, C., and R. Medzhitov. 2004. Toll-like receptors: linking innate and adaptive immunity. Microbes Infect. 6:1382–1387.
Peek, H. W. 2010. Resistance to anticoccidial drugs: alternative strategies to control coccidiosis in broilers. Doctoral Dissertation. Utrecht University.
Pham, V. H., L. Kan, J. Huang, Y. Geng, W. Zhen, Y. Guo, W. Abbas, and Z. Wang. 2020. Dietary encapsulated essential oils and organic acids mixture improves gut health in broiler chickens challenged with necrotic enteritis. J. Anim. Sci. Biotechnol. 11:1–18.
Rath, N. C., G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Factors regulating bone maturity and strength in poultry. Poult. Sci. 79:1024–1032.
Rengaraj, D., A. D. Truong, S. H. Lee, H. S. Lillehoj, and Y. H. Hong. 2016. Expression analysis of cytosolic DNA-sensing pathway genes in the intestinal mucosal layer of necrotic enteritis-induced chicken. Vet. Immunol. Immunopathol. 170:1–12.
Rothwell, L., J. R. Young, R. Zoorob, C. A. Whittaker, P. Hesketh, A. Archer, A. L. Smith, and P. Kaiser. 2004. Cloning and characterization of chicken IL-10 and its role in the immune response to *Eimeria maxima*. J. Immunol. 173:2675–2682.
Schmidt, C. J., M. E. Persia, E. Feierstein, B. Kingham, and W. W. Saylor. 2009. Comparison of a modern broiler line and a heritage line unselected since the 1950s. Poult. Sci. 88:2610–2619.
Shah, B. R., W. A. Hakeem, R. Shanmugasundaram, and R. K. Selvaraj. 2023. Effect of synbiotic supplementation on production performance and severity of necrotic enteritis in broilers during an experimental necrotic enteritis challenge. Poult. Sci. 102:102959.
Shojadoost, B., A. R. Vince, and J. F. Prescott. 2012. The successful experimental induction of necrotic enteritis in chickens by *Clostridium perfringens*: a critical review. Vet. Res. 43:1–12.
Skinner, J. T., S. Bauer, V. Young, G. Pauling, and J. Wilson. 2010. An economic analysis of the impact of subclinical (mild) necrotic enteritis in broiler chickens. Avian Dis. 54:1237–1240.
Song, B., H. Li, Y. Wu, W. Zhen, Z. Wang, Z. Xia, and Y. Guo. 2017. Effect of microencapsulated sodium butyrate dietary supplementation on growth performance and intestinal barrier function of broiler chickens infected with necrotic enteritis. Anim. Feed Sci. Tech. 232:6–15.
Stark, G. R., I. M. Kerr, B. R. Williams, R. H. Silverman, and R. D. Schreiber. 1998. How cells respond to interferons. Annu. Rev. Biochem. 67:227–264.
Su, S., Y. Wang, C. Chen, M. Suh, M. Azain, and W. K. Kim. 2020. Fatty acid composition and regulatory gene expression in late-term embryos of ACRB and COBB broilers. Front. Vet. Sci. 7:317.
Sumners, L. H., K. B. Miska, M. C. Jenkins, R. H. Fetterer, C. M. Cox, S. Kim, and R. A. Dalloul. 2011. Expression of Toll-like receptors and antimicrobial peptides during Eimeria praecox infection in chickens. Exp. Parasitol. 127:714–718.
Takehara, M., S. Seike, Y. Sonobe, H. Bandou, S. Yokoyama, T. Takagishi, K. Miyamoto, K. Kobayashi, and M. Nagahama. 2019. *Clostridium perfringens* α-toxin impairs granulocyte colony-stimulating factor receptor-mediated granulocyte production while triggering septic shock. Commun. Biol. 2:45.
Takeuchi, O., and S. Akira. 2010. Pattern recognition receptors and inflammation. Cell 140:805–820.
Tan, J., T. J. Applegate, S. Liu, Y. Guo, and S. D. Eicher. 2014. Supplemental dietary L-arginine attenuates intestinal mucosal disruption during a coccidial vaccine challenge in broiler chickens. Br. J. Nutr. 112:1098–1109.
Tau, G., and P. Rothman. 1999. Biologic functions of the IFN-γ receptors. Allergy 54:1233.
Teng, P. Y., S. Yadav, F. L. de Souza Castro, Y. H. Tompkins, A. L. Fuller, and W. K. Kim. 2020. Graded *Eimeria* challenge linearly regulated growth performance, dynamic change of gastrointestinal permeability, apparent ileal digestibility, intestinal morphology, and tight junctions of broiler chickens. Poult. Sci. 99:4203–4216.
Tian, X., Y. Shao, Z. Wang, and Y. Guo. 2016. Effects of dietary yeast β-glucans supplementation on growth performance, gut morphology, intestinal *Clostridium perfringens* population and immune response of broiler chickens challenged with necrotic enteritis. Anim. Feed Sci. Tech. 215:144–155.
Townes, C. L., G. Michailidis, C. J. Nile, and J. Hall. 2004. Induction of cationic chicken liver-expressed antimicrobial peptide 2 in response to *Salmonella enterica* infection. Infect. Immun. 72:6987–6993.
Van Immerseel, F., J. D. Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. 2004. *Clostridium perfringens* in poultry: an emerging threat for animal and public health. Avian Pathol. 33:537–549.
Van Immerseel, F., J. I. Rood, R. J. Moore, and R. W. Titball. 2009. Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens. Trends Microbiol. 17:32–36.
Wade, B., and A. Keyburn. 2015. The true cost of necrotic enteritis. World Poult. 31:16–17.
Wei, X., Z. Yang, F. E. Rey, V. K. Ridaura, N. O. Davidson, J. I. Gordon, and C. F. Semenkovich. 2012. Fatty acid synthase modulates intestinal barrier function through palmitoylation of mucin 2. Cell Host Microbe 11:140–152.
Zhou, X., L. Wang, Z. Wang, P. Zhu, Y. Chen, C. Yu, S. Chen, and Y. Xie. 2023. Impacts of Eimeria coinfection on growth performance, intestinal health and immune responses of broiler chickens. Vet. Parasitol. 322:110019.
Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. Poult. Sci. 93:2970–2982.