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Evaluating performance, intestinal lesions, and immunity related genes in heritage and modern broiler breeds during a necrotic enteritis challenge

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ABSTRACT In this comparative study, the differential responses of heritage (ACRB; Athens Canadian Random Bred) and modern (Cobb) broilers to a necrotic enteritis (**NE**) challenge were evaluated. The design was a 2×2 factorial with breed (ACRB and Cobb) and challenge (non-challenged and NE-challenged) as main factors. On day (d) of hatch, 96 male chicks (48 ACRB and 48 Cobb) were allocated to 4 experimental groups with 8 replicate cages and 3 birds/cage. On d 14, birds in the NE-challenged groups were orally gavaged with 3,000 *Eimeria maxima* sporulated oocysts followed by 2 doses of ∼1×10^8 CFU of *Clostridium perfringens* on d 19 and 20. On d 21, 2 birds/cage were necropsied to score NE lesions, and spleen and cecal tonsils (**CT**) samples were collected from 1 bird/cage for assessing mRNA abundance. Challenged ACRB birds exhibited reduced growth performance and relative growth performance compared to challenged Cobb birds. There was no significant interaction between breed and challenge during the challenge period (d 14−21) for mortality. However, there was a challenge main effect ($P \leq 0.05$) on mortality as manifested by greater NE-associated mortality compared to non-challenged birds. No significant breed × challenge interaction or breed main effect on lesion scores were observed in the duodenum, jejunum, and ileum. NE-challenged Cobb birds exhibited greater mRNA abundance of IL-18, TNFα, TLR1.2, TLR2.1, CCR5, CCR6, CCL20, and AvBD1 in CT compared to NE challenged ACRB birds. There was a significant breed × challenge interaction effect on mRNA abundance of IL-10, AvBD13, NK-Lysin, and LEAP2 in the spleen. Moreover, a main effect of breed was observed in IL-1β, IL-18, TNFα, TLR2.1, CCR5, CCL20, and NK-Lysin where ACRB birds had higher mRNA abundance than Cobb birds ($P \leq 0.05$). The observed differences in performance, pathology, and mRNA abundance between ACRB and Cobb broilers during the NE challenge highlight the distinct immune response profiles of heritage and modern breeds, emphasizing the need for breed-specific nutritional, managerial, and genetic selection programs for modulating immune responses during enteric disease challenges.

Key words: necrotic enteritis, immune response, host defense peptide, ACRB, broiler

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INTRODUCTION

In the broiler industry, there is an ongoing genetic selection process aimed at enhancing production performance and feed efficiency, along with the development of more precise diet formulations. Selection for rapid growth could lead to certain alterations in metabolic and physiological traits including reduced immune response and disease resistance (Cheema et al., 2003; Zou et al., 2020), poor skeletal development and integrity (McDevitt et al., 2006; Siegel et al., 2019), ascites (Baghbanzadeh and Decuypere, 2008), and cardiovascular issues (Olkowski et al., 2008). Additionally, modern broilers exhibit a higher mortality rate compared to heritage lines due to frequent exposure to infectious diseases or metabolic syndromes under intensive production systems (Yassin et al., 2009; Hartcher and Lum, 2020). Considering the current industrial dynamics, infectious diseases, especially enteric diseases, present a significant challenge in commercial farms. While there is a great effort in the development of non-drug dietary additives to mitigate the negative impacts of such enteric problems, improving birds' resistance and immune response could play a crucial role in poultry production.

Necrotic enteritis (**NE**) is an enteric disease caused by toxins produced by *Clostridium perfringens* and is

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estimated to cost the global poultry industry $6 billion annually (Wade and Keyburn, 2015). *C. perfringens* is ubiquitous in the poultry environment (soil, litter, feces); therefore, birds are continuously exposed to these bacteria and their spores. In healthy birds, it is typical to find approximately 10^2-10^4 CFU/g digesta of *C. perfringens*; however, birds can have as high of 10^7-10^9 CFU/g digesta during an NE outbreak (Shojadoost et al., 2012). The presence of *C. perfringens* and its spores in the intestinal tract of broilers do not always cause disease, but several predisposing factors including *Eimeria* spp., nature and composition of the diet (e.g. mycotoxin presence, non-starch polysaccharides level, fish meal), and stressors that suppress immunity contribute to NE development (Cravens et al., 2013; Emami and Dalloul, 2021).

The Athens Canadian Random Bred (**ACRB**) is a heritage breed of the modern broiler. This breed is maintained at the University of Georgia Department of Poultry Science, and is representative of the 1957 meat-type broilers (Collins et al., 2016). There are indications towards breed-specific differences in immune response of ACRB vs. modern broilers. Namely, in a study involving this genetic line and 2001 modern broilers, the ACRBs had greater relative lymphoid organ weights (excluding thymus) and greater antibody responses against sheep red blood cells compared to modern broiler line (Cheema et al., 2003). Also, reduction in growth performance at the expense of immune function in a commercial broiler compared to 1957 and 1978 non-selected strains has been documented (Zou et al., 2020). Given that the ACRB strain has not undergone any selective breeding for performance parameters, it presents a valuable opportunity for a side-by-side comparison. This facilitates an examination of the underlying differences between heritage and modern broilers in response to enteric diseases, potentially helping develop targeted strategies for improving the health and resilience of modern broilers. However, to our knowledge, published data on the response of heritage and modern broilers against intestinal pathogens are very limited. In this context, the objective of this study was to evaluate growth performance, mortality rate, intestinal pathology, and mRNA abundance of immune response-related genes in a modern (Cobb) broiler and heritage (ACRB) breed during a NE challenge.

MATERIALS AND METHODS

Birds, Management, and Diet

This project was approved and conducted under the guidelines of the Institutional Animal Care and Use Committee (AUP # A2021-10-020). On day (**d**) of hatch, a total of 48 ACRB males and 48 Cobb males (n = 96) were acquired from the UGA ACRB breeding flock and local Cobb hatchery, respectively. The study was a 2 × 2 factorial design with genetic lines (ACRB and Cobb) and challenge state (no challenge and NE challenge) as main factors. Upon arrival, birds were randomized, individually weighed, wing-tagged, and assigned to 1 of the 4 experimental groups each with 8 replicate battery cages and 3 birds/cage. All birds were fed a corn-soybean basal diet formulated to meet or exceed the nutrient recommendations for commercial Cobb broilers. Birds were housed in clean battery cages for the duration of the trial and allowed access to feed and water *ad libitum*.

Necrotic Enteritis Challenge

On d 14, ACRB and Cobb birds designated to be in the NE challenge groups received 3,000 sporulated *E. maxima* oocysts by oral gavage of 1 mL/bird. On d 19 and d 20, birds in the NE challenge groups received approximately 1 × 10^8 CFU/bird of *C. perfringens* by oral gavage. Birds in the NC groups received 1 mL of sterile water on all inoculation days.

Performance

Upon arrival (d 0), birds were individually weighed and assigned to each pen. Birds were individually weighed on d 14 before *E. maxima* inoculation and d 21 at trial termination. Additionally, feed intake (**FI**) was recorded on a per pen basis on d 14 and d 21. Any mortality was removed and recorded (including bird weight) twice daily. Mortality-corrected body weight gain (**BWG**), feed conversion ratio (**FCR**), and FI were calculated for the pre-challenge period (d 0−14), post-challenge (d 14−21), and cumulative experimental period (d 0-21). Additionally, relative BW (**RBW**), relative BWG (**RBWG**), relative FI (**RFI**), and relative FCR (**RFCR**) were determined by setting the values of the non-challenged group for each strain as the baseline (100%).

Lesion Scores

On d 21, 2 birds from each cage (16 birds/treatment) were selected, euthanized by cervical dislocation, and the 3 sections of the small intestine were inspected for NE lesions based on a 0 to 4 scale system (Prescott et al., 1978). The lesion scoring system used was as follows: 0 = no gross lesions, normal intestinal appearance; 1 = thin-walled or friable, gray appearance; 2 = thin-walled, focal necrosis, gray appearance, small amounts of gas production; 3 = thin-walled, sizable patches of necrosis, gas-filled intestine, small flecks of blood; 4 = severe extensive necrosis, marked hemorrhage, large amounts of gas in the intestine by personnel blinded to the treatments.

Total RNA Extraction and Reverse Transcription

On d 21, 1 bird/pen was randomly selected (the first bird selected for lesion scoring), euthanized by cervical dislocation, and cecal tonsils (**CT**) and spleen samples

were snap frozen in liquid nitrogen and stored at −80°C until analysis. Approximately 50 mg aliquot of CT and spleen tissues were weighed into a 2 mL screw-capped tube and homogenized in 1 mL TRI Reagent (Zymo Research, Irvine, CA) by Bead Mill (VWR, Radnor, PA). After homogenization, 200 μL of chloroform were added, and samples were centrifuged at 12,000 × g for 15 minutes at 4°C. After centrifugation, total RNA settled in the upper aqueous liquid was precipitated with isopropanol and rinsed with ethanol. The extracted RNA was resuspended in DEPC-H_2O. Total RNA concentration was determined at optical density (**OD**) of 260 (NanoDrop One^C spectrophotometer, Thermo Fisher Scientific, Waltham, MA), and RNA purity was verified by evaluating the 260/280 OD ratios. RNA samples with a ratio in the range of 1.8-2.0 were considered high quality and were used to synthesize cDNA. After extraction, 2 μg total RNA were used to synthesize first-strand cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA) according to manufacturer's recommendation, and the cDNA was stored at −20°C.

Quantitative Real-Time PCR

The mRNA abundance of cytokines [interleukin (**IL**)-1β, IL-10, IL-18, and tumor necrosis factor (**TNF**)α)], chemokines [chemokine ligand (**CCL**)5 and CCL20)], chemokine receptors [(chemokine receptor (**CCR**)5 and CCR6), Toll-like receptors (**TLR**)1.2 and TLR2.1)], liver-expressed antimicrobial peptide 2 (**LEAP2**), NK-Lysin, avian β-defensins (**AvBD**)1, 8, 10, 12, 13, and 14, cathelicidins (**CATH**)1, 3, and B1 was determined by quantitative real-time PCR (QuantStudio 3, Applied Biosystems, Foster City, CA) using PowerTrack^{TM} SYBR Green Master Mix (Applied Biosystems, Foster City, CA). Primer details and NCBI accession numbers are shown in Table 1. Each reaction was completed in duplicate wells of a 96-well PCR plate with a total volume of 20 μL. mRNA abundance was analyzed using 18S as the endogenous control. Average mRNA abundance relative to 18S for each sample was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The calibrator for each gene was the average ΔCt value of the ACRB non-challenged group.

Statistical Analyses

Statistical analysis for pre-challenge period (d 0-14) was performed by student t-test and challenge period (d 14-21) was analyzed by 2-way ANOVA procedure of JMP software (Pro 17) followed by Fisher's least significant difference (**LSD**) tests. Additionally, a chi-squared test was conducted to further analyze NE lesion scores. The probability $P \leq 0.05$ was considered significant unless otherwise noted.

RESULTS

Performance and Mortality

Pre-challenge and post-challenge performance, relative performance, and mortality results are presented in Table 2, Table 3, Figure 1, and Figure 2, respectively. As expected, Cobb birds exhibited greater BWG and FI and lower FCR ($P < 0.001$) compared to ACRB birds during pre-challenge period (Table 2). During the challenge period (d 14−21), a significant interaction for BWG ($P < 0.001$), FI ($P = 0.005$), and FCR ($P = 0.005$) was observed for d 14-21. There was a significant breed main effect on growth performance with Cobb birds having a higher BWG and FI and lower FCR ($P < 0.001$) compared to ACRB birds during d 14-21. Indicative of the successful challenge, birds in the NE groups exhibited compromised growth performance compared to their NC counterparts ($P < 0.01$) during the challenge period (d 14−21). Overall, a significant breed × challenge interaction effect on FCR was observed during d 0-21 ($P = 0.026$). In terms of main effects, Cobb birds demonstrated better growth performance compared to ACRB birds during the overall experimental period ($P < 0.001$). As presented in Figure 1, challenged ACRB birds exhibited lower RBWG, higher RFI, and higher RFCR compared to challenged Cobb birds during the challenge period (d 14-21). Additionally, challenged birds showed lower relative growth performance when compared to their non-challenged counterparts. There was no significant interaction between breed and challenge during the challenge period (d 14−21) for mortality. However, there was a challenge main effect ($P < 0.001$) on mortality with NE-challenged birds having significantly greater NE-associated mortality compared to non-challenged birds.

Lesion Scores

There were no NE lesions exhibited in non-challenged birds. No significant breed × challenge interaction effect on lesion scores was observed in the duodenum, jejunum, and ileum. There was a challenge main effect with NE-challenged birds having higher NE lesion scores in the duodenum ($P < 0.001$) and jejunum ($P = 0.012$) compared to non-challenged birds. There were no significant differences in lesion scores in the duodenum, jejunum, and ileum of NE-challenged ACRB and Cobb birds (Table 4).

mRNA Abundance in Cecal Tonsils and Spleen

The effects of breed and challenge on mRNA abundance of cytokines, TLRs, chemokines, and host defense peptides (**HDPs**) in the CT and spleen are presented in Tables 5 and 6, respectively. A significant breed × challenge interaction was observed in mRNA abundances of IL-1β, IL-10, IL-18, TNFα,

Table 1. Sequences of primer pairs used for amplification of target and reference genes.[1]

Gene[2]	Primer Sequence	Size	Acc. # (Reference)
Cytokines			
IL-1β	F-CGAGGAGCAGGGACTTTGC R-GAAGGTGACGGGCTCAAAAA	71	NM_204524.2
IL-10	F-CGCTGTCACCGCTTCTTCA R-CGTCTCCTTGATCTGCTTGATG	63	NM_001004414.4
IL-18	F-TGCAGCTCCAAGGCTTTTAAG R-CTCAAAGGCCAAGAACATTCCT	63	NM_204608.3
TNFα	F-CCCATCCCTGGTCCGTAAC R-ATACGAAGTAAAGGCCGTCCC	77	MF000729.1
Toll-like Receptors			
TLR 1.2	F-AGCTGCAGGACTTCCTGCGC R-TTGTCTGCGTCCACTGCCAC	264	NM_001007488.5
TLR 2.1	F-TTAAAAGGGTGTGCCAGGAG R-GTCCAAACCCATGAAAGAGC	271	AB050005.2
Chemokines			
CCR5	F-GTGGTCAACTGCAAAAAGCA R-GCCCGTTCAACTGTGTCG	190	NM_001271141.1
CCR6	F-TTATGCTGCTGACAAATGGA R-TAAAATAGCCGATGCCCACA	217	NM_001398176.1
CCL5	F-TTTCTACACCAGCAGCAAATGC R-GCCCCTTCCTGGTGATGAA	59	NM_001045832.2
CCL20	F-TGCTGCTTGGAGTGAAAATGC R-CAGCAGAGAAGCCAAAATCAAA	62	NM_204438.2
Host Defense Peptides			
AvBD1	F-TACCTGCTCCTCCCCTTCAT R-GATGAGAGTGAGGGAAGGGC	135	NM_204993.1
AvBD8	F-ATGCGCGTACCTAACAACGA R-TGCCCAAAGGCTCTGGTATG	95	NM_001001781.1
AvBD10	F-CAGACCCACTTTTCCCTGACA R-CCCAGCACGGCAGAAATT	62	NM_001001609.3
AvBD12	F-ATCTTCATCTCCCTGCTCGC R-TCAGGTCTTGGTGGGAGTTG	174	NM_001397753.2
AvBD13	F-CAGCTGTGCAGGAACAACCA R-CAGCTCTCCATGTGGAAGCA	59	NM_001001780.2
AvBD14	F-TGGGCATATTCCTCCTGTTTCT R-TTGCCAGTCCATTGTAGCAG	156	NM_001348511.2
CATH1	F-TCTACCGGGCGATCAAGAAGA R-GCAGCAAAGGAACAGTGCTAA	66	NM_001001605.3
CATH3	F-GCACAACCTCAACTTCACCA R-CGTGTTGATGGCCACTGG	223	NM_001311177.2
CATHB1	F-CCGATCACCTACCTGGATGC R-CCTGAGATCCTCTGGTTGAGC	62	NM_001271172.1
NK-Lysin	F-AGATGAAGGGGACGCAATGG R-CTTCACCAGACTGACGCAGA	67	NM_001044680.2
LEAP2	F-TGTGCTTCCCTGCACCAA R-GGCGTCATCCGCTTCAGT	53	NM_001001606.2
Housekeeping Gene			
18S	F-TCCCCTCCCGTTACTTGGAT R-GCGCTCGTCGGCATGTA	60	AF173612.1

[1]For each gene, the sequence for forward (F) and reverse (R) (5′-3′) primers, the amplicon size (bp) and the NCBI Accession number (Acc) used for the primer designs are listed.

[2]IL: Interleukin; TNFα: tumor necrosis factor alpha; CCR: C-C chemokine receptor; CCL: C-C motif chemokine ligand; AvBD: avian β-defensin; CATH: cathelicidin; LEAP2: Liver-expressed antimicrobial peptide 2; 18S: 18S ribosomal RNA gene.

Table 2. Body weight gain (BWG, g/bird), feed intake (FI, g/bird), and feed conversion ratio (FCR) during d 0-14.[1]

	Treatments[2]		Statistics	
Item[3]	ACRB	Cobb	*SEM*	*P-value*
d 0-14				
BWG (g)	83[b]	326[a]	*13.21*	*<0.001*
FI (g)	185[b]	410[a]	*21.47*	*<0.001*
FCR	2.25[a]	1.27[b]	*0.09*	*<0.001*

[a-b]Means followed by different superscript letters are significantly ($P \leq 0.05$) different by student *t*-test.

[1]Data represent mean values of 8 replicate cages per treatment.

[2]ACRB: Athens Canadian Random Bred; Cobb: Cobb500.

[3]BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio.

Table 3. Body weight gain (BWG, g/bird), feed intake (FI, g/bird), and feed conversion ratio (FCR) from d 14−21 and 0−21 according to breed (ACRB or Cobb) and challenge status (non-challenged or NE-challenged).[1]

	Treatments[2]									Probabilities		
	ACRB		Cobb		Breed		Challenge			*P*-value		
Item[3]	NC	NE	NC	NE	ACRB	Cobb	NC	NE	SEM	*Breed*	*Challenge*	*B* × *C*
d 14-21												
BWG (g)	74[c]	39[d]	388[a]	251[b]	56[b]	309[a]	227[a]	145[b]	*15.44*	*<0.001*	*<0.001*	*<0.001*
FI (g)	192[c]	170[c]	500[a]	397[b]	181[b]	448[a]	346[a]	283[b]	*25.74*	*0.001*	*0.001*	*0.005*
FCR	2.63[b]	4.77[a]	1.29[c]	1.65[bc]	3.70[a]	1.47[b]	1.98[b]	3.21[a]	*0.30*	*<0.001*	*0.002*	*0.005*
d 0-21												
BWG (g)	159	120	707	585	139[b]	649[a]	433[a]	352[b]	*28.05*	*<0.001*	*<0.001*	*0.122*
FI (g)	379	353	899	813	365[b]	856[a]	639[a]	583[b]	*44.49*	*<0.001*	*0.025*	*0.379*
FCR	2.42[a]	2.97[a]	1.28[b]	1.40[b]	2.70[a]	1.34[b]	1.85[b]	2.19[a]	*0.13*	*<0.001*	*0.001*	*0.026*

[a-d]Means followed by different superscript letters are significantly ($P \leq 0.05$) different by LSD test.
[1]Data represent mean values of 8 replicate cages per treatment.
[2]ACRB: Athens Canadian Random Bred; Cobb: Cobb500; NC: non-challenged; NE: necrotic enteritis challenged.
[3]BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio.

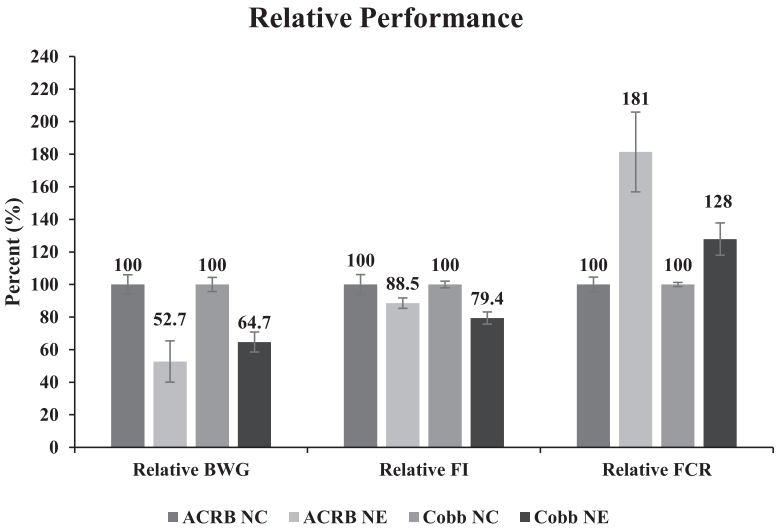

Figure 1. Effects of NE challenge on relative growth performance of ACRB and Cobb broilers during challenge period (d 14−21). BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio. Each bar represents the mean ± SEM.

TLR1.2, TLR2.1, CCR5, CCR6, CCL20, AvBD1, AvBD12, and LEAP2 ($P \leq 0.05$) in CT tissues. Accordingly, challenged Cobb birds exhibited a higher mRNA abundance of IL-18, TNFα, TLR1.2, TLR2.1, CCR5, CCR6, CCL20, and AvBD1 compared to challenged ACRB birds ($P \leq 0.05$). In terms of main effects, Cobb birds exhibited higher IL-18, TNFα, TLR1.2, TLR2.1, CCR5, CCL20, and AvBD1 mRNA abundance compared to ACRB birds. The mRNA abundances of IL-1β, IL-10, IL-18, CCR6, AvBD1, and AvBD12 were significantly higher in non-challenged ACRB birds compared to

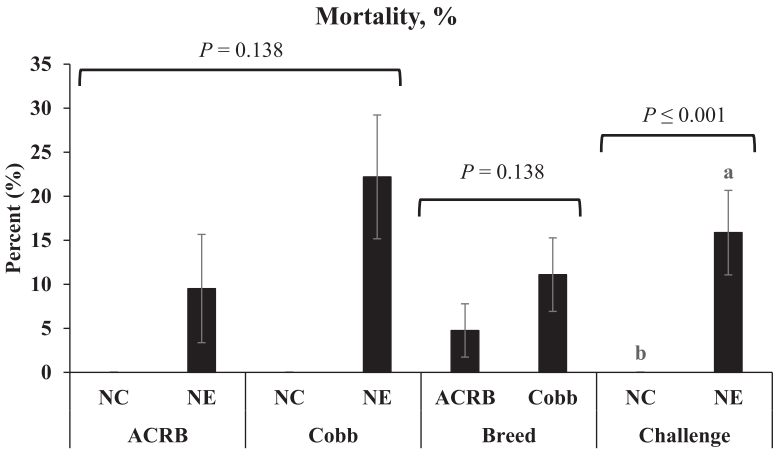

Figure 2. Effects of NE challenge on mortality (%) during the challenge period (d 14−21). NC: non-challenged; NE: necrotic enteritis challenged. Each bar represents the mean ± SEM.

Table 4. Necrotic enteritis lesion scores in the duodenum, jejunum, and ileum on d 21.[1]

	Treatments[2]									Probabilities		
	ACRB		Cobb		Breed		Challenge			*P*-value		
Item	NC	NE	NC	NE	ACRB	Cobb	NC	NE	SEM	*Breed*	*Challenge*	*B* × *C*
Duodenum	0	0.30	0	0.32	0.15	0.13	0[b]	0.31[a]	*0.05*	*0.915*	*<0.001*	*0.915*
Jejunum	0	0.50	0	0.59	0.24	0.24	0[b]	0.54[a]	*0.11*	*0.830*	*0.012*	*0.830*
Ileum	0	0.20	0	0.36	0.10	0.10	0	0.27	*0.09*	*0.639*	*0.110*	*0.639*

[a,b]Means followed by different superscript letters are significantly ($P \leq 0.05$) different by LSD test.
[1]Data represent mean values of 16 birds per treatment.
[2]ACRB: Athens Canadian Random Bred; Cobb: Cobb500; NC: non-challenged; NE: necrotic enteritis challenged.

Table 5. Effects of NE challenge on mRNA abundance of cytokines, TLRs, chemokines, HDPs in cecal tonsils.[1]

	Treatments[2]									Probabilities		
	ACRB		Cobb		Breed		Challenge			*P*-value		
Item	NC	NE	NC	NE	ACRB	Cobb	NC	NE	SEM	*Breed*	*Challenge*	*B* × *C*
Cytokines												
IL-1β	1.00[a]	0.87[a]	0.57[b]	0.88[a]	0.94[a]	0.73[b]	0.79	0.88	*0.05*	*0.029*	*0.354*	*0.027*
IL-10	1.00[a]	0.72[b]	0.76[b]	0.89[ab]	0.86	0.83	0.88	0.80	*0.03*	*0.622*	*0.226*	*0.002*
IL-18	1.00[a]	0.69[b]	0.64[b]	1.04[a]	0.85	0.84	0.82	0.87	*0.04*	*0.924*	*0.437*	*<0.001*
TNFα	1.00[bc]	1.11[b]	0.69[c]	1.62[a]	1.05	1.16	0.85[b]	1.36[a]	*0.07*	*0.413*	*<0.001*	*0.002*
Toll-like Receptors												
TLR 1.2	1.00[b]	0.82[b]	1.08[b]	2.53[a]	0.91[b]	1.80[a]	1.04[b]	1.68[a]	*0.14*	*<0.001*	*0.001*	*<0.001*
TLR 2.1	1.00[b]	0.87[b]	1.09[b]	1.93[a]	0.94[b]	1.51[a]	1.05[b]	1.40[a]	*0.09*	*<0.001*	*0.017*	*0.002*
Chemokines												
CCR5	1.00[ab]	0.51[c]	0.69[bc]	1.33[a]	0.76	1.01	0.85	0.92	*0.11*	*0.101*	*0.614*	*<0.001*
CCR6	1.00[a]	0.60[b]	0.76[b]	1.16[a]	0.80[b]	0.96[a]	0.88	0.88	*0.05*	*0.049*	*0.981*	*<0.001*
CCL5	1.00	0.93	1.09	1.21	0.97[b]	1.15[a]	1.04	1.07	*0.05*	*0.048*	*0.779*	*0.302*
CCL20	1.00[b]	0.88[b]	0.85[b]	1.36[a]	0.94	1.10	0.92[b]	1.12[a]	*0.06*	*0.098*	*0.048*	*0.002*
Host Defense Peptides												
AvBD1	1.00[a]	0.34[c]	0.67[b]	1.21[a]	0.67[b]	0.94[a]	0.83	0.78	*0.07*	*0.011*	*0.580*	*<0.001*
AvBD8	1.00	1.27	0.84	0.88	1.14[a]	0.86[b]	0.92	1.07	*0.07*	*0.037*	*0.244*	*0.362*
AvBD10	1.00	1.23	1.20	1.02	1.12	1.11	1.10	1.13	*0.10*	*0.969*	*0.875*	*0.250*
AvBD12	1.00[a]	0.82[ab]	0.49[c]	0.65[bc]	0.91[a]	0.57[b]	0.75	0.74	*0.04*	*<0.001*	*0.897*	*0.029*
AvBD13	1.00	1.28	0.90	1.02	1.14	0.96	0.95	1.15	*0.08*	*0.199*	*0.157*	*0.580*
AvBD14	1.00	1.32	0.87	1.63	1.16	1.25	0.94[b]	1.47[a]	*0.10*	*0.649*	*0.007*	*0.259*
CATH1	1.00	1.52	0.98	1.47	1.26	1.22	0.99[b]	1.49[a]	*0.07*	*0.794*	*<0.001*	*0.924*
CATH3	1.00	1.30	0.65	1.35	1.15	1.00	0.83[b]	1.32[a]	*0.10*	*0.456*	*0.012*	*0.287*
CATHB1	1.00	1.27	1.41	1.81	1.14[b]	1.61[a]	1.21[b]	1.54[a]	*0.07*	*<0.001*	*0.008*	*0.607*
NK-Lysin	1.00	1.10	1.37	1.59	1.05[b]	1.48[a]	1.19	1.34	*0.06*	*0.001*	*0.182*	*0.615*
LEAP2	1.00[b]	1.39[a]	1.49[a]	1.20[ab]	1.20	1.35	1.25	1.30	*0.09*	*0.366*	*0.760*	*0.045*

[a-c]Means followed by different superscript letters are significantly ($P \leq 0.05$) different by LSD test.
[1]Data represent mean values of 8 birds per treatment.
[2]ACRB: Athens Canadian Random Bred; Cobb: Cobb500; NC: non-challenged; NE: necrotic enteritis challenged.

non-challenged Cobb birds. While a main effect was observed in mRNA abundance for IL-1β, AvBD8, and AvBD12 being higher in ACRB birds, Cobb birds demonstrated higher mRNA abundance of TLR1.2, TLR2.1, CCR6, CCL5, AvBD1, CATHB1, and NK-Lysin in CT.

There was a significant breed × challenge interaction effect on IL-10, AvBD13, NK-Lysin, and LEAP2 mRNA abundance in the spleen. NC-Cobb birds exhibited greater IL-10 ($P < 0.001$) and lower LEAP2 ($P = 0.008$) mRNA abundance compared to other treatments. Moreover, AvBD13 ($P = 0.029$) and NK-Lysin ($P = 0.004$) mRNA abundances were significantly greater in NE-Cobb and NE-ACRB birds, respectively, compared to other treatments. Additionally, a main effect of breed was observed in IL-1β, IL-18, TNFα, TLR2.1, CCR5, CCL20, and NK-Lysin where ACRB birds had higher mRNA abundance than Cobb birds ($P \leq 0.05$). However, Cobb birds showed greater IL-10 ($P = 0.002$) and AvBD8 ($P = 0.003$) mRNA abundance in the spleen compared to ACRB birds. The mRNA levels of AvBD1, AvBD14, and CATH1 were significantly lower ($P \leq 0.05$) in the spleen of the NE-challenged group compared to the NC group while the mRNA abundances of AvBD8 and NK-Lysin were significantly greater.

DISCUSSION

Poultry meat is one of the most important sources of high-quality and affordable animal-derived protein to meet the increasing global demand. To address this need, the poultry industry has implemented intensive breeding programs aimed at achieving rapid growth, improved feed efficiency, and increased muscle mass. However, early selection for performance traits over generations may have inadvertently affected the susceptibility of modern broilers to various metabolic and health

Table 6. Effects of NE challenge on mRNA abundance of cytokines, TLRs, chemokines, and HDPs in the spleen.[1]

	Treatments[2]									Probabilities		
	ACRB		Cobb		Breed		Challenge			*P*-value		
Item	NC	NE	NC	NE	ACRB	Cobb	NC	NE	SEM	*Breed*	*Challenge*	*B* × *C*
Cytokines												
IL-1β	1.00	0.84	0.32	0.33	0.92[a]	0.33[b]	0.66	0.59	*0.09*	*<0.001*	*0.514*	*0.503*
IL-10	1.00[c]	1.59[b]	2.00[a]	1.30[bc]	1.29[b]	1.65[a]	1.50	1.44	*0.08*	*0.002*	*0.600*	*<0.001*
IL-18	1.00	1.04	0.44	0.51	1.02[a]	0.48[b]	0.72	0.78	*0.08*	*<0.001*	*0.628*	*0.921*
TNFα	1.00	0.97	0.70	0.65	0.99[a]	0.67[b]	0.85	0.81	*0.04*	*<0.001*	*0.623*	*0.871*
Toll-like receptors												
TLR 1.2	1.00	1.07	1.21	1.10	1.04	1.16	1.11	1.09	*0.06*	*0.324*	*0.860*	*0.484*
TLR 2.1	1.00	0.87	0.62	0.75	0.94[a]	0.69[b]	0.81	0.81	*0.06*	*0.014*	*0.982*	*0.197*
Chemokines												
CCR5	1.00	0.93	0.69	0.52	0.97[a]	0.61[b]	0.85	0.73	*0.05*	*<0.001*	*0.178*	*0.557*
CCL5	1.00	0.99	1.39	1.02	1.00	1.21	1.20	1.01	*0.06*	*0.080*	*0.116*	*0.136*
CCL20	1.00	0.99	0.82	0.63	1.00[a]	0.73[b]	0.91	0.81	*0.05*	*0.002*	*0.242*	*0.270*
Host Defense Peptides												
AvBD1	1.00	0.34	1.07	0.73	0.67	0.90	1.04[a]	0.54[b]	*0.10*	*0.240*	*0.012*	*0.419*
AvBD8	1.00	2.90	2.03	4.36	1.95[b]	3.20[a]	1.52[b]	3.63[a]	*0.30*	*0.003*	*<0.001*	*0.581*
AvBD10	1.00	4.36	0.66	0.99	2.68	0.83	0.83	2.68	*0.62*	*0.076*	*0.078*	*0.144*
AvBD12	1.00	1.66	1.65	1.50	1.33	1.57	1.32	1.58	*0.19*	*0.499*	*0.470*	*0.253*
AvBD13	1.00[b]	0.96[b]	0.88[b]	1.41[a]	0.98	1.15	0.95	1.19	*0.07*	*0.193*	*0.055*	*0.029*
AvBD14	1.00	0.58	1.00	0.54	0.79	0.77	1.00[a]	0.56[b]	*0.06*	*0.864*	*<0.001*	*0.866*
CATH1	1.00	0.62	0.97	0.83	0.81	0.90	0.99[a]	0.72[b]	*0.05*	*0.368*	*0.011*	*0.236*
CATH3	1.00	0.76	0.98	0.83	0.88	0.91	0.99	0.79	*0.06*	*0.806*	*0.078*	*0.699*
CATHB1	1.00	0.97	1.02	1.21	0.99	1.12	1.01	1.09	*0.07*	*0.391*	*0.577*	*0.460*
NK-Lysin	1.00[c]	2.62[a]	1.10[bc]	1.59[b]	1.81[a]	1.35[b]	1.05[b]	2.11[a]	*0.13*	*0.018*	*<0.001*	*0.004*
LEAP2	1.00[a]	0.98[a]	0.77[b]	1.11[a]	1.01	0.94	0.91	1.04	*0.04*	*0.337*	*0.063*	*0.008*

[a-c]Means followed by different superscript letters are significantly ($P \leq 0.05$) different by LSD test.
[1]Data represent mean values of 8 birds per treatment.
[2]ACRB: Athens Canadian Random Bred; Cobb: Cobb500; NC: non-challenged; NE: necrotic enteritis challenged.

challenges (Cheema et al., 2003; Olkowski et al., 2008; Siegel et al., 2019; Zou et al., 2020). Consequently, the industry's current perspective is to maintain poultry health and welfare to minimize economic losses while maximizing their potential growth rates. Therefore, comprehending any indirect outcomes of selection on disease susceptibility and immune competency of modern broilers against common pathogens or stressors is essential for devising breed-specific nutritional and managerial programs to mitigate health and disease challenges. In this context, the current study evaluated intestinal NE lesions and the mRNA abundance of cytokines, chemokines, TLRs, and HDPs in CT and spleen tissues of the ACRB heritage breed and the modern Cobb broiler under normal conditions or during a NE challenge.

Cobb birds showed better growth performance compared to ACRB birds during both the pre- and post-challenge period. Both ACRB and Cobb birds exhibited lower BWG and greater FCR under NE challenge conditions. Such reduced performance is an expected consequence of the challenge model as previously reported where the modern and heritage lines are compared under disease challenge conditions (Aggrey et al., 2019). However, it is not scientifically accurate to make direct performance comparisons between modern broilers and heritage lines due to the inherent genetic potential of modern broilers for higher productivity. Therefore, evaluating relative growth performance provides more direct information to understand the impact of NE in modern and heritage lines. RBWG and RFI decreased and RFCR was increased in both genetic lines in response to NE challenge. However, ACRB birds exhibited a greater reduction in RBWG (52.7% vs 64.7%) and a higher RFCR (181% vs 128%) compared to Cobb birds during d 14-21. In agreement with these results, a previous study reported a similar reduction in performance incidents of ACRB and modern broiler lines under *Eimeria* challenge (Aggrey et al., 2019). However, differences in body mass, feed efficiency, and metabolism of modern and heritage lines should be considered when comparing relative performance. Mortality is also an important parameter that directly impacts the flock profitability. Modern broilers have higher mortality rates compared to heritage lines under standard practices (Collins et al., 2014) likely due to their higher metabolic rate, susceptibility to metabolic disorders, and less efficient immune responses (Hartcher and Lum, 2020; Aylward et al., 2022). In the current study, even though there were no significant differences in lesion scores between challenged ACRB and Cobb birds, ACRB birds exhibited numerically lower mortality compared to Cobb birds under NE challenge. Low mortality in ACRB birds could be related to their lower metabolic rate, more consistent immune profile in response to immune stimulation (Aylward et al., 2022), or enriched predicted functions involved in the immune system and endocrine system in ACRB microbiota (Emami et al., 2022). However, to our knowledge, published data on the response of heritage and modern broilers against intestinal pathogens are limited.

The efficiency of innate and adaptive immune responses in modern broilers has long been debated by industry professionals and researchers. This concern arises from the higher metabolic activity rate and selective breeding practices, which could have impacted the

inflammatory status of the host in the present study. The cells involved in the innate immune response are less energetically demanding compared to those involved in the adaptive immune response (Klasing, 2007). Modern broilers have been selectively bred for rapid weight gain, potentially diverting resources away from developing a robust adaptive immune system (Aylward et al., 2022). This difference in energy requirements may contribute to variations in immune responses observed between modern and heritage broiler birds. Modern broilers may exhibit lower adaptive immune response compared to their heritage counterparts (Qureshi and Havenstein, 1994; Cheema et al., 2003). However, there is limited information regarding the impact of NE challenge on the innate immune response in modern and heritage broilers. According to this study, the mRNA abundance of cytokines and chemokines showed differential patterns in the CT and, to a lesser extent, the spleen, suggesting biological differences between ACRB and Cobb broilers during a NE challenge. Challenged Cobb birds exhibited higher proinflammatory cytokine (IL-18 and TNFα) and chemokine (CCR5, CCR6, and CCL20) responses in CT, which corresponds with higher TLR1.2 and TLR2.1 mRNA levels compared to NE-challenged ACRB birds. However, the mRNA abundance of cytokines and chemokines in the spleen did not exhibit a similar response to that observed in CT. This difference could be due to the nature of the *E. maxima* and *C. perfringens* involved in this NE model, which primarily causes tissue damage in the small intestine (Emami and Dalloul, 2021). It is known that TLR activation modulates inflammatory response through the activation of downstream signaling pathways, such as NF-κB and MAPK pathways, leading to the production of proinflammatory cytokines and chemokines (Takeuchi and Akira, 2010; Paul et al., 2013). Aylward et al. (2022) observed a significant increase in mRNA levels of potent inflammatory cytokines in modern broilers compared to ACRB broilers in response to intra-abdominal CpG oligonucleotide inoculation at d 3 post-hatch. This increase was accompanied by the activation of PI3K-Akt, JAK-STAT, and NF-kB signaling pathways, as determined by kinome array analysis of blood samples. The authors further reported that ACRB broilers had a more consistent immune response to CpG throughout the 34-d period of the study. Overall, modern broilers seem to exhibit a heightened proinflammatory response compared to heritage birds, likely due to selective breeding that may have favored production parameters over adaptive immune system development. More comprehensive studies utilizing RNA-seq and other global screening tools are warranted to extend our understanding of the genetic and molecular bases for these observed differences in immune responses between modern broilers and heritage lines.

HDPs play a crucial role in the first-line defense against antigens, possessing antimicrobial and immunomodulatory characteristics (van Dijk et al., 2008). Among avian species, β-defensins and cathelicidins represent 2 primary families of HDPs with potent antimicrobial properties, synthesized primarily in host phagocytic and mucosal epithelial cells in response to microbial infection (Zhang and Sunkara, 2014; van Dijk et al., 2023). Additionally, TLRs and proinflammatory cytokines, such as IL-1β, IL-6, and TNFα, are known to be potent inducers and up-regulators of HDPs (Hong et al., 2012). In the present study, challenged Cobb birds exhibited greater mRNA levels of AvBD1 in the CT and AvBD13 in the spleen, while showing lower mRNA abundance of NK-Lysin in the spleen compared to challenged ACRB birds. Regardless of broiler strain, the CT exhibited increased levels of AvBD14, CATH1, CATH3, and CATHB1, while the spleen showed elevated mRNA levels of AvBD8 and NK-Lysin in response to NE. Our findings are consistent with previous reports of a positive association between several AvBDs and CATHs and an elevated inflammatory response following pathogen infections (Akbari et al., 2008; Hong et al., 2012; Tian et al., 2016). However, AvBD1, AvBD14, and CATH1 mRNA levels were lower in spleen tissues of NE-challenged birds. Differential expression patterns observed in HDPs were also evident in previous studies. This result suggests that AvBDs and CATHs may show differential expression patterns in response to NE challenge. Additionally, observed differences between spleen and CT could be due to the tissue-specific expression patterns (Zhang and Sunkara, 2014; Garcia et al., 2021). Overall, the AvBDs and CATHs response to NE challenge was not consistent and requires further examination to elucidate the underlying mechanisms and potential implications for host defense.

LEAP2, a cationic antimicrobial peptide, plays a crucial role within the innate immune system, serving as a vital defense against infections (Townes et al., 2004; Casterlow et al., 2011). *Eimeria* infection is known to downregulate LEAP2 mRNA abundance in different sections of the small intestine in modern broilers (Su et al., 2014; Su et al., 2017). Additionally, as reflected by lesion scores, a more severe *Eimeria* infection leads to a greater downregulation of LEAP2 in the jejunum (Casterlow et al., 2011). Despite similar LEAP2 mRNA abundance between challenged and non-challenged Cobb broilers, we observed a numerical decrease in CT LEAP2 mRNA abundance of challenged Cobb broilers compared with non-challenged Cobb broilers. This difference in LEAP2 mRNA abundance observed in the current study could be attributed to factors such as the severity of the infection or the specific tissue response. The relative reduction in LEAP2 expression is believed to be caused by NE challenge, which could disrupt the innate immune system and increase the host's vulnerability to pathogenic bacterial infection (Yin et al., 2015; Tian et al., 2016). Interestingly, we observed a greater LEAP2 mRNA abundance in challenged ACRB birds compared to non-challenged counterparts. This response could be attributed to more metabolic flexibility in terms of resource allocation between growth and innate immune responses to the NE challenge (Aylward et al., 2022). Therefore, such response may confer enhanced

resistance to infection and lower tissue damage in the challenged ACRB birds.

In conclusion, these results indicate that ACRB birds showed a lower proinflammatory response to the NE challenge possibly due to their lower growth potential. In contrast to Cobb broilers, challenged ACRB birds exhibited greater LEAP2 mRNA abundance compared to non-challenged ACRB birds. This distinction could play a crucial role in regulating inflammation and the immune response to intestinal infections such as NE. Nevertheless, additional research is warranted to explore both innate and adaptive immune responses along with the changes in the microbiota in response to acute/clinical disease models in various broiler breeds.

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DISCLOSURES

The authors declare no conflicts of interest.

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