

## Supplemental materials

**Supplementary Table 1.** Oligonucleotides used for quantitative RT-PCR

cDNA Target	Primer Sequence (5'-3')		Accession no. <sup>1</sup>	Annealing temp (°C)	References
	Forward	Reverse			
28S	GGCGAAGCCAGAGGAAACT	GACGACCGATTTGCACGTC	AH001604	61	(1)
GAPDH	TGTGACTTCAATGGTGACAGC	GCTATATCCAACTCATTGTGCATACC	NM_204305	55	(2)
TATA-BP	TAGCCCGATGATGCCGTAT	GTTCCCTGTGTGCGTTGC	D83127	58	(3)
IFN $\gamma$	GTGAAGAAGGTGAAAGATATCATGGA	GCTTTGCGCTGGATTCTCA	Y07922	59	(4)
iNOS	TTGGAAACCAAAGTGTGTAATATCTTG	CCCTGGCCATGCGTACAT	U46504	59	(1)
IL-1 $\beta$	GCTCTACATGTCGTGTGTGATGAG	TGTCGATGTCCCGCATGA	AJ245728	59	(4)
IL-2	TTGGAAAATATCAAGAACAAGATTCATC	TCCCAGGTAACACTGCAGAGTTT	AJ009800	59	(4)
IL-6	GCTCGCCGGCTTCGA	GGTAGGCTGAAAGGCGAACAG	AJ250838	59	(1)
IL-10	CATGCTGCTGGGCCTGAA	CGTCTCCTTGATCTGCTTGATG	AJ621614	59	(5)
IL-13	CACCCAGGGCATCCAGAA	TCCGCAGGTAGATCTCAT	AJ621250	55	(6)
Muc2	ATGCGATGTTAACACAGGACTC	GTGGAGCACAGCAGACTTTG	JX284122	60	(2)
Muc5ac	TGTGGTTGCTATGAGAATGGA	TTGCCATGGTTTGTGCAT	XM_015286693	60	(2)
Muc13	GCATTCTCAAGCAGAGGTG	CTCAGGCTGCCGTGATATTT	XM_003641585	60	(2)

<sup>1</sup>Genomic DNA sequence (NCBI GenBank)

## Supplemental materials and methods

Influence of dietary *N*-acetylcysteine supplementation: Following quantification of the impact of *E. tenella* infection on *C. jejuni* load in the caeca, liver, and spleen, the mucus-thinning food supplement N-acetylcysteine (NAC, Sigma-Aldrich) (7, 8) was mixed with the standard diet to investigate association between *Eimeria* induced mucogenesis and *C. jejuni* load. Briefly, groups of eight individually caged Light Sussex chickens were either left unexposed to NAC (Groups 1-3; NAC-) or received 5 g NAC per Kg feed from 13 days of age (Groups 4-6; NAC+ (9)). Co-infection schedules included mock challenge with sterile MH broth at 18 days of age (Groups 1 and 4; E-/C-),  $\sim 10^8$  CFU *C. jejuni* at 18 days of age (Groups 2 and 5; E-/C+) or 4,000 sporulated *E. tenella* oocysts at 13 days of age plus  $\sim 10^8$  CFU *C. jejuni* at 18 days of age (Groups 3 and 6; nE+/C+). All chickens were culled three days post bacterial challenge (21 days of age) and samples collected as described for trial 1. Intestinal sections were collected for periodic acid–Schiff (PAS) staining to assess the influence of NAC supplementation on mucosubstances within the intestinal lumen.

**Supplementary Table 2.** *Campylobacter jejuni* and *Eimeria tenella* dose regimes and viable counts from single and co-infection of chickens fed diets with or without *N*-acetylcysteine (NAC).

Group	NAC	<i>E. tenella</i> (dose; age at dosing)	Log <sub>10</sub> <i>C. jejuni</i> CFU dose (d18)	Log <sub>10</sub> output oocysts/bird	Log <sub>10</sub> CFU/g Day 21 (three days post <i>C. jejuni</i> )		
					Caeca	Liver	Spleen
nE+/C+	-	Wis (4,000; d13)	8.27	6.89 ± 0.08 <sup>a</sup>	7.51 ± 0.68 <sup>b</sup>	2.14 ± 0.54 <sup>b</sup>	2.05 ± 0.43 <sup>b</sup>
nE-/C+	-	None	8.27	nd	6.55 ± 0.22 <sup>a</sup>	3.17 ± 0.11 <sup>a</sup>	2.89 ± 0.25 <sup>a</sup>
nE-/C-	-	None	Mock	nd	nd	nd	nd
nE+/C+	+	Wis (4,000; d13)	8.27	6.91 ± 0.10 <sup>a</sup>	5.91 ± 0.66 <sup>a</sup>	1.32 ± 0.38 <sup>c</sup>	1.69 ± 0.38 <sup>c</sup>
nE-/C+	+	None	8.27	nd	5.82 ± 0.58 <sup>a</sup>	2.49 ± 0.19 <sup>b</sup>	2.30 ± 0.23 <sup>b</sup>
nE-/C-	+	None	Mock	nd	nd	nd	nd

nE = non-attenuated *E. tenella* Wis, C = *C. jejuni*, + = administered, - = not administered, nd = none detected. Averages (± SD, shown in italics) that were significantly different within each trial are identified by a different superscript letter (p < 0.05). Mock = no bacterial control.

## References

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