Expression of nutrient transporters and host defense peptides in *Campylobacter* challenged broilers

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ABSTRACT *Campylobacter* is a bacterium that colonizes the lower gastrointestinal tract of poultry and may influence the intestinal environment to promote its survival. The objective of this study was to characterize the effects of *Campylobacter* challenge on the mRNA abundance of nutrient transporters and host defense peptides (HDP), such as the avian β-defensins (AvBD) and liver expressed antimicrobial peptide 2 (LEAP2). On the day of hatch, broiler chicks were challenged with one of three (10^6, 10^7, 10^8 colony-forming units, cfu) levels of *Campylobacter jejuni*. Quantitative PCR analysis revealed that there were dose-, tissue-, and age-specific changes in gene expression for both nutrient transporters and HDP. Expression of zinc transporter 1 (ZnT1) mRNA increased on d 7 in the duodenum, ileum, and cecum of birds challenged with 10^6 cfu of *C. jejuni*. At d 14, there was upregulation of the amino acid transporter $b^{o,+}$AT mRNA in the duodenum, jejunum, and ileum of birds challenged with 10^6 cfu of *C. jejuni*. Other transporters such as EAAT3, GLUT2, SGLT1, and ZnT1 showed upregulation of mRNA in the ileum of the 10^6 cfu challenged group. There was a delayed response of the HDP to the *C. jejuni* challenge, with only a few HDP changed at d 7 but all HDP changed at d 14. At d 7, there was upregulation of AvBD10 mRNA in the duodenum of the 10^6 cfu challenged group but downregulation of AvBD10 in the ileum and AvBD12 and LEAP2 in the cecum of the 10^8 cfu challenged group. At d 14, there was upregulation of AvBD1, AvBD6, AvBD8, AvBD10, AvBD11, AvBD12, and AvBD13 mRNA in the ileum and cecum of the 10^6 cfu challenged group but not the 10^7 and 10^8 cfu challenged groups compared to control. These results indicated that at a low dose (10^6 cfu) of *C. jejuni*, intestinal cells increased nutrient transporter and AvBD mRNA abundance to try to counter the infection, but that at higher doses the cellular response was suppressed.

Key words: *Campylobacter*, nutrient transporter, host defense peptides, avian β-defensins, LEAP2

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INTRODUCTION

Campylobacter is one of the most common causes of bacterial foodborne illness in the United States (Ghareeb et al., 2012). Commonly found in the gastrointestinal tract of poultry, birds may carry high loads of bacteria without showing any clinical signs of infection (Newell and Fearnley, 2003; Van Deun et al., 2008). However, colonization by *Campylobacter* negatively impacts the production of poultry by affecting the well-being of the birds (Humphrey, 2006; Williams et al., 2013). The majority of broiler flocks are colonized within 2 to 4 wk of age and remain colonized until slaughter, which may lead to contamination of meat during processing (van Gerwe et al., 2009).

The small intestine plays an important role in the absorption of nutrients, which is mediated by a number of nutrient transporters. These transporters are members of the solute carrier (**SLC**) gene family and are responsible for transporting a variety of nutrients such as amino acids, peptides, sugars, and minerals across the brush border or basolateral membranes of the enterocytes lining the intestinal villi (Hediger et al., 2013). At the brush border membrane, the sodium-glucose transporter SGLT1 (SLC5A1) is responsible for the active transport of glucose (Wright, 2013). The excitatory amino acid transporter EAAT3 (SLC1A1) transports anionic amino acids such as glutamate, which is the main energy source for the enterocytes (Brosnan and Brosnan, 2013; Kanai et al., 2013). The amino acid transporter $b^{o,+}$AT (SLC7A9) mediates the uptake of cationic amino acids in exchange for the export of neutral amino acids. At the basolateral membrane, GLUT2 (SLC2A2) facilitates the transport of glucose, fructose, galactose, and mannose (Mueckler and Thorens, 2013). The zinc transporter 1 (ZnT1) (SLC22A18) is responsible for the efflux of zinc out

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of the enterocyte (Tako et al., 2005; Koepsell, 2013). The amino acid transporters CAT2 (SLC7A2) and LAT1 (SLC7A5) facilitate the transport of cationic and neutral amino acids, respectively (Fotiadis et al., 2013). Downregulation of some nutrient transporters in the duodenum, jejunum, and ceca have been reported in *Campylobacter*-infected chickens (Awad et al., 2014).

Avian beta-defensins (**AvBD**) and liver-expressed antimicrobial peptide 2 (**LEAP2**) are two classes of host defense peptides (**HDP**) that belong to the innate immune system (Cuperus et al., 2013). Defensins are cysteine-rich cationic peptides, with the beta-defensins being the sole defensin family found in birds. The expression of HDP is altered during bacterial infections. Expression of AvBD mRNA was upregulated in the gastrointestinal and reproductive tracts of chickens during a *Salmonella* challenge (Yoshimura et al., 2006; Milona et al., 2007; Akbari et al., 2008). LEAP2 mRNA was also found to be upregulated in the intestine of *Salmonella* infected chickens (Townes et al., 2004).

The objective of this study was to profile the mRNA expression of nutrient transporters, AvBD and LEAP2 in the small intestine and ceca of broilers challenged with three doses of *C. jejuni*.

MATERIALS AND METHODS

Campylobacter Challenge and Tissue Sampling

This study was approved by the Southern Plains Research Center Animal Care and Use Committee and was conducted at the Food and Feed Safety Research Unit (USDA Southern Plains Agricultural Research Center, College Station, TX). Day of hatch (**doh**) chicks (Ross 308 cross broilers) were obtained from a local hatchery and transported to the USDA-ARS facility. The paper chick tray liners were collected for *Campylobacter* testing (Musgrove et al., 1997; Byrd et al., 2007). For the challenge, a wild-type strain of *C. jejuni* was used and grown overnight in Bolton's broth (Byrd et al., 1998). The wild-type *C. jejuni* strain was recovered from a market aged chicken (7 to 8 wk of age). This strain was taken as part of an on-farm National Antimicrobial Resistance Monitoring System (**NARMS**) and should meet the standards of a natural strain of *Campylobacter*. The titer of viable *C. jejuni* was determined by dilution in Butterfield's solution and incubation on Campy-Cefex plates (Stern et al., 1992) for 48 h at 42°C in a microaerophilic environment (85% N_2, 10% CO_2, and 5% O_2). The doh chicks were orally gavaged with 0.5 mL of Butterfield's solution containing 10^6, 10^7, or 10^8 cfu *C. jejuni* or gavaged with just 0.5 mL of sterile Butterfield's solution (control). These doses were similar to the doses (10^6 to 10^7 cfu) previously used by Cox et al. (2006) and Stern (2008). Chicks were placed into pens and provided feed and water *ad libitum*. The feed was a non-medicated corn-soybean meal starter diet that was formulated to meet or exceed the National Research Council nutrient requirements for poultry (National Research Council, 1994).

On d 7 and 14 post-challenge, chickens were euthanized by cervical dislocation and samples were collected. Cecal contents were collected and tested for *C. jejuni* by plating on Campy-Cefex plates. One to 2 cm sections of duodenum, jejunum, ileum, and cecum were collected, rinsed in phosphate buffered saline and stored in RNAlater (Qiagen, Germantown, MD). Samples were frozen at −80°C, until shipment to Virginia Tech.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from duodenal, jejunal, ileal, and cecal samples (n = 5) collected on d 7 and 14, using the Direct-zol RNA Miniprep protocol (Zymo Research, Irvine, CA). The RNA concentration for each sample was quantified using a Nanodrop 1000 (Thermo Scientific, Waltham, MA). The nutrient transporter genes analyzed included neutral (LAT1), Na^+-independent ($b^{o,+}AT$), anionic (EAAT3), and cationic (CAT2) amino acid transporters, facilitated (GLUT2) and Na^+-dependent (SGLT1) sugar transporters and the zinc (ZnT1) transporter. The HDP analyzed included LEAP2 and the avian beta-defensins AvBD1, AvBD6, AvBD8, AvBD10, AvBD11, AvBD12, and AvBD13. The cDNA was synthesized from 500 ng of total RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Waltham, MA). Each of the qPCR wells contained 5 μL of Fast SYBR Green Mastermix (Applied Biosystems), 1 μL of forward primer (5 μM), 1 μL of reverse primer (5 μM), 2 μL of diethyl pyrocarbonate treated water, and 1 μL of cDNA (diluted 1:30) and the reactions were run in a 7500 Fast Real-time PCR instrument (Applied Biosystems). The primers for each of the genes plus β-actin are listed in Tables 1 and 2.

Fold change was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) with β-actin serving as the reference gene to calculate ΔCt. For each individual day, the average ΔCt of the control duodenum, jejunum, ileum, and cecum were used as the calibrator to calculate $\Delta\Delta$Ct of the corresponding challenge group. For example, the average ΔCt of the five control samples for duodenum at d 7 was used to calculate the $\Delta\Delta$Ct of the 10^6, 10^7, and 10^8 cfu samples (n = 5) as well as the same control samples for duodenum at d 7, to assess variance of both the control and treatment samples.

Statistical Analysis

Campylobacter recovery levels (cfu/g of cecal contents, n = 6 per treatment) were analyzed by ANOVA using JMP Pro 13 (SAS Institute Inc., Cary, NC) software. The model included the main effect of log10

Table 1. PCR primers for nutrient transporter genes.

Gene	Gene name	Function/Location[a]	Forward/reverse primer	Amplicon (bp)	Accession #
$b^{o,+}AT$	Solute carrier family 7, member 9 (SLC7A9)	Na^+-independent neutral/cystine, cationic amino acid exchanger/BB	CAGTAGTGAATTCTCT GAGTGTGAAGCT/ GCAATGATTGCCACAACTACCA	88	NM_001199133.1
CAT2[b]	Cationic amino acid transporter-2 (SLC7A2)	Transports lysine, arginine and histidine/BL	TGCTCGCGTTCCCAAGA/ GGCCCACAGTTCACCAACAG	67	NM_001199102.1
EAAT3[b]	Excitatory amino acid transporter 3 (SLC1A1)	Transports aspartate, glutamate and cysteine/BB	TGCTGCTTTGGATTCCAGTGT/ AGCATGACTGTAGTGCAGAA GTAATATAT	76	XM_424930.5
LAT1[b]	L type amino acid transporter-1 (SLC7A5)	Transports hydrophobic amino acids/BL	GATTGCAACGGGTGATGTGA/ CCCCACACCCACTTTTGTTT	70	CD217821
GLUT2[b]	Glucose transporter-2 (SLC2A2)	Transports fructose, mannose, galactose, glucose and glucosamine/BL	CACACTATGGGCGCATGCT/ ATTGTCCCTGGAGGTGTTGGTG	67	NM_207178.1
SGLT1[b]	Sodium glucose transporter-1 (SLC5A1)	Transports low concentrations of D-glucose/BB	GCCATGGCCAGGGCTTA/ CAATAACCTGATCTGTGCACCAGTA	71	XM_415247.4
ZnT1[c]	Zinc transporter-1 (SLC22A18)	Efflux of Zn^{2+}/BL	TCCGGGAGTAATGGAAATCTTC/ AATCAGGAACAAACCTATGGGAAA	67	XM_015286677.1
β-actin[b]	Beta-actin	Reference gene/C	GTCCACCGCAAATGCTTCTAA/ TGCGCATTTATGGGTTTTGTT	78	L08165.1

[a]BB = brushborder, BL = basolateral, C = cytosol.
[b]Primer sequence designed by Gilbert et al. (2007).
[c]Primer sequence designed by Paris and Wong (2013).

Table 2. PCR primers for host defense peptide genes.

Gene	Gene name	Forward/reverse primer	Amplicon (bp)	Accession #
AvBD1[a]	Avian β-defensin 1	GAGTGGCTTCTGTGCATTTCTG/ TTGAGCATTTCCCACTGATGAG	62	NM_204993.1
AvBD6[a]	Avian β-defensin 6	GCCCTACTTTTCCAGCCCTATT/ GGCCCAGGAATGCAGACA	63	NM_001001193.1
AvBD8[a]	Avian β-defensin 8	ATGCGCGTACCTAACAACGA/ TGCCCAAAGGCTCTGGTATG	95	NM_001001781.1
AvBD10[a]	Avian β-defensin 10	CAGACCCACTTTTCCCTGACA/ CCCAGCACGGCAGAAATT	64	NM_001001609.2
AvBD11[a]	Avian β-defensin 11	GGTACTGCATCCGTTCCAAAG/ GCATGTTCCAAATGCAGCAA	56	NM_001001779.1
AvBD12[a]	Avian β-defensin 12	TGTAACCACGACAGGGGATTG/ GGGAGTTGGTGACAGAGGTTT	114	NM_001001607.2
AvBD13[a]	Avian β-defensin 13	CAGCTGTGCAGGAACAACCA/ CAGCTCTCCATGTGGAAGCA	59	NM_001001780.1
LEAP2[b]	Liver-expressed antimicrobial peptide 2	TTGCTGGCTTTGGGTTGTG/ GGAGGTTGAGGGCCAAAGTC	66	NM_001001606.1

[a]Primer sequence designed by Su et al. (2017).
[b]Primer sequence designed by Casterlow et al. (2011).

cfu, sorted by day. Significant differences ($P < 0.05$) were further separated using Tukey's test. Chi-Square analysis was performed to determine significant differences between groups in *C. jejuni* recovery levels.

Changes in gene expression of nutrient transporters and HDP (n = 5 per treatment) were analyzed by ANOVA using JMP Pro 13 (SAS Institute Inc.) software. The pooled standard error was derived from ANOVA. The model included the main effect of treatment, sorted by tissue and genes. Significant differences ($P < 0.05$) were further separated using Tukey's test.

RESULTS

Campylobacter Challenge of Broilers

The cecal contents were collected from broilers challenged with 10^6, 10^7, or 10^8 cfu of *C. jejeuni* at d 7 and 14 post-challenge and the titer of *C. jejuni* was determined. At d 7, there were no *C. jejuni* colonies detected in the control chicks, whereas there were greater than 10^6 colonies recovered for each of the three groups challenged with 10^6, 10^7, or 10^8 cfu of *C. jejuni* (Figure 1). There were a greater number of colonies at

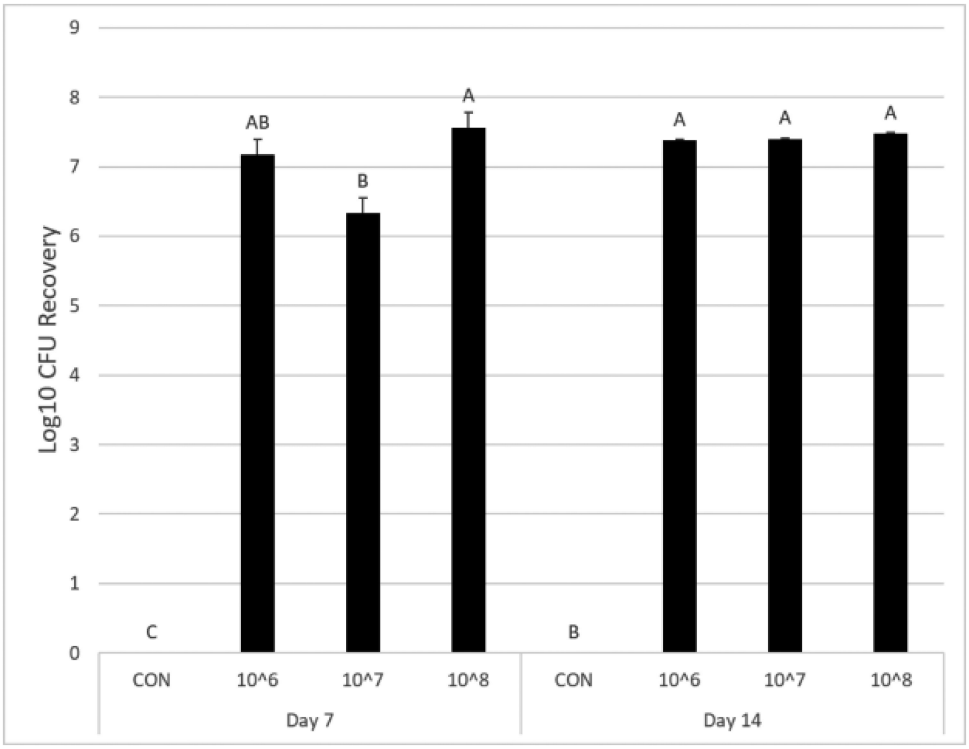

Figure 1. Recovery of *Campylobacter* in the ceca of broilers. Cecal contents were collected from broilers 7 and 14 d post-challenge. The presence of *C. jejuni* was determined by plating serial dilutions on Campy-Cefex plates. CON = Control, $10^{\wedge 6} = 10^6$ cfu, $10^{\wedge 7} = 10^7$ cfu, and $10^{\wedge 8} = 10^8$ cfu of *C. jejuni* per chicken. ^{A,B,C}Means with no common superscript within a day differ significantly ($P < 0.05$).

10^8 than 10^7 cfu. At d 14, there were again no colonies in the control and greater than 10^7 colonies recovered for each of the three *C. jejuni* challenged groups, with no difference among these groups.

Nutrient Transporter Gene Expression

The mRNA expression profiles of seven amino acid, sugar, and mineral transporters following *C. jejuni* challenge were found to be tissue-, age-, and dose-dependent. These transporters included the amino acid transporters EAAT3 and $b^{o,+}$AT and the sugar transporter SGLT1, which are located at the brush border membrane, and the amino acid transporters CAT2 and LAT1, the sugar transporter GLUT2 and the ZnT1, which are located at the basolateral membrane.

Amino Acid Transporter mRNA Abundance—7 d Post-Challenge with C. jejuni There were changes in mRNA abundance for $b^{o,+}$AT, EAAT3, and LAT1, but no changes for CAT2 (Table 3). $b^{o,+}$AT mRNA was upregulated in the duodenum of the 10^7 cfu group compared to the control ($P = 0.01$). EAAT3 mRNA was upregulated in the duodenum of the 10^6 cfu group compared to the 10^8 cfu group ($P = 0.03$), but the 10^6 cfu group was not different from the control. LAT1 mRNA was upregulated in the duodenum of the 10^8 cfu group compared to the control ($P = 0.05$) and upregulated in the jejunum of the 10^7 cfu group compared to the control ($P = 0.03$).

Sugar Transporter mRNA Abundance—7 d Post-Challenge with C. jejuni Changes in mRNA abundance for GLUT2 and SGLT1 are shown in Table 3. GLUT2 mRNA was upregulated in the ileum of the 10^7 cfu group compared to the control ($P = 0.01$). In the cecum, GLUT2 mRNA was upregulated in the 10^6 cfu group compared to the 10^8 cfu group ($P = 0.02$), but the 10^6 cfu group was not different from the control. SGLT1 mRNA was downregulated in the duodenum of the 10^6 cfu group compared to the control ($P = 0.02$).

Zinc Transporter mRNA Abundance —7 d Post-Challenge with C. jejuni ZnT1 mRNA was upregulated in the duodenum ($P = 0.001$) and ileum ($P = 0.001$) of the 10^6 and 10^7 cfu groups compared to the control and upregulated in the cecum ($P = 0.01$) of the 10^6 cfu group compared to the control (Table 3).

Amino Acid Transporter mRNA Abundance —14 d Post-Challenge with C. jejuni There were changes in mRNA abundance for $b^{o,+}$AT, EAAT3, and CAT2, but no changes for LAT1 (Table 4). $b^{o,+}$AT mRNA was upregulated in the duodenum ($P = 0.02$), jejunum ($P = 0.001$), and ileum ($P = <0.001$) of the 10^6 cfu group compared to the 10^7 and 10^8 cfu groups, but only in the jejunum and ileum was the 10^6 cfu group greater than control. EAAT3 mRNA was upregulated in the jejunum of the 10^6 cfu group compared to the 10^8 cfu group ($P = 0.03$), but the 10^6 cfu group was not different from the control. In the ileum, EAAT3 mRNA was upregulated in the 10^6 cfu group compared to the

Table 3. Fold change of nutrient and sugar transporter mRNA in *Campylobacter jejuni* challenged (10^6, 10^7, and 10^8 cfu) and non-challenged (CON) broilers at d 7 post-challenge.

Tissue	Treatment	$b^{o,+}AT$	EAAT3	CAT2	LAT1	GLUT2	SGLT1	ZnT1
Duodenum	CON	1.02^{B}	$1.04^{A,B}$	1.10	1.08^{B}	1.13	1.02^{A}	1.12^{B}
	10^6 cfu	$1.47^{A,B}$	1.52^{A}	1.56	$2.78^{A,B}$	1.38	0.56^{B}	4.46^{A}
	10^7 cfu	1.56^{A}	$0.96^{A,B}$	2.07	$2.55^{A,B}$	1.25	$0.80^{A,B}$	4.29^{A}
	10^8 cfu	$1.16^{A,B}$	0.79^{B}	2.15	3.46^{A}	1.21	$0.62^{A,B}$	2.01^{B}
Pooled SEM		0.11	0.16	0.36	0.56	0.33	0.10	0.55
P-value		0.01	0.03	0.18	0.05	0.96	0.02	0.001
Jejunum	CON	1.05	1.09	1.07	1.09^{B}	1.04	1.12	1.16
	10^6 cfu	1.19	1.25	1.32	$1.74^{A,B}$	1.17	0.73	3.14
	10^7 cfu	1.17	1.04	2.39	3.21^{A}	1.11	0.63	3.31
	10^8 cfu	1.14	1.06	1.93	$2.46^{A,B}$	0.87	1.05	2.27
Pooled SEM		0.17	0.17	0.36	0.47	0.16	0.15	0.56
P-value		0.93	0.80	0.07	0.03	0.57	0.08	0.053
Ileum	CON	0.80	1.10	1.06	1.10	1.07^{B}	1.21	1.13^{B}
	10^6 cfu	1.26	1.72	0.81	1.72	$2.48^{A,B}$	0.76	5.11^{A}
	10^7 cfu	1.46	1.33	1.00	1.61	2.83^{A}	0.99	4.76^{A}
	10^8 cfu	1.19	1.09	0.79	1.33	$1.43^{A,B}$	0.73	$3.6^{A,B}$
Pooled SEM		0.19	0.29	0.18	0.24	0.38	0.27	0.78
P-value		0.13	0.39	0.65	0.23	0.01	0.56	0.001
Cecum	CON	1.02	1.17	1.04	1.05	$1.07^{A,B}$	1.19	1.05^{B}
	10^6 cfu	0.78	0.58	0.71	2.61	2.08^{A}	0.49	4.07^{A}
	10^7 cfu	0.70	0.61	2.87	2.49	$0.95^{A,B}$	0.69	$2.62^{A,B}$
	10^8 cfu	1.34	0.77	1.26	1.41	0.37^{B}	1.32	$2.32^{A,B}$
Pooled SEM		0.26	0.23	0.56	0.52	0.35	0.29	0.52
P-value		0.34	0.29	0.06	0.13	0.02	0.12	0.01

Fold change was determined using the $2^{-\Delta\Delta Ct}$ method.

^{A,B}Means with no common superscript within a gene and a tissue differ significantly ($P < 0.05$).

$b^{o,+}AT$ = Na^+ -independent neutral amino acid transporter; EAAT3 = Excitatory Amino Acid Transporter 3; CAT2 = Cationic amino acid transporter-2; LAT1 = L-type amino acid transporter; GLUT2 = Glucose Transporter 2; SGLT1 = Sodium glucose transporter-1; ZnT1 = Zinc Transporter 1.

control ($P = 0.03$). CAT2 mRNA was upregulated in the duodenum of the 10^7 cfu group compared to the control ($P = 0.03$).

Sugar Transporter mRNA Abundance —14 d Post-Challenge with C. jejuni Changes in mRNA abundance for GLUT2 and SGLT1 are shown in Table 4. GLUT2 mRNA was upregulated in the jejunum ($P = 0.004$) and cecum ($P = 0.02$) of the 10^6 cfu group compared to the 10^8 cfu group, but the 10^6 cfu group was not different from the control. In the ileum, GLUT2 mRNA was upregulated in the 10^6 cfu group compared to the control and the 10^7 and 10^8 cfu groups ($P = 0.01$). SGLT1 mRNA was upregulated in the ileum of the 10^6 and 10^8 cfu groups compared to the control ($P = 0.01$).

Zinc Transporter mRNA Abundance—14 d Post-Challenge with C. jejuni ZnT1 mRNA was upregulated in the ileum ($P = 0.04$) of the 10^6 cfu group compared to the control (Table 4). There was no change in ZnT1 mRNA in the duodenum, jejunum, and cecum.

HDP Gene Expression

The mRNA expression profiles of eight HDP (AvBD and LEAP2) following *C. jejuni* challenge were also found to be tissue-, age-, and dose-dependent, with a greater number of changes at d 14 than d 7 post-challenge.

AvBD mRNA Abundance—7 d Post-Challenge with C. jejuni There were changes in mRNA abundance for AvBD10, AvBD12, and AvBD13, but no changes for AvBD1, AvBD6, AvBD8, and AvBD11 (Table 5). For AvBD11, ANOVA revealed a significant difference ($P = 0.04$), but there were no differences by Tukey's test. AvBD10 mRNA was upregulated in the duodenum of the 10^6 cfu group compared to the control ($P = 0.02$). In the ileum, AvBD10 mRNA was downregulated in the 10^7 and 10^8 cfu groups compared to the control ($P = 0.02$), whereas in the cecum AvBD10 mRNA was upregulated in the 10^6 cfu group compared to the 10^8 cfu group ($P = 0.02$), but the 10^6 cfu group was not different from the control. AvBD12 mRNA was downregulated in the cecum of the 10^8 cfu group compared to the control ($P = 0.03$). AvBD13 mRNA was upregulated in the cecum of the 10^6 cfu group compared to the 10^7 and 10^8 cfu groups ($P = 0.01$), but the 10^6 cfu group was not different from the control.

LEAP2 mRNA Abundance—7 d Post-Challenge with C. jejuni In the cecum, LEAP2 mRNA was downregulated in the 10^7 and 10^8 cfu groups compared to the control ($P = 0.01$; Table 6). There was no change in LEAP2 mRNA in the duodenum, jejunum, or ileum.

AvBD mRNA Abundance—14 d Post-Challenge with C. jejuni There were changes in mRNA abundance for all AvBD (Table 6). AvBD1 mRNA was upregulated in the duodenum of the 10^6 and 10^7 cfu groups compared to the 10^8 group ($P = 0.01$), but both the 10^6

Table 4. Fold change of nutrient and sugar transporter mRNA in *Campylobacter jejuni* challenged (10^6, 10^7, and 10^8 cfu) and non-challenged (CON) broilers at d 14 post-challenge.

Tissue	Treatment	$b^{o,+}AT$	EAAT3	CAT2	LAT1	GLUT2	SGLT1	ZnT1
Duodenum	CON	$1.03^{A,B}$	1.08	1.02^{B}	1.03	1.06	1.05	1.04
	10^6 cfu	1.81^{A}	1.31	$1.36^{A,B}$	1.43	1.57	1.21	0.97
	10^7 cfu	0.82^{B}	0.95	1.85^{A}	1.56	0.74	1.38	0.93
	10^8 cfu	0.97^{B}	0.66	$1.59^{A,B}$	1.65	0.55	1.68	1.44
Pooled SEM		0.21	0.16	0.18	0.25	0.26	0.24	0.16
P-value		0.02	0.07	0.03	0.34	0.07	0.33	0.17
Jejunum	CON	1.04^{B}	$1.05^{A,B}$	1.01	1.01	$1.03^{A,B}$	1.02	1.09
	10^6 cfu	2.07^{A}	1.22^{A}	0.96	1.15	1.46^{A}	1.74	1.65
	10^7 cfu	1.17^{B}	$0.89^{A,B}$	0.97	0.98	$1.07^{A,B}$	1.62	1.34
	10^8 cfu	1.16^{B}	0.59^{B}	1.54	1.27	0.65^{B}	1.50	1.50
Pooled SEM		0.16	0.14	0.19	0.19	0.13	0.25	0.28
P-value		0.001	0.03	0.13	0.67	0.004	0.24	0.53
Ileum	CON	1.04^{B}	1.09^{B}	1.11	1.05	1.05^{B}	1.08^{B}	1.07^{B}
	10^6 cfu	3.39^{A}	2.29^{A}	1.33	1.19	2.66^{A}	2.71^{A}	2.42^{A}
	10^7 cfu	1.26^{B}	$1.45^{A,B}$	1.26	1.21	0.99^{B}	$2.46^{A,B}$	$2.09^{A,B}$
	10^8 cfu	1.54^{B}	$1.79^{A,B}$	1.51	1.34	0.77^{B}	2.97^{A}	$2.07^{A,B}$
Pooled SEM		0.25	0.26	0.19	0.13	0.34	0.38	0.31
P-value		<0.001	0.03	0.53	0.49	0.01	0.01	0.04
Cecum	CON	1.08	1.06	1.08	1.01	$1.09^{A,B}$	1.13	1.11
	10^6 cfu	1.43	1.46	0.75	1.32	1.30^{A}	2.34	1.72
	10^7 cfu	1.16	1.48	0.91	0.94	$0.53^{A,B}$	2.44	1.64
	10^8 cfu	0.98	0.99	0.96	1.22	0.48^{B}	2.30	2.53
Pooled SEM		0.19	0.2	0.13	0.16	0.20	0.50	0.40
P-value		0.39	0.24	0.41	0.32	0.02	0.24	0.14

Fold change was determined using the $2^{-\Delta\Delta Ct}$ method.

^{A,B}Means with no common superscript within a gene and a tissue differ significantly ($P < 0.05$).

$b^{o,+}AT$ = Na^+ -independent neutral amino acid transporter; EAAT3 = Excitatory Amino Acid Transporter 3; CAT2 = Cationic amino acid transporter-2; LAT1 = L-type amino acid transporter; GLUT2 = Glucose Transporter 2; SGLT1 = Sodium glucose transporter-1; ZnT1 = Zinc Transporter 1.

and 10^7 cfu groups were not different from the control. In the jejunum, AvBD1 mRNA was downregulated in the 10^8 cfu group compared to the control ($P = 0.01$). AvBD1 mRNA was upregulated in the ileum ($P = 0.03$) and cecum ($P = 0.01$) of the 10^6 cfu group compared to the control. AvBD6 mRNA was upregulated in the duodenum of the 10^6 cfu group compared to the 10^8 cfu group ($P = 0.01$), but the 10^6 cfu group was not different from the control. In the jejunum, AvBD6 mRNA was downregulated in the 10^8 cfu group compared to the control ($P = 0.02$). In the ileum and cecum, AvBD6 mRNA was upregulated in the 10^6 cfu group compared to the control and the 10^7 and 10^8 cfu groups ($P = 0.001$). AvBD8 mRNA in the duodenum was downregulated in the 10^8 cfu group compared to the control and the 10^6 and 10^7 cfu groups ($P < 0.001$) and downregulated in the jejunum of the 10^8 cfu group compared to the control ($P = 0.02$). AvBD8 mRNA was upregulated in the ileum ($P = 0.01$) and cecum ($P = 0.003$) of the 10^6 cfu group compared to the control and the 10^7 and 10^8 cfu groups. AvBD10 ($P = 0.02$), AvBD11 ($P = 0.04$), and AvBD13 ($P = 0.02$) mRNA were upregulated in the duodenum of the 10^6 cfu group compared to the 10^8 cfu group, but the 10^6 cfu group was not different from the control. In the ileum, AvBD10 ($P = 0.001$) and AvBD11 ($P = 0.001$) mRNA were upregulated in the 10^6 cfu group compared to the control and the 10^7 and 10^8 cfu groups. In addition, AvBD12 mRNA was upregulated in the 10^6 cfu group compared to the control ($P = 0.02$) and AvBD13 mRNA was upregulated in the 10^6 cfu group compared to the control and 10^7 cfu group ($P = 0.01$). In the cecum, AvBD10 mRNA was upregulated in the 10^6 and 10^8 cfu groups compared to the control ($P < 0.001$), and AvBD11 mRNA was upregulated in all three challenged groups compared to the control ($P < 0.001$). AvBD12 mRNA was upregulated in the 10^6 and 10^8 cfu groups compared to the control ($P = 0.01$), whereas AvBD13 mRNA was upregulated in all challenged groups compared to the control ($P < 0.001$).

LEAP2 mRNA Abundance—14 d Post-Challenge with C. jejuni In the cecum, LEAP2 mRNA was upregulated in the 10^8 cfu group compared to the 10^7 cfu group ($P = 0.02$), but the 10^7 cfu group was not different from the control (Table 6). There was no change in LEAP2 mRNA in the duodenum, jejunum, or ileum.

DISCUSSION

Although *C. jejuni* is generally considered a commensal bacterium in poultry, its presence in the gastrointestinal tract still influenced expression of intestinal genes. In our study, there was the upregulation of selected nutrient transporters in the *C. jejuni* challenged groups compared to the control. This was in contrast to the downregulation of sugar (SGLT1 and GLUT2) and amino acid/peptide transporters (CAT2,

Table 5. Fold change of avian β-defensin and LEAP2 mRNA in *Campylobacter jejuni* challenged (10^6, 10^7, and 10^8 cfu) and non-challenged (CON) broilers at d 7 post-challenge.

Tissue	Treatment	AvBD1	AvBD6	AvBD8	AvBD10	AvBD11	AvBD12	AvBD13	LEAP2
Duodenum	CON	1.08	1.13	1.19	1.08^{B}	1.17	1.13	1.22	1.03
	10^6 cfu	1.19	1.84	1.16	2.03^{A}	1.82	1.54	1.19	0.92
	10^7 cfu	1.59	1.63	1.05	$1.18^{A,B}$	1.33	0.96	0.66	0.73
	10^8 cfu	1.25	1.42	0.96	$1.64^{A,B}$	1.72	1.12	0.88	0.44
Pooled SEM		0.56	0.52	0.32	0.22	0.38	0.19	0.22	0.18
P-value		0.93	0.80	0.95	0.02	0.59	0.21	0.25	0.16
Jejunum	CON	1.13	1.19	1.20	1.31	1.19	1.14	1.28	1.04
	10^6 cfu	1.21	0.75	0.94	1.32	1.84	1.41	1.20	0.75
	10^7 cfu	1.28	0.58	1.01	0.58	0.88	0.64	0.55	0.69
	10^8 cfu	0.62	0.39	0.60	0.47	1.98	0.59	0.46	0.52
Pooled SEM		0.39	0.21	0.33	0.29	0.52	0.23	0.25	0.13
P-value		0.64	0.07	0.51	0.051	0.11	0.40	0.07	0.06
Ileum	CON	1.10	1.40	1.24	1.13^{A}	1.03	1.03	1.14	1.01
	10^6 cfu	0.80	1.28	0.81	$0.59^{A,B}$	0.64	0.95	0.87	1.36
	10^7 cfu	0.77	0.77	0.76	0.42^{B}	0.43	0.50	0.44	1.33
	10^8 cfu	0.66	0.70	0.54	0.49^{B}	1.00	0.68	0.61	0.44
Pooled SEM		0.17	0.39	0.24	0.15	0.15	0.16	0.20	0.25
P-value		0.32	0.51	0.24	0.02	0.04*	0.11	0.11	0.07
Cecum	CON	1.05	0.76	1.03	$1.10^{A,B}$	1.30	1.12^{A}	$1.11^{A,B}$	1.18^{A}
	10^6 cfu	1.10	0.84	2.29	1.19^{A}	1.06	$0.85^{A,B}$	1.55^{A}	$0.71^{A,B}$
	10^7 cfu	1.93	1.81	1.56	$0.71^{A,B}$	0.60	$0.58^{A,B}$	0.77^{B}	0.34^{B}
	10^8 cfu	0.66	0.49	0.53	0.40^{B}	0.38	0.39^{B}	0.53^{B}	0.19^{B}
Pooled SEM		0.47	0.38	0.50	0.18	0.24	0.16	0.18	0.19
P-value		0.30	0.11	0.12	0.02	0.06	0.03	0.01	0.01

Fold change was determined using the $2^{-\Delta\Delta Ct}$ method.

^{A,B}Means with no common superscript within a gene and a tissue differ significantly ($P < 0.05$).

AvBD1 = Avian β-defensin 1; AvBD6 = Avian β-defensin 6; AvBD8 = Avian β-defensin defensin 8; AvBD10 = Avian β-defensin 10; AvBD11 = Avian β-defensin 11; AvBD12 = Avian β-defensin 12; AvBD13 = Avian β-defensin 13; LEAP2 = Liver-Expressed Antimicrobial Peptide 2.

*indicates significance by ANOVA but not by Tukey's.

PepT1, EAAT3, and y^+LAT2) in the intestine of 14 d old specific pathogen free (SPF) chickens challenged with *C. jejuni* (10^8 cfu/bird) reported by Awad et al. (2014). Although we examined a different set of genes, there were four genes (i.e., SGLT1, GLUT2, CAT2, and EAAT3) that were in common between the two studies. Awad et al. (2014) reported downregulation of SGLT1 and PepT1 mRNA in duodenum, jejunum, and cecum, downregulation of GLUT2 and CAT2 mRNA in the jejunum and downregulation of y^+LAT2 mRNA in the duodenum at d 14 post-challenge. For EAAT3 mRNA, there was upregulation in the duodenum and downregulation in the jejunum. In contrast, we observed the downregulation of SGLT1 mRNA at d 7 post-challenge in the duodenum of only the 10^6 cfu group, but upregulation at d 14 in the ileum of the 10^6 and 10^8 cfu groups. GLUT2 and EAAT3 mRNA, as well as ZnT1 and GLUT2 mRNA, were upregulated in the ileum of the 10^6 cfu group at d 14. This difference in gene expression is likely due to the dose of *C. jejuni* used. We observed upregulation using 10^6 cfu *C. jejuni* per chick, whereas Awad et al. (2014) observed downregulation using 10^8 cfu *C. jejuni* per chick. They concluded that the downregulation of glucose and amino acid transporters would result in an accumulation of nutrients in the intestinal lumen that may favor *C. jejuni* replication and colonization. Our results showing upregulation at the lower dose (10^6 cfu) would suggest that at this dose, the intestinal epithelial cells may be increasing the uptake of nutrients to try to counter the infection, whereas at the same time depleting nutrients in the lumen available for *C. jejuni* replication.

Changes in mRNA abundance of nutrient transporters may result in changes in nutrient influx or efflux. Upregulation of mRNA for $b^{o,+}$AT, EAAT3, and SGLT1 could lead to increased uptake of important nutrients such as lysine, glutamate, and glucose, respectively. When higher doses of *C. jejuni* were used (10^7 or 10^8 cfu), however, upregulation of $b^{o,+}$AT and EAAT3 mRNA was not observed, suggesting that the higher dose may have overwhelmed the ability of the enterocytes to increase nutrient uptake. At the basolateral membrane, there was an increase in ZnT1 and GLUT2 mRNA abundance in the 10^6 or 10^7 cfu groups that was not observed in the 10^8 cfu group. This could result in an increase in the export of zinc and sugars into the blood that may be utilized by other tissues. This again indicates that at a low or medium *C. jejuni* dose, the enterocytes may have enhanced ability to transport some nutrients into the blood but at a high dose transport was suppressed.

In addition to challenge dose, the difference in gene expression results may be due to the type of chicken and *C. jejuni* strains used for the challenge, as well as the timeline for the challenge protocol. The *C. jejuni* strain that was used in this study was a natural,

Table 6. Fold change of avian β-defensin and LEAP2 mRNA in *Campylobacter jejuni* challenged (10^6, 10^7, and 10^8 cfu) and non-challenged (CON) broilers at d 14 post-challenge.

Tissue	Treatment	AvBD1	AvBD6	AvBD8	AvBD10	AvBD11	AvBD12	AvBD13	LEAP2
Duodenum	CON	$1.16^{A,B}$	$1.12^{A,B}$	1.05^{A}	$1.05^{A,B}$	$1.02^{A,B}$	1.03	$1.09^{A,B}$	1.04
	10^6 cfu	1.29^{A}	1.54^{A}	1.51^{A}	1.64^{A}	1.33^{A}	1.35	1.37^{A}	0.87
	10^7 cfu	1.30^{A}	$1.10^{A,B}$	1.01^{A}	$1.02^{A,B}$	$1.17^{A,B}$	1.78	$1.21^{A,B}$	0.64
	10^8 cfu	0.34^{B}	0.37^{B}	0.38^{B}	0.56^{B}	0.49^{B}	0.5	0.55^{B}	1.30
Pooled SEM		0.19	0.20	0.13	0.20	0.19	0.41	0.17	0.22
P-value		0.01	0.01	<0.001	0.02	0.04	0.20	0.02	0.25
Jejunum	CON	1.06^{A}	1.09^{A}	1.10^{A}	1.01	1.04	1.06	1.01	1.06
	10^6 cfu	$0.66^{A,B}$	$0.72^{A,B}$	$0.87^{A,B}$	1.06	0.96	0.75	1.23	1.09
	10^7 cfu	$0.74^{A,B}$	$0.67^{A,B}$	$0.75^{A,B}$	0.80	0.60	0.90	1.19	1.09
	10^8 cfu	0.28^{B}	0.32^{B}	0.35^{B}	0.48	0.40	0.32	0.47	1.26
Pooled SEM		0.14	0.15	0.15	0.15	0.17	0.20	0.26	0.26
P-value		0.01	0.02	0.02	0.051	0.050	0.09	0.18	0.95
Ileum	CON	1.13^{B}	1.16^{B}	1.17^{B}	1.06^{B}	1.11^{B}	1.09^{B}	1.13^{B}	1.09
	10^6 cfu	3.18^{A}	3.43^{A}	3.61^{A}	3.82^{A}	3.92^{A}	3.38^{A}	4.11^{A}	2.18
	10^7 cfu	$1.47^{A,B}$	1.37^{B}	1.39^{B}	1.57^{B}	1.59^{B}	$1.55^{A,B}$	1.36^{B}	2.53
	10^8 cfu	$1.24^{A,B}$	1.29^{B}	1.49^{B}	1.87^{B}	1.94^{B}	$1.99^{A,B}$	$2.21^{A,B}$	0.89
Pooled SEM		0.50	0.37	0.47	0.41	0.41	0.47	0.56	0.48
P-value		0.03	0.001	0.01	0.001	0.001	0.02	0.01	0.08
Cecum	CON	1.16^{B}	1.02^{B}	1.20^{B}	1.08^{C}	1.08^{C}	1.07^{B}	1.09^{B}	$1.14^{A,B}$
	10^6 cfu	4.45^{A}	4.44^{A}	4.91^{A}	7.02^{A}	5.66^{A}	4.88^{A}	5.94^{A}	$3.35^{A,B}$
	10^7 cfu	$3.30^{A,B}$	1.89^{B}	2.28^{B}	$3.62^{B,C}$	$4.09^{A,B}$	$3.82^{A,B}$	3.9^{A}	1.01^{B}
	10^8 cfu	$2.20^{A,B}$	2.01^{B}	2.34^{B}	$5.44^{A,B}$	3.36^{B}	4.18^{A}	4.28^{A}	3.97^{A}
Pooled SEM		0.63	0.46	0.59	0.75	0.56	0.75	0.57	0.72
P-value		0.01	0.001	0.003	<0.001	<0.001	0.01	<0.001	0.02

Fold change was determined using the $2^{-\Delta\Delta Ct}$ method.

^{A,B,C} Means with no common superscript within a gene and a tissue differ significantly ($P < 0.05$).

AvBD1 = Avian β-defensin 1; AvBD6 = Avian β-defensin 6; AvBD8 = Avian β-defensin defensin 8; AvBD10 = Avian β-defensin 10; AvBD11 = Avian β-defensin 11; AvBD12 = Avian β-defensin 12; AvBD13 = Avian β-defensin 13; LEAP2 = Liver-Expressed Antimicrobial Peptide 2.

wild-type strain recovered from a market aged chicken as part of an on-farm NARMS and thus represents a contemporary strain. The SPF chickens used by Awad et al. (2014) are from laying hens bred for production of pathogen free eggs. These chickens are genetically different from the Ross 308 broilers used in our study and thus likely have physiological differences. The challenge strain of *C. jejuni* could also differentially affect gene expression. Young et al. (1999) showed that colonization of the ceca varied with different isolates of *C. jejuni*. The age of *C. jejuni* challenge likely also plays an important role. We challenged chicks at doh and collected samples at d 7 or 14 post-challenge, whereas Awad et al. (2014) challenged at d 14 and collected samples 14 d post-challenge (i.e., d 28). *C. jejuni* colonization of the gut at doh before the microbiome of the gut has been established and the innate immune system has been fully developed could have a very different outcome than colonization at d 14 when both the microbiome and immune system have been established. Changes in the gut microbiome of broiler chickens at different ages have been documented (Awad et al., 2016; Ding et al., 2017). Thus, challenge at doh vs. d 14 would be expected to influence colonization by *C. jejuni*. Han et al. (2017) showed that the composition of the gut microbiota affected the colonization rate in chickens inoculated with *C. jejuni* at d 18 post-hatch.

The expression profiles of HDP following *C. jejuni* challenge have been reported. Meade et al. (2009) showed that AvBD3, AvBD4, AvBD8, AvBD12, and AvBD14 mRNA as well as cathelicidin-2 and-3 mRNA were downregulated in peripheral blood lymphocytes after *C. jejuni* challenge of 4-wk old Ross broilers. Van Dijk et al. (2012) reported the downregulation of cathelicidin-2 in the small intestine of 4 d old Ross broilers at 48 but not 8 h post-challenge but did not examine expression of the AvBDs. Our study showed that there was a delayed response to the *C. jejuni* challenge with only a few changes in HDP mRNA at d 7 but by d 14 all HDP were affected. In many cases, there was a dose response for expression of the AvBD mRNA similar to that seen for the nutrient transporters. There was upregulation of the AvBD mRNA in the ileum and cecum at the 10^6 cfu dose compared to control with the 10^7 and 10^8 cfu doses showing no difference from control. Increased expression of AvBD could be an attempt by the host to prevent the bacteria from spreading systemically.*C. jejuni* has been reported to induce a response from the immune system, with the release of pro-inflammatory cytokines (Vaezirad et al., 2016). AvBD can induce pro-inflammatory cytokine expression in macrophages, indicating that AvBD may play an important role in the immune response (Semple et al., 2011).

Chicken LEAP2 is a 40 amino acid cationic peptide that has broad-spectrum antimicrobial activities against bacteria such as *Salmonella*, *Streptococcus*, *and Staphylococcus* (Townes et al., 2004, 2009). In the small intestine, the enterocytes lining the villi were shown to

express LEAP2 mRNA (Su et al., 2017). In our study, *C. jejuni* caused a downregulation of LEAP2 mRNA at d 14 in the 10^7 and 10^8 cfu groups compared to control, but there was no change in the 10^6 cfu group. This was a different pattern from the upregulation of AvBD observed in the 10^6 cfu group compared to control at d 14. Townes et al. (2004) showed that LEAP2 mRNA was upregulated in the small intestine of 5-d old chicks, 3 d after challenge with 10^6 cfu of *Salmonella*, another commensal bacterium in poultry. Thus, challenge with both *C. jejuni* and *Salmonella* affects intestinal LEAP2 mRNA expression.

In summary, the mRNA abundance of intestinal nutrient transporters and HDP, showed dose-, tissue-, and age-dependent changes following challenge with *C. jejuni*. At the low dose (10^6 cfu per chicken) there was upregulation of many of the nutrient transporter and AvBD mRNA, which was likely part of the mechanism employed by the intestinal epithelial cells to counter the *C. jejuni* infection. However, when the dose was increased to 10^7 or 10^8 cfu per bird, the transporter and HDP mRNA were no longer upregulated, suggesting that the response of the intestinal epithelial cells was suppressed by the higher dose of *C. jejuni*.

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