# Differential expression of intestinal nutrient transporters and host defense peptides in *Eimeria maxima*-infected Fayoumi and Ross chickens

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**ABSTRACT** Fayoumi chickens are believed to be more disease resistant compared to commercial broiler chickens. The objective of this study was to compare mRNA expression of intestinal nutrient transporters, digestive enzymes, and host defense peptides (HDP) between *Eimeria maxima*-challenged Fayoumi and Ross broiler chickens. At 21 d of age, Ross broilers and Fayoumi lines M5.1 and M15.2 were challenged with 1,000 *E. maxima* oocysts. Control birds were not challenged. Duodenum, jejunum, and ileum were sampled (n = 6) at 7 d post challenge. Gene expression was analyzed using relative quantification PCR. Data were analyzed by ANOVA and significance level was set at $P < 0.05$. There was numerical, but not statistically significant, differential weight gain depression for Ross (15%) and Fayoumi lines M5.1 (21%) and M15.2 (22%) and significant line-specific changes in gene expression. For nutrient transporters, there was downregulation of mRNA for the brush border membrane, amino acid transporters $b^{0,+}$AT/rBAT, $B^{o}$AT, and EAAT3 in different segments of the small intestine of Ross and both lines of Fayoumi chickens, indicating that *E. maxima* challenge likely caused a decrease in nutrient uptake. For HDP, there was downregulation of avian beta defensin (AvBD) 1, 6, 10, 12, and 13 mRNA in the jejunum of the 2 Fayoumi lines, but no change in the Ross broilers. In the duodenum, there was upregulation of AvBD10 mRNA in the Ross and both Fayoumi lines and additionally upregulation of AvBD11, 12, and 13 mRNA in only Fayoumi line M15.2. Liver expressed antimicrobial peptide 2 (LEAP2) mRNA was downregulated in the duodenum and jejunum of Ross and Fayoumi line M5.1 but not in Fayoumi line M15.2. The homeostatic, non-challenged levels of AvBD mRNA were greater in Fayoumi line M15.2 than Ross and Fayoumi line M5.1 in the duodenum and ileum. This study demonstrates tissue- and genetic line-specific transcriptional responses to *E. maxima*, highlights novel potential candidate genes for response to coccidiosis, and confirms a role for several previously reported genes in response to coccidiosis.

**Key words:** *Eimeria*, Fayoumi, Ross, nutrient transporters, host defense peptides

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## INTRODUCTION

Fayoumi chickens originated in Egypt where they are used for both meat and table egg production (Hassan et al., 2002). These chickens were imported into the USA in 1954, primarily because of their resistance to avian leukosis (Kim et al., 2008). The Fayoumi breed is more resistant to coccidiosis compared to commercial breeds (Pinard-van der Laan et al., 1998). Two congenic Fayoumi lines (M5.1 and M15.2) have been developed that are genetically distant from commercial broiler and Leghorn lines, highly inbred, and differ only at the major histocompatibility complex (Zhou and Lamont, 1999). The Fayoumi line M5.1 showed greater resistance to *Eimeria maxima* infection compared to line M15.2, based on difference in body weight (**BW**) gain after challenge (Kim et al., 2008).

The weight gain depression observed following *Eimeria* infection is likely due to damage to the intestinal epithelial layer and the resultant decrease in expression of digestive enzymes and nutrient transporters. The mRNA expression profiles of several digestive enzymes



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and amino acid, peptide, monosaccharide, and mineral transporters in the intestine of layers and broilers challenged with *E. acervulina*, *E. maxima*, *E. tenella*, or *E. praecox* have been reported (Paris and Wong, 2013; Fetterer et al., 2014; Su et al., 2014, 2015; Yin et al., 2015; Miska and Fetterer, 2017). These genes showed tissue- and age-specific expression profiles depending upon the chicken breed and *Eimeria* species used. There was, however, a common set of mRNA that was downregulated in all of these studies. There was downregulation of mRNA for the amino acid transporters $b^{0,+}$AT and EAAT3, which are located at the brush border membrane and downregulation of the zinc transporter ZnT1 located at the basolateral membrane. The amino acid transporter $b^{0,+}$AT mediates the exchange of extracellular cationic amino acids and cystine for intracellular neutral amino acids (Fotiadis et al., 2013), whereas the amino acid transporter EAAT3 mediates the uptake of anionic amino acids, such as glutamate (Kanai et al., 2013). The zinc transporter ZnT1 mediates the efflux of zinc out of the cell (Huang and Tepaamorndech, 2013). These changes in gene expression may lead to a decrease in the intracellular pools of key nutrients and disruption of zinc balance, which may represent a cellular mechanism to inhibit *Eimeria* replication in the intestinal enterocytes.

Innate immunity is the first line of host defense against pathogens in vertebrate animals (Dziarski, 2013). Host defense peptides (**HDP**) are important components of innate immunity, have antimicrobial and immunomodulatory properties, and show broad-spectrum activity against a range of bacteria, fungi, and enveloped viruses (Robinson et al., 2015). In aves, the HDP consist of 14 avian beta-defensins (**AvBD**), 4 cathelicidins, and liver expressed antimicrobial peptide-2 (**LEAP2**) (Cuperus et al., 2013; Zhang and Sunkara, 2014). The AvBD consist of 59 to 104 amino acids, contain 6 conserved cysteines, and show biological activity against a broad range of Gram-positive and Gram-negative bacteria (Zhang and Sunkara, 2014). Expression of AvBD mRNA in the intestine of *Eimeria*-challenged chickens is variable (Su et al., 2017). LEAP2 is a 40-amino-acid cationic peptide that has broad-acting antimicrobial activity against several bacterial pathogens (Townes et al., 2009). In broilers and layers, *E. acervulina*, *E. maxima*, *E. tenella*, or *E. praecox* challenge resulted in downregulation of LEAP2 mRNA in the small intestine (Casterlow et al., 2011; Sumners et al., 2011; Paris and Wong, 2013; Fetterer et al., 2014; Su et al., 2014, 2015, 2017). This suggests that upon invasion of the intestinal epithelia, *Eimeria* causes downregulation of LEAP2, which may enhance its survival.

The objective of this study was to compare the mRNA expression of digestive enzymes, nutrient transporters, and HDP in *E. maxima*-infected Ross broilers and 2 lines of Fayoumi chickens.

## MATERIAL AND METHODS

### Chickens and Eimeria Infection

Male Ross Heritage broilers were obtained from Longenecker's Hatchery (Elizabethtown, PA), and Fayoumi line M5.1 and M15.2 chicks were obtained from the Iowa State University flock. The Fayoumi chicks were shipped on day of hatch from Iowa State University to the USDA-ARS facility (Beltsville, MD), where they were housed and maintained in coccidia-free housing. Chickens were provided ad libitum access to water and a standard starter-type corn-soybean meal that was formulated to meet NRC requirements for poultry (National Research Council, 1994). At 21 d of age, chickens were inoculated with 1,000 *E. maxima* oocysts (USDA APU1 isolate) via oral gavage. Oocysts were maintained and isolated as previously described by Fetterer and Barfield (2003). Seven days post infection (**dpi**), duodenum, jejunum, and ileum were collected from control (n = 6) and challenged birds (n = 6). The contents of the intestine were removed, and tissue segments (2 to 3 cm) were immediately stored individually in RNAlater (Thermo-Fisher Scientific, Waltham, MA). Body weight was recorded at 21 and 28 d (7 dpi) for weight gain calculation. This study was carried out under a protocol approved by the Beltsville Research Center Animal Care and Use Committee and conducted at the Animal Parasitic Disease Laboratory (USDA Agricultural Research Service, Beltsville, MD).

### RNA Extraction and Quantitative Real-Time PCR

A 20 to 30 mg sample of frozen tissue was homogenized in TriReagent (Molecular Research Center Inc., Cincinnati, OH), and total RNA was extracted following the manufacturer's instruction for the Directzol RNA MiniPrep Kit (Zymo Research, Irvine, CA). RNA quantity and purity were determined using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific), and RNA quality was assessed by agarose-formaldehyde gel electrophoresis. The cDNA was synthesized from total RNA (500 ng) using the high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific) and then diluted 1:30 for real-time PCR analysis. The forward and reverse primers for all genes analyzed in this study are shown in Table 1. Primers for $\beta$-actin and the ribosomal proteins (cRPL4, cRPLP0, and cRPLP1) were tested as reference genes. Following geNorm analysis, the 2 most stable genes (cRPL4 and cRPLP1) were identified and the geometric mean of these 2 genes was used as the reference gene (Vandesompele et al., 2002). qPCR was performed on an Applied Biosystems 7500 system using Fast SYBR green (Thermo Fisher Scientific) and the following conditions for all genes: 95°C for 20 s followed by 40 cycles of 95°C for 3 s and 60°C for

**Table 1.** Forward and reverse primers for quantitative PCR.

| Gene | Description[1] | Forward primer (5′→3′) | Reverse primer (5′→3′) | Amplicon size (bp) | Accession no. |
|---|---|---|---|---|---|
| APN | | AATACGCGCTCGAGAAAACC | AGCGGGTACGCCGTGTT | 70 | NM_204861.1 |
| $b^{0,+}$AT | SLC7A9 | CAGTAGTGAATTCTCTGAGTG TGAAGCT | GCAATGATTGCCACAACTACCA | 88 | NM_001199133.1 |
| rBAT | SLC3A1 | CCCGCCGTTCAACAAGAG | AATTAAATCCATCGACTCCTTTGC | 70 | XM_004935370.2 |
| $B^0$AT | SLC6A19 | GGGTTTTGTGTTGGCTTAGGAA | TCCATGGCTCTGGCAGAGAT | 60 | XM_419056.5 |
| EAAT3 | SLC1A1 | TGCTGCTTTGGATTCCAGTGT | AGCAATGACTGTAGTGCAGAAG TAATATATG | 79 | XM_424930.5 |
| SI | | CGCAAAAGCACAGGGACAGT | TCGATACGTGGTGTGCTCAGTT | 138 | XM_015291762.1 |
| GLUT2 | SLC2A2 | CACACTATGGGCGCATGCT | ATTGTGCCTGGAGGTGTTGGT | 68 | NM_207178 |
| SGLT1 | SLC5A1 | GCCATGGCCAGGGCTTA | CAATAACCTGATCTGTGCACCAGTA | 71 | NM_001293240.1 |
| ZnT1 | SLC22A18 | TCCGGGAGTAATGGAAATCTTC | AATCAGGAACAAACCTATGGGAAA | 67 | XM_421021.4 |
| AvBD1 | | GAGTGGCTTCTGTGCATTTCTG | TTGAGCATTTCCCACTGATGAG | 62 | NM_204993.1 |
| AvBD6 | | GCCCTACTTTTCCAGCCCTATT | GGCCCAGGAATGCAGACA | 63 | NM_001001193 |
| AvBD10 | | CAGACCCACTTTTCCCTGACA | CCCAGCACGGCAGAAATT | 64 | NM_001001609.2 |
| AvBD11 | | GGTACTGCATCCGTTCCAAAG | GCATGTTCCAAATGCAGCAA | 56 | NM_001001779.1 |
| AvBD12 | | TGTAACCACGACAGGGGATTG | GGGAGTTGGTGACAGAGGTTT | 114 | NM_001001607.2 |
| AvBD13 | | CAGCTGTGCAGGAACAACCA | CAGCTCTCCATGTGGAAGCA | 59 | NM_001001780.1 |
| LEAP2 | | CTCAGCCAGGTGTACTGTGCTT | CGTCATCCGCTTCAGTCTCA | 66 | NM_001001606.1 |
| $\beta$-actin | | GTCCACCGCAAATGCTTCTAA | TGCGCATTTATGGGTTTTGTT | 78 | NM_205518.1 |
| cRPL4 | | TCAAGGCGCCCATTCG | TGCGCAGGTTGGTGTGAA | 55 | NM_001007479.1 |
| cRPLP0 | | GCGATTGCTCCCTGTGATG | TCTCAGGTCCGAGACCAGTGT | 59 | NM_204987.2 |
| cRPLP1 | | TCTCCACGACGACGAAGTCA | CCGCCGCCTTGATGAG | 63 | NM_205322.1 |

[1]SLC is the Solute carrier name for the transporter.

30 s. Samples were run in duplicate and relative gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), as described in Su et al. (2015). The mean ΔCt of the uninfected samples was used to calculate the ΔΔCt value, which was performed separately for each line.

### Statistical Analysis

All data were analyzed by ANOVA using JMP® Statistical Discovery Software (SAS Institute, Cary, NC). For *Eimeria*-induced gene expression of each line, the model included the main effects of infection, sorted by tissue and genes. Significance level was set at $P < 0.05$ when compared with the control. For homeostatic HDP gene expression, the ΔCt values were analyzed by gene within a tissue. Means were separated using Tukey's test and significance level was set at $P < 0.05$.

## RESULTS

### Body Weight Gain During Eimeria Challenge

The BW of the Fayoumi line M5.1 and M15.2 chickens used for the challenged and unchallenged groups was 17.2% and 20.6% of the BW of the Ross chickens, on the day of challenge (day 21). Seven days post challenge with 1,000 *E. maxima* oocysts per chicken, there was a 15% weight gain depression in Ross chickens, a 21% weight gain depression in Fayoumi line M5.1, and a 22% weight gain depression in Fayoumi line M15.2 (Table 2). This difference in weight gain depression was not significantly different between the Ross and Fayoumi lines M5.1 and M15.2.

### Gene Expression *in* E. maxima-*Challenged Chickens*

The mRNA abundance of digestive enzymes, nutrient transporters, and HDP during *E. maxima* infection was assessed by qPCR in the small intestine of Ross chickens and Fayoumi lines M5.1 and M15.2. Changes in gene expression were analyzed separately for duodenum, jejunum, and ileum.

In the duodenum, Ross broilers challenged with *E. maxima* showed downregulation of $b^{0,+}$AT, EAAT3, SI, and GLUT2 mRNA compared to uninfected controls (Figure 1A). Fayoumi line M5.1 challenged with *E. maxima* showed decreased mRNA expression of the digestive enzymes APN and SI and all transporters ($b^{0,+}$AT, rBAT, $B^0$AT, EAAT3, GLUT2, SGLT1, and ZnT1) compared to control, whereas challenged Fayoumi line M15.2 showed downregulation of $b^{0,+}$AT, rBAT, $B^0$AT, EAAT3, and ZnT1 mRNA compared to control. For the HDP in comparison to the control (Figure 1B), *E. maxima* challenge caused a downregulation of AvBD1, 6, 13, and LEAP2 mRNA in Fayoumi line M5.1 and downregulation of only LEAP2 mRNA in Ross chickens. There was upregulation of AvBD10, 11, 12, and 13 mRNA in challenged Fayoumi line M15.2. In addition, there was upregulation of AvBD10 mRNA in both Ross and Fayoumi line M5.1.

In the jejunum, *E. maxima*-challenged Ross broilers showed decreased mRNA expression of APN and nutrient transporters $b^{0,+}$AT, rBAT, $B^0$AT, and EAAT3 (Figure 2A). Challenged Fayoumi line M5.1 showed downregulation of APN, SI, and all transporter ($b^{0,+}$AT, rBAT, $B^0$AT, EAAT3, GLUT2, SGLT1, and ZnT1) mRNA, whereas challenged Fayoumi line M15.2

**Table 2.** Body weight gain of *Eimeria* challenged broilers.

| Breed | Ross | | | Fayoumi M5.1 | | | Fayoumi M15.2 | | |
|---|---|---|---|---|---|---|---|---|---|
| Measurement[1] | BW day 21 | BW day 28 | BWG | BW day 21 | BW day 28 | BWG | BW day 21 | BW day 28 | BWG |
| Non-infected | 817 ± 24 | 1311 ± 47 | 494 ± 28 | 138 ± 7 | 208 ± 7 | 70 ± 3 | 161 ± 6 | 233 ± 6 | 72 ± 2 |
| Infected | 725 ± 58 | 1144 ± 79 | 418 ± 24 | 127 ± 11 | 182 ± 14 | 55 ± 3 | 157 ± 4 | 213 ± 3 | 56 ± 2 |
| Weight gain depression | | | 15% | | | 21% | | | 22% |
| *P* value | | | <0.05 | | | <0.01 | | | <0.001 |

[1]BW = Body weight and BWG = Body weight gain; all body weight measurements were expressed as mean ± SE; BW and BWG were measured in gram.

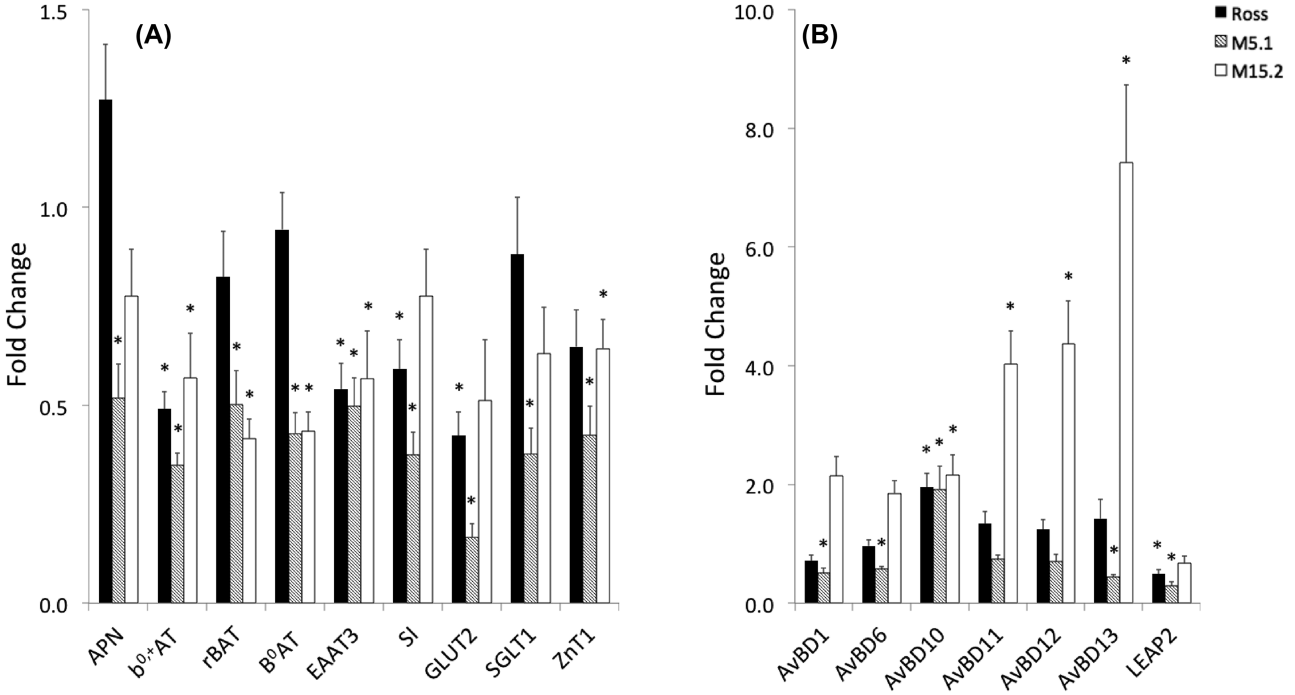


**Figure 1.** mRNA expression of digestive enzymes, nutrient transporters, and host defense peptides in the duodenum of Ross and Fayoumi lines M5.1 and M15.2 chickens challenged with 1,000 *E. maxima* oocysts. (A) Digestive enzymes and nutrient transporters include aminopeptidase N (APN), the dimeric neutral/cationic amino acid transporter $b^{0,+}$AT/rBAT, the neutral amino acid transporter $B^0$AT, the anionic amino acid transporter EAAT3, sucrase isomaltase (SI), the facilitated monosaccharide transporter GLUT2, the sodium glucose transporter SGLT1, and the zinc transporter ZnT1. (B) Host defense peptides include the avian beta defensins (AvBD) and liver expressed antimicrobial peptide 2 (LEAP2). Controls (non-challenged) are equal to a fold change of 1.0 for each gene. * indicates statistical significance from control at $P < 0.05$.

showed decreased $b^{0,+}$AT, $B^0$AT, and EAAT3 mRNA. For the HDP, challenged Fayoumi lines M5.1 and M15.2 showed downregulation of all AvBD mRNA, but there were no changes in Ross chickens (Figure 2B). For LEAP2 mRNA, there was downregulation in Ross and Fayoumi line M5.1, but no changes in Fayoumi line M15.2.

In the ileum, *E. maxima*-challenged Ross broilers showed downregulation of APN and the nutrient transporters $b^{0,+}$AT, rBAT, $B^0$AT, EAAT3, GLUT2, and ZnT1 mRNA (Figure 3A). Challenged Fayoumi line M5.1 showed only decreased EAAT3 mRNA, whereas challenged Fayoumi line M15.2 showed downregulation of all genes examined (APN, $b^{0,+}$AT, rBAT, $B^0$AT, EAAT3, SI, GLUT2, SGLT1, and ZnT1). For the HDP, challenged Ross broilers showed only decreased LEAP2 mRNA and no changes for AvBD mRNA (Figure 3B). Challenged Fayoumi line M5.1 showed decreased mRNA expression of all AvBD, whereas challenged Fayoumi line M15.2 showed downregulation of AvBD6, 11, 12, and LEAP2 mRNA.

To better visualize differences in gene expression between Ross chickens and the Fayoumi lines, a graphical summary is shown in Table 3. A few general patterns emerge. For the transporters, there was downregulation of mRNA for all digestive enzymes (APN and SI) and nutrient transporters (amino acids, sugars, zinc) in the duodenum and jejunum of Fayoumi line M5.1 and ileum of Fayoumi line M15.2. There was downregulation of APN and the amino acid transporters in the jejunum and ileum of Ross chickens. For the HDP, there was downregulation of all AvBD in the jejunum and ileum of Fayoumi line M5.1 and the jejunum of Fayoumi line M15.2, but not in Ross chickens. Only a few AvBD mRNA were upregulated following *E. maxima* challenge. AvBD10 mRNA was upregulated in the duodenum of Ross chickens and both Fayoumi lines and AvBD11, 12, and 13 mRNA were upregulated in the

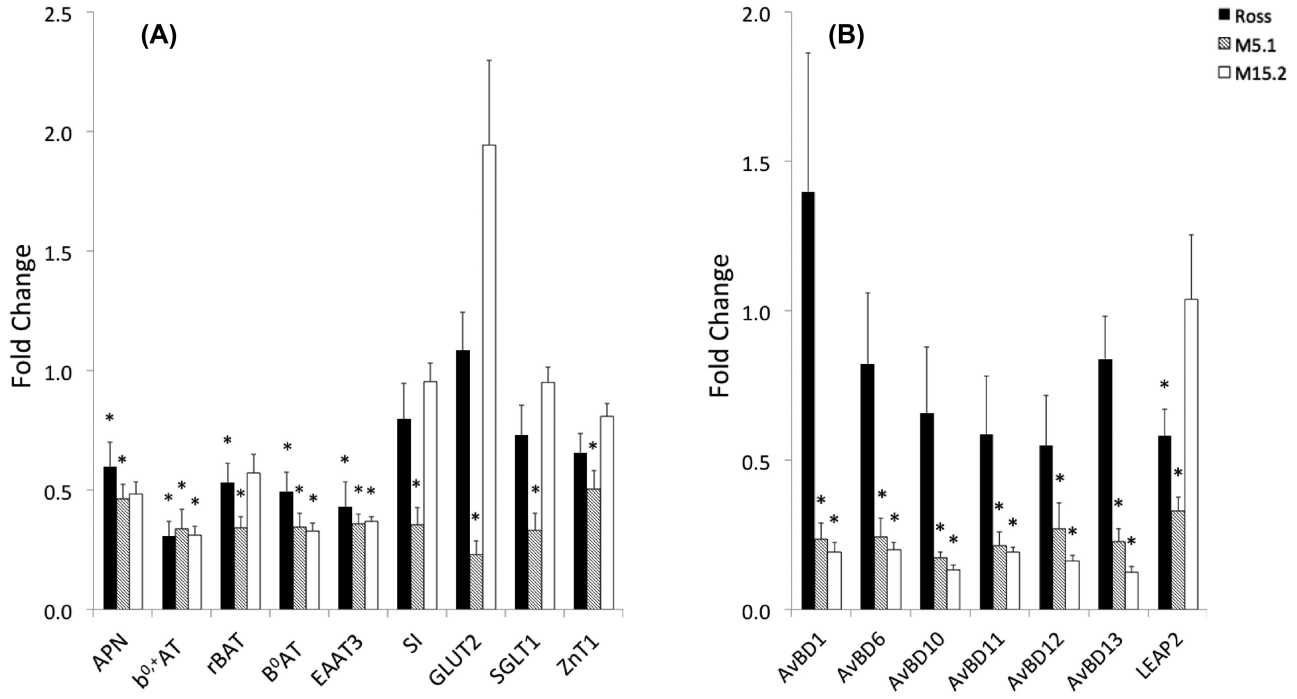


**Figure 2.** mRNA expression of digestive enzymes, nutrient transporters, and host defense peptides in the jejunum of Ross and Fayoumi lines M5.1 and M15.2 chickens challenged with 1,000 *E. maxima* oocysts. (A) Digestive enzymes and nutrient transporters include aminopeptidase N (APN), the dimeric neutral/cationic amino acid transporter $b^{0,+}$AT/rBAT, the neutral amino acid transporter $B^0$AT, the anionic amino acid transporter EAAT3, sucrase isomaltase (SI), the facilitated monosaccharide transporter GLUT2, the sodium glucose transporter SGLT1, and the zinc transporter ZnT1. (B) Host defense peptides include the avian beta defensins (AvBD) and liver expressed antimicrobial peptide 2 (LEAP2). Controls (non-challenged) are equal to a fold change of 1.0 for each gene. * indicates statistical significance from control at $P < 0.05$.

**Table 3.** Summary of changes in gene expression.

| | Ross | | | Fayoumi M5.1 | | | Fayoumi M15.2 | | |
|---|---|---|---|---|---|---|---|---|---|
| Gene[1] | Duo | Jej | Ile | Duo | Jej | Ile | Duo | Jej | Ile |
| APN | | ↓ | ↓ | ↓ | ↓ | | | | ↓ |
| $b^{o,+}$AT | ↓ | ↓ | ↓ | ↓ | ↓ | | ↓ | ↓ | ↓ |
| rBAT | | ↓ | ↓ | ↓ | ↓ | | ↓ | | ↓ |
| $B^o$AT | | ↓ | ↓ | ↓ | ↓ | | ↓ | ↓ | ↓ |
| EAAT3 | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ |
| SI | ↓ | | | ↓ | ↓ | | | | ↓ |
| GLUT2 | ↓ | | ↓ | ↓ | ↓ | | | | ↓ |
| SGLT1 | | | | ↓ | ↓ | | | | ↓ |
| ZnT1 | | | ↓ | ↓ | ↓ | | ↓ | | ↓ |
| AvBD1 | | | | ↓ | ↓ | ↓ | | ↓ | |
| AvBD6 | | | | ↓ | ↓ | ↓ | | ↓ | ↓ |
| AvBD10 | ↑ | | | ↑ | ↓ | ↓ | ↑ | ↓ | |
| AvBD11 | | | | | ↓ | ↓ | ↑ | ↓ | ↓ |
| AvBD12 | | | | | ↓ | ↓ | ↑ | ↓ | ↓ |
| AvBD13 | | | | | ↓ | ↓ | ↑ | ↓ | |
| LEAP2 | ↓ | ↓ | ↓ | ↓ | ↓ | | | | ↓ |

[1]↓ and ↑ indicate downregulation and upregulation, respectively, of mRNA abundance relative to control in the duodenum (Duo), jejunum (Jej), and ileum (Ile).

duodenum of Fayoumi line M15.2. LEAP2 mRNA was downregulated in all 3 intestinal segments in Ross chickens, but was downregulated in only selected intestinal segments in Fayoumi lines M5.1 and M15.2.

The homeostatic expression levels of HDP mRNA may also play an important role in regulating an *Eimeria* infection. The relative homeostatic levels (ΔCt) of AvBD and LEAP2 mRNA for the Ross and Fayoumi lines are shown in Figure 4. In the duodenum, Fayoumi line M15.2 had greater expression of all AvBD and LEAP 2 mRNA compared to Ross and Fayoumi line M5.1. For AvBD10 and AvBD13, Fayoumi line M5.1 had greater homeostatic expression than Ross chickens. In the jejunum, Fayoumi line M15.2 had lower AvBD10, 11, and 13 mRNA than Ross chickens, with Fayoumi line M5.1 intermediate. In contrast, expression of LEAP2 mRNA was greater in Fayoumi line M15.2 than line M5.1, with Ross chickens intermediate. In the ileum, there was a similar pattern as seen in the duodenum. Fayoumi line M15.2 had greater expression

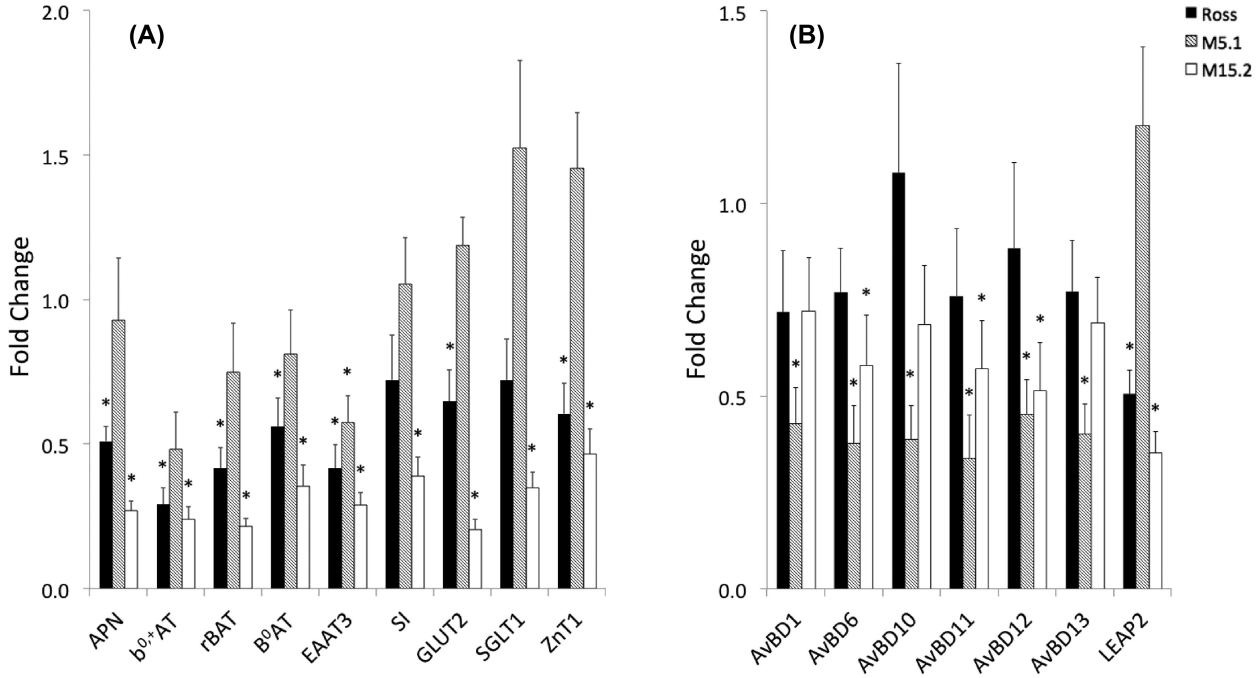


**Figure 3.** mRNA expression of digestive enzymes, nutrient transporters, and host defense peptides in the ileum of Ross and Fayoumi lines M5.1 and M15.2 chickens challenged with 1,000 *E. maxima* oocysts. (A) Digestive enzymes and nutrient transporters include aminopeptidase N (APN), the dimeric neutral/cationic amino acid transporter $b^{0,+}$AT/rBAT, the neutral amino acid transporter $B^0$AT, the anionic amino acid transporter EAAT3, sucrase isomaltase (SI), the facilitated monosaccharide transporter GLUT2, the sodium glucose transporter SGLT1, and the zinc transporter ZnT1. B. Host defense peptides include the avian beta defensins (AvBD) and liver expressed antimicrobial peptide 2 (LEAP2). Controls (non-challenged) are equal to a fold change of 1.0 for each gene. * indicates statistical significance from control at $P < 0.05$.

of all AvBD mRNA compared to Ross and Fayoumi line M5.1. For LEAP2 mRNA, Fayoumi line M5.1 was greater than line M15.2 and Ross chickens.

## DISCUSSION

Fayoumi chickens have been reported to be more resistant to *Eimeria* infection when compared to other chicken breeds. For example, Pinard-van der Laan et al. (1998) compared the resistance of 5 lines of chickens (2 Egyptian lines: Mandarah and Fayoumi, Rhode Island Red and 2 White Leghorn lines: WLB21 and WLDW) to challenge with 150,000 *E. tenella* oocysts at 26 d of age. The Fayoumi line was the most resistant, showing no mortality, less severe lesions, and only a 30% weight gain depression 10 d after infection. The WLDW line was the most susceptible and showed an 85% weight gain depression. Kim et al. (2008) compared Fayoumi lines M5.1 and M15.2 following challenge with 10,000 *E. maxima* oocysts at 4 wk of age. At 9 dpi, Fayoumi line M5.1 was more resistant to *E. maxima* infection compared to line M15.2. Fayoumi line M5.1 showed no change in BW, whereas line M15.2 showed a 20% weight gain depression. Lee et al. (2016) compared the susceptibility of Fayoumi lines M5.1 and M15.2 to an initial challenge with $5 \times 10^4$ *E. tenella* oocysts at 4 wk of age, followed by a second challenge with $5 \times 10^6$ *E. tenella* oocysts 10 d after the initial challenge. At 9 d following the initial challenge, there was no difference in weight gain between infected and uninfected Fayoumi line M5.1, but there was a significant weight loss for infected compared to uninfected Fayoumi line M15.2. After the secondary challenge, however, both Fayoumi lines M5.1 and M15.2 showed weight losses relative to their respective controls, with the weight loss of line M15.2 (39%) greater than line M5.1 (32.7%).

In our experiment, we did not observe the expected differences in BW gain between the Fayoumi lines M5.1 and M15.2 and the Ross broilers or between Fayoumi line M5.1 and line M15.2 in response to an *E. maxima* infection. The Ross and Fayoumi chickens all showed a similar (15 to 22%) weight gain depression following challenge with 1,000 *E. maxima* oocysts at 3 wk of age. Because the Ross chickens were approximately 5 times heavier than the Fayoumi chickens at the time of challenge, a similar dosage might have been expected to affect the Fayoumi lines more than the Ross broilers, but it did not. The alternative approach would have been to challenge the Ross and Fayoumi chickens at the same weight, but then they would have been of different age. We opted for challenge at the same age, rather than weight. It is not clear why we did not observe a differential response of the 2 Fayoumi lines to *E. maxima* infection as reported by Kim et al. (2008). The different results could be due to differences in the *E. maxima* dosage (1,000 vs. 10,000 oocysts), age at challenge (3 vs. 4 wk), time of infection (7 vs. 9 d,) or the potency or pathogenicity of the *E. maxima* strain used. The *E. maxima* APU1 strain that was used in this study is a very pathogenic strain compared to

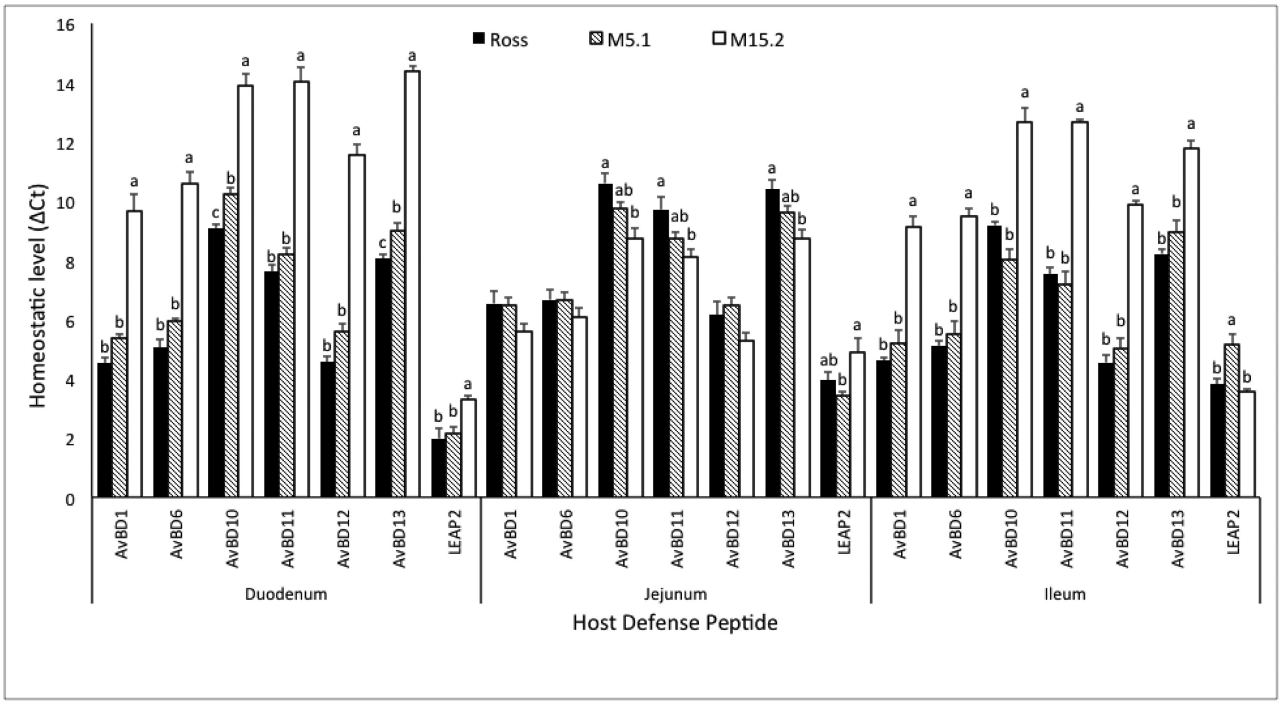


**Figure 4.** Homeostatic mRNA levels of the host defense peptides in the small intestine of Ross and Fayoumi lines M5.1 and M15.2 chickens. Host defense peptides include the avian beta defensins (AvBD) and liver expressed antimicrobial peptide 2 (LEAP2). ΔCt = Ct (HDP)-Ct (reference gene). Bars with a different letter (a–c) are significantly different ($P < 0.05$) within a gene and intestinal segment, when analyzed with Tukey's test.

most field isolates. Jenkins et al. (2017) showed that *E. maxima* strain APU1 caused a greater reduction in BW, higher lesion scores, and increased oocyst output compared to the less pathogenic *E. maxima* strain APU2.

Expression of intestinal amino acid, peptide, sugar, and mineral transporters has been previously studied in chickens challenged with various species of *Eimeria*. In the current study, we analyzed both males and females for the 2 Fayoumi lines because the sexes cannot be visually distinguished at the age they were tested, but only male Ross broilers. Kaminski and Wong (2018) reported that there was no difference between male and female Aviagen chickens in the expression of several nutrient transporters, including the amino acid transporters $b^{0,+}$AT, ASCT1, and $y^+$LAT2, the peptide transporter PepT1, and the sugar transporters SGLT1 and GLUT2 at day 7 and 14 post hatch, although there was a difference at day of hatch.

Downregulation of the mRNA for the amino acid transporters $b^{0,+}$AT and EAAT3 and the zinc transporter ZnT1 have been reported following challenge with different *Eimeria* species in both layers and broilers (Paris and Wong, 2013; Fetterer et al., 2014; Su et al., 2014, 2015; Yin et al., 2015; Miska and Fetterer, 2017). The results of the current study show a similar pattern with downregulation of EAAT3 in all small intestinal segments of Ross broilers and both Fayoumi lines and downregulation of $b^{0,+}$AT mRNA in all intestinal segments of Ross broilers and Fayoumi line M15.2 but only the duodenum and jejunum of Fayoumi line M5.1. Furthermore, there was tissue-specific expression of transporter mRNA between challenged Fayoumi lines M5.1 and M15.2, which demonstrated that these 2 lines responded differently to an *Eimeria* challenge. A model has been previously proposed to describe these changes in transporter expression during an *Eimeria* infection (Paris and Wong, 2013; Su et al., 2014, 2015). Downregulation of $b^{0,+}$AT and EAAT3 mRNA could lead to decreased uptake of cationic amino acids such as lysine and anionic amino acids, such as glutamate, of which the latter is important because it serves as the major energy source for intestinal epithelial cells (Brosnan and Brosnan, 2013). The changes in transporter gene expression that occur following an *Eimeria* challenge in the Fayoumi lines are similar to those documented in broilers and layers, suggesting that there is a common cellular response to an *Eimeria* infection in chickens.

In addition to glutamate, $b^{0,+}$AT also transports cystine, the oxidized form of cysteine (Fotiadis et al., 2013). Downregulation of $b^{0,+}$AT and the anionic (EAAT3) and neutral ($B^0$AT) amino acid transporters would lead to a decreased uptake of cysteine, glutamate, and glycine, which combined produce the antioxidant glutathione. To counter this potential reduction in glutathione, there may be a cellular response to downregulate expression of the basolateral zinc transporter to increase the intracellular pool of zinc, which while acting as an antioxidant, can protect the host against

*Eimeria*-induced oxidative damage (Georgieva et al., 2011).

The HDP are members of the innate immune system and possess both antimicrobial and immunomodulatory activities (reviewed in Cuperus et al., 2013; Zhang and Sunkara, 2014). The AvBD show antibacterial activity against a number of Gram-positive and Gram-negative bacteria. To our knowledge, there are no published reports showing AvBD activity against protozoa, such as *Eimeria*. The HDP also play an important role in regulating the host immune response to infections by binding directly to chemokine receptors, inducing proinflammatory cytokine expression and showing chemotactic properties.

In our study, there was differential expression of AvBD mRNA in the Ross and 2 Fayoumi lines. There were many changes in AvBD mRNA expression in the Fayoumi lines compared to the Ross chickens. In the duodenum, AvBD10, 11, 12, and 13 mRNA were upregulated in Fayoumi line M15.2, whereas only AvBD10 mRNA was upregulated in Ross chickens and Fayoumi line 5.1. In contrast, in the jejunum there was downregulation of all 6 AvBD mRNA in both Fayoumi lines M5.1 and M15.2. *E. maxima* preferentially affects the jejunum, so it was reasonable to expect that there might be downregulation of AvBD mRNA in the jejunum to promote survival of *E. maxima*. Because the AvBD are secreted peptides, the upregulation of AvBD mRNA in the duodenum of Fayoumi line M15.2 could represent a compensatory response to the downregulation of AvBD mRNA in the jejunum. This could result in enhanced resistance to *E. maxima* challenge compared to Fayoumi line M5.1; however, Kim et al. (2008) reported the opposite result, i.e., Fayoumi line M5.1 was more resistant to *E. maxima* challenge than Fayoumi line M15.2 based on BW gain depression. In our study, we did not observe a difference in BW gain depression between Fayoumi lines M5.1 and M15.2. Thus, it appears that the increase in AvBD mRNA expression in the duodenum of Fayoumi line M15.2 had no significant effect on BW gain. The changes in AvBD mRNA expression may play a more important role in modulating the immune system.

Chicken LEAP2 is a 40-amino-acid cationic peptide that has broad-acting antimicrobial activity against a number of bacterial pathogens (Townes et al., 2009). Su et al. (2017) showed that LEAP2 mRNA was expressed in the epithelial cells lining the intestinal villi and thus might be affected by *Eimeria* in the intestinal lumen. LEAP2 mRNA was consistently downregulated following challenge with *E. acervulina*, *E. maxima*, *E. tenella*, or *E. praecox* in broilers and layers (Casterlow et al., 2011; Sumners et al., 2011; Paris and Wong, 2013; Fetterer et al., 2014; Su et al., 2014, 2015, 2017). In the model proposed by Paris and Wong (2013), LEAP2 was hypothesized to play a role in modulating an *Eimeria* infection, such that a more resistant chicken would be expected to show less downregulation of LEAP2 mRNA. Because there was no difference in BW gain depression between the Ross and Fayoumi lines, the precise role for LEAP2 in mediating an *Eimeria* infection remains to be determined.

In the current study, we also observed downregulation of LEAP2 mRNA in all 3 intestinal segments of Ross broilers, in the duodenum and jejunum of Fayoumi line M5.1 and in only the ileum of Fayoumi line M15.2. One proposed explanation for the downregulation of LEAP2 following an *Eimeria* challenge is shortening of the villi and a generalized effect on gene expression (Su et al., 2017). This is a possible explanation for the jejunum in Fayoumi line M5.1, where all genes examined were downregulated. However, in the jejunum and ileum of Ross chickens, there was downregulation of amino acid transporter and LEAP2 mRNA, but no changes for AvBD mRNA. Furthermore, in the duodenum of Fayoumi line M5.1 there was downregulation of all transporter and LEAP2 mRNA, but upregulation of AvBD10 mRNA. Thus, a simple shortening of the villi cannot be the sole reason for downregulation of gene expression.

In most pathogen challenge studies, the focus is on the change in HDP expression following challenge. The homeostatic level of HDP expression, however, may also play an important role in mediating the response to a pathogen. In our study, Fayoumi line M15.2 had greater relative expression of the AvBD mRNA in the duodenum and ileum compared to the Ross and Fayoumi line M5.1. In contrast, Fayoumi line M15.2 showed lower homeostatic expression of AvBD10, 11, and 13 in the jejunum compared to Ross chickens, with Fayoumi line M5.1 intermediate. Because *E. maxima*, which causes lesions mainly in the jejunum, was used for the challenge, the lack of a difference in BW depression between Ross and the Fayoumi lines may be related to the similar homeostatic AvBD mRNA expression levels in the jejunum. Challenge with different *Eimeria* species such as *E. acervulina* or *E. tenella*, which cause lesions mainly in the duodenum or ileum and cecum, respectively, might be expected to affect Fayoumi line M15.2 less than Ross and Fayoumi line M5.1 due to greater homeostatic AvBD mRNA levels. Consistent with this idea, Pinard-van der Laan et al. (1998) showed that Fayoumi chickens showed less severe lesions and reduced weight gain depression compared to Rhode Island Red and White Leghorn chickens following challenge with *E. tenella*. The response to a pathogen may be a combination of the homeostatic and pathogen-induced levels of HDP mRNA expression. Fayoumi line M15.2 had not only the greatest homeostatic AvBD mRNA but also the greatest *E. maxima*-induced AvBD mRNA levels in the duodenum.

In summary, although a reduction in BW gain was similar among Ross chickens and the 2 Fayoumi lines, homeostatic HDP mRNA expression and *Eimeria*-induced transporter and HDP mRNA expression showed tissue- and genetic line-specific differences. Fayoumi line M15.2 had the greatest homeostatic and *Eimeria*-induced expression of AvBD mRNA in the duodenum. Common downregulation of the mRNA for

the amino acid transporters $b^{0,+}$AT and EAAT3 and the HDP LEAP2 in the Ross and Fayoumi lines supports our previous conclusion on the importance of these genes in the host response to *Eimeria* challenge.

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