# Expression of Digestive Enzymes and Nutrient Transporters in the Intestine of *Eimeria*-challenged Chickens

Shengchen Su

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
In
Animal and Poultry Sciences

Eric A. Wong, Chair
Rami A. Dalloul
Elizabeth R. Gilbert

Aug 08, 2013
Blacksburg, VA

Keywords: chicken, *Eimeria*, transporter, LEAP2

**Expression of Digestive Enzymes and Nutrient Transporters in the Intestine of *Eimeria*-challenged Chickens**

Shengchen Su

## ABSTRACT

Avian coccidiosis is caused by the intestinal protozoa *Eimeria*. The parasite's site of infection in the intestine is site specific. *Eimeria acervulina* infects the duodenum, *E. maxima* the jejunum, and *E. tenella* the ceca. Lesions in the intestinal mucosa cause reduced feed efficiency and body weight gain in *Eimeria*-challenged chickens. The growth reduction may be due to changes in expression of digestive enzymes and nutrient transporters in the intestine. The objective of this thesis was to examine the expression of digestive enzymes: APN and SI, peptide and amino acid transporters: Pept1, ASCT1, $b^{o,+}$AT/rBAT, $B^0$AT, CAT1/2, EAAT3, LAT1 and $y^+$LAT1/2, sugar transporters: GLUT1, GLUT2, GLUT5 and SGLT1, mineral transporter: ZNT1 and an immune factor: LEAP2 in the duodenum, jejunum, ileum and ceca of *Eimeria*-challenged layers and broilers. Comparisons were made between *E. acervulina*-challenged layers and broilers and *E. acervulina, E. maxima* and *E. tenella*-challenged broilers to examine the effect of chicken breeds and *Eimeria* species, respectively, on digestive enzymes and nutrient transporter expression. *E. acervulina*-challenged layers and broilers showed downregulation of APN, $b^{o,+}$AT/rBAT, $B^0$AT, CAT2, EAAT3, GLUT2, SI, ZNT1 and LEAP2 in the duodenum, but not in the jejunum and ileum. *E. acervulina*-challenged duodenum, *E. maxima*-challenged jejunum and *E. tenella*-challenged ceca samples showed common downregulation of APN, GLUT5 and ZNT1. These results demonstrate that there are common changes in intestinal gene expression in response to *E. acervulina* in broilers and layers, and common changes in response to challenge by different *Eimeria* species in broilers.

## ACKNOWLEDGEMENTS

**My family.** Even though none of you can read what I write here, I still want to say thank you for all your unconditional love and support. Without you, I most certainly would not be the person I am today. I miss all of you very deeply.

**Dr. Wong.** Thank you for being incredibly patient with me at every time I struggle with writing and preparing presentations. You have been kind and supportive to me ever since I came for the interview. Thank you for giving me the opportunity to come to Virginia Tech for graduate school and having me stay for the PhD.

**Dr. Gilbert.** I am so lucky to have you as my committee member. The smile on your face just made me less nervous every time I gave a presentation. Thank you for the valuable feedback on my research.

**Dr. Dalloul.** Thank you for being my committee member and giving all the great advice with my project. I appreciate all of your help with my thesis and defense.

**Dr. Kate Miska, Dr. Raymond Fetterer and Dr. Mark Jenkins.** Thank you for doing the chicken experiments and sampling and everything that made this project possible. I cannot thank you enough for sending me the samples and answering all my questions.

**Dr. Dunnington.** Thank you for giving me the opportunity to be a teaching assistant in your class, I have learned so much about teaching from you. I was so nervous because I have never been a TA before. Thank you for all the encouragement and help.

**Dr. Siegel.** Thank you for the hundreds of eggs you gave me for the *in-ovo* feeding study. Unfortunately it didn't work out. Your everlasting smile and stories always made my day.

**Pat Williams.** You are amazing. I don't know how you found time taking care of the orders and helping me keep an eye on the fish at the same time. Thank you for checking on lab supplies and sending those memos regarding the fish.

**My fellow graduate students and all of the Wong Lab.** You are a big part of my life in Litton-Reaves hall. It is my pleasure to work with all of you. Thank you for your help with my research and classes.

**Mui and Juan.** You are not just my friends, you are my sisters. Mui, thank you for taking care of me back at Indiana and calling me every other day after I moved to Virginia. You made me feel I have a home in the US. Juan, thank you for sharing all your crazy stories with me, I often hope my life can be as exciting as yours.

**Jingjing and Ning.** I am so lucky to have you in my life here at Virginia. Thank you for inviting me to all the lunch and dinner parties, and considered me as one of the gang. Jingjing, I can't believe we never actually talked to each other even though we were neighbors in the dorm when we were at China Agricultural University. You are such an amazing person, I am so glad we now became friends. Ning, thank you for all your help on the statistical analysis, and being my makeup and fashion advisor.

## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

# CHAPTER I. REVIEW OF LITERATURE

## Morphology of the intestine

### Sections of the gastrointestinal tract

The majority of nutrient absorption occurs in the small intestine (Leeson, et al., 2001). Structures such as plicae circularis (mucosal folds), villi and microvilli present at the small intestine increase the surface for absorption. In an adult chicken, the small intestine is about 1.3m in length and can be divided into three sections: duodenum, jejunum and ileum. Figure I-1. illustrates the anatomical structure of part of the chicken digestive system.

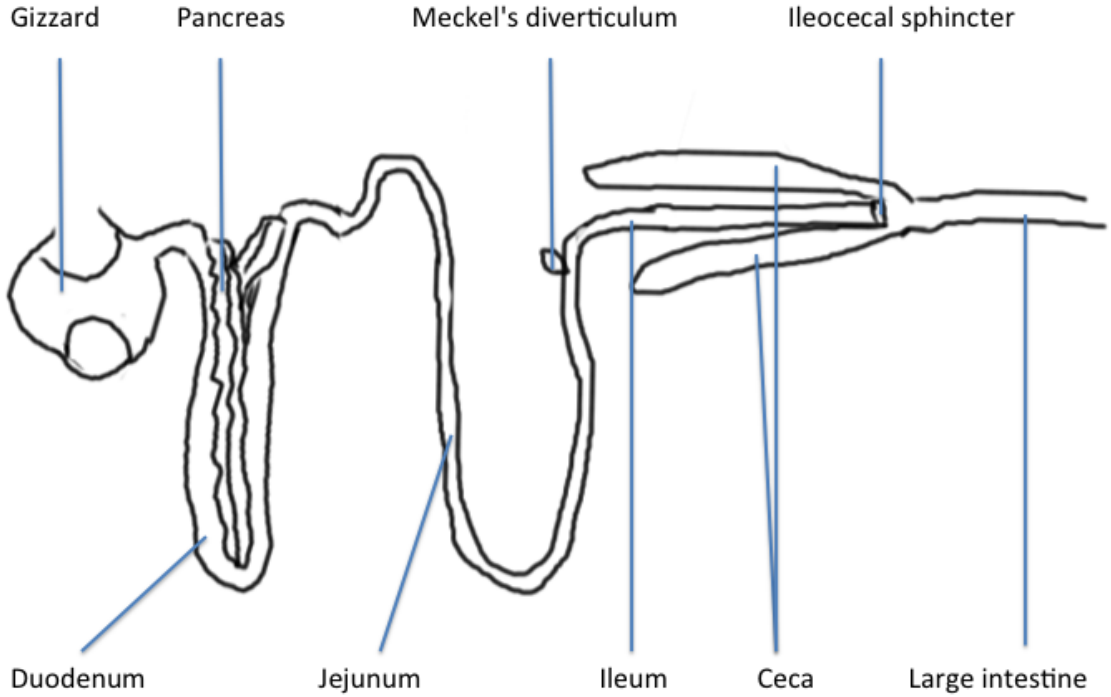


**Figure I-1. Anatomy of the chicken digestive system.** (not drawn to scale) (Su, 2013).

The duodenum is the first section of the small intestine (Figure I-1). The duodenum loops around the pancreas, which is called the duodenal loop. The primary function of the duodenum is to mix food chyme with digestive enzymes secreted from the liver, pancreas and the duodenal wall, this process also results in neutralizing the acid in the food chyme from the stomach (Smith and Morton, 2010). The duodenal section is larger in diameter compared with the other regions of the small intestine.

The jejunum has a very similar anatomical structure to the duodenum, but there is a reduction of the mucosal folds compared to the duodenum (Smith and Morton, 2010). The jejunum makes up about 40% in human and up to 90% in other species of the total small intestine length (Twietmeyer and McCracken, 2001). In many species there is no distinct anatomical feature that separates the jejunum and the ileum. In chicken, Meckel's diverticulum marks the end of the jejunum and the start of the ileum (Noy and Sklan, 2001). Right before hatch, the yolk sac is absorbed into the naval cavity of the chicken embryo, and the residual tiny yolk sac stalk is Meckel's diverticulum.

There is further reduction of the mucosal folds in the ileum, the distal part of the small intestine. The mucosal folds are absent at the end of the ileum. The ileum section has abundant lymph node like structures, called Peyer's patches, which are located in the mucosa and submucosal layer of the ileum (Smith and Morton, 2010). The ileocecal sphincter separates the ileum and the large intestine, which functions in reduction of reflux from the colon (Smith and Morton, 2010). In addition to nutrients, the ileum also absorbs bile acid, vitamin B12 and other intrinsic factors to be recycled in the body (Lazaridis, et al., 1997; Shaw, et al., 1989).

Birds have two ceca located below the junction of the small intestine and the large intestine (Moreto and Planas, 1989; Smith and Morton, 2010). The wall of the ceca has mucosal folds but not villus structures. The main function of the ceca is fermentation of dietary fiber, absorption of water, sugar and amino acids (Salanitro, et al., 1976; Whittow, 2000).

**Structure of the intestinal wall**

***Villus.*** The mucosal side of the intestine is covered with tiny projections, known as villi. The villus is considered the unit of absorption. In an adult chicken, the height of a villus is about 1mm, which varies depending on its location in the small intestine. The villi present in the duodenum are longer and tongue shaped. In the jejunum and ileum, there are reduced number and size of villi and more finger shaped villi (Smith and Morton, 2010).

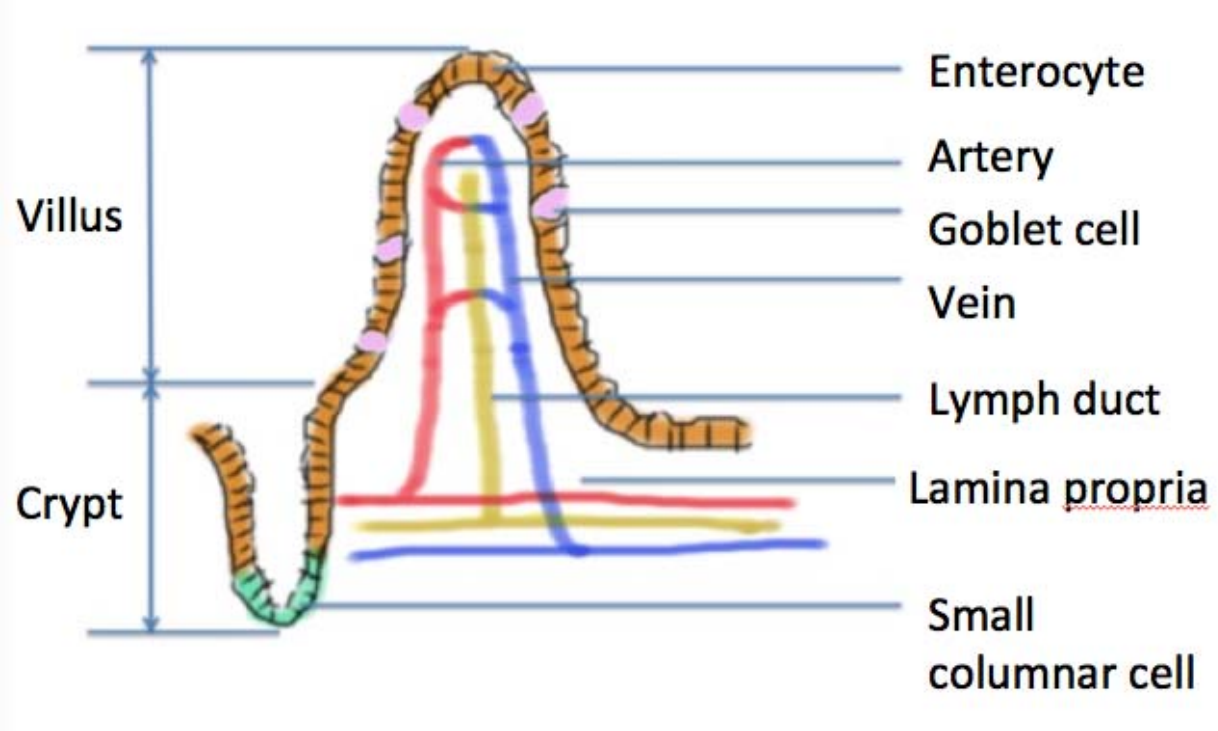


**Figure I-2. Structure of an intestinal villus and crypt.** (not drawn to scale) (Su, 2013).

A single simple columnar epithelium layer covers the villi (Figure I-2.) (Smith and Morton, 2010). Most of these cells are enterocytes with numerous cytoplasmic extensions, known as the microvilli, for nutrient digestion and absorption. Most of the rest of the cells are goblet cells and about less than 0.5% are endocrine cells. The goblet cells produce mucus, which serves as the primary barrier between the luminal environment and the epithelial layer. Tight junctions between the cells also form a physical barrier that is impermeable to fluids, nutrients and waste and thus protect the body from the harmful environment (Ivanov, 2012). The entero-endocrine cells are sensors of the luminal contents and regulate postprandial secretion and motility of the small intestine (Moran, et al., 2008). Underneath the epithelial cells, there is a layer of mucosa tissue called the lamina propria, which contains the capillary network and a sac like lymph vessel (Smith and Morton, 2010).

***Crypts of Lieberkuhn.*** Between adjacent villi, there are cell depressions into the lamina propria that form the crypts of Lieberkuhn. The cells in the crypts are the only cells of the villus that undergo cell division. They gave rise to enterocytes, goblet cells, entero-endocrine cells, and paneth cells (Green and Greene, 1984). Paneth cells stay within the crypts; they secrete lysozyme by releasing granules into the lumen by exocytosis (Smith and Morton, 2010). The other three

types of cells migrate up the villus to replace the extruded old cells at the villus tip. The replacement time (cell turnover) is dependent on the age of the animal, section of the intestine and height of the villus (Green and Greene, 1984; Reece and Reece, 2005; Smith and Morton, 2010). In chicken, this process takes about 3 to 4 d (Uni, et al., 2000).

***Enterocyte.*** The enterocytes are highly specialized and polarized cells (Figure I-3). Like other polarized cells, cell type specific proteins are expressed at the brush border membrane, while non-cell type specific proteins are expressed at the basolateral membrane (Van Beers, et al., 1995). The tight junction formed between adjacent enterocytes separates these two types of membranes. The specialized brush border membrane contains many cytoplasmic extensions, which characterize the brush border membrane and is essential for nutrient digestion and absorption (Van Beers, et al., 1995). Membrane bound glycoproteins like mucins at the brush border membrane protect the host against intestinal pathogens (Belley, et al., 1999).

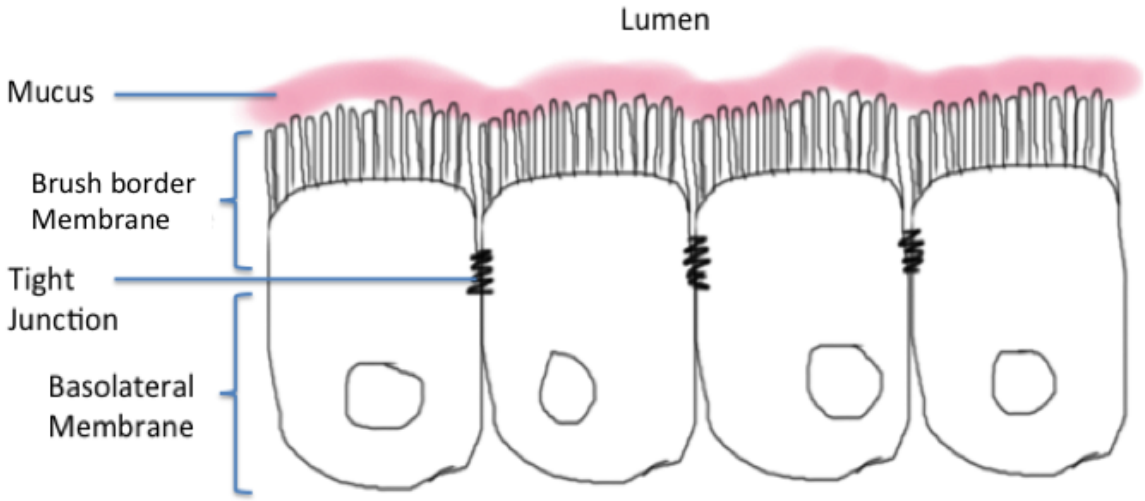


**Figure I-3. Tight junction between enterocytes.** (Su, 2013).

**Nutrient digestion and absorption at enterocyte**

The process of digestion, chemical breakdown of food by digestive enzymes, begins in the mouth. The final stage of digestion and nutrient absorption takes place in the intestinal lumen at the enterocyte surface (Johnson, 2007). At the brush border membrane, disaccharides are degraded into monosaccharides by saccharidases, and small peptides are further broken down to di- and tri-peptides or amino acids by peptidases (Van Beers, et al., 1995). Digestion is accomplished by hydrolysis by membrane bound enzymes produced by enterocytes. Transporters

located at the brush border mediate absorption of monosaccharides, amino acids and di- and tri-peptides (Johnson, 2007). Only a small fraction of the absorbed nutrients is used within the enterocyte. Most of the nutrients exit the cell via transporters located at the basolateral membrane. Once the nutrients are passed into the blood, they are exported via the portal vein to the liver and the rest of the body (Van Beers, et al., 1995).

**Protein digestion and absorption**

Dietary protein is required to supply the essential amino acids, which the body cannot produce or cannot synthesize rapidly enough, and replace nitrogen lost in the urine (Smith and Morton, 2010). Degradation of protein into di- and tri-peptides and free amino acids is accomplished by two kinds of proteolytic enzymes: endopeptidases and exopeptidases. Endopeptidases are digestive enzymes like pepsin produced by the stomach and pancreas secreted trypsin, chymotrypsin and elastase. These proteases secreted by the pancreas cleave peptide bonds in the center of the peptides. Exopeptidases cleave peptide bonds at the ends of the peptides, while carboxypeptidases break the peptide bond at the C-terminus and aminopeptidases at the N-terminus.

Di- and tri-peptides are transported across the brush border membrane via peptide transporters (SLC15 family members) (Daniel and Kottra, 2004). Most of the small peptides that enter the enterocyte are hydrolyzed by intracellular peptidases, and transported out of the cell via amino acid transporters at the basolateral membrane. Free amino acids cross the brush border membrane via different types of transporters depending on the size and the electrical property of the amino acids. Most amino acids that enter the enterocyte are transported out of the cell via different types of amino acid transporters, except glutamate is retained and mainly used by the enterocyte as an energy source (Smith and Morton, 2010). Transporters, their location and function discussed in this review are summarized in Table I-1.

| Abbreviation | Gene full name | Location | Function |
|---|---|---|---|
| APN | Aminopeptidase N | Brush border | Final digestion of peptides by N terminus cleavage |
| ASCT1 | Alanine, serine, cysteine and threonine transporter (SLC1A4) | Basolateral | $Na^+$-dependent neutral amino acid transporter |
| $b^{o,+}AT$ | Solute carrier family 7, member 9 (SLC7A9) | Brush border | $Na^+$-independent neutral/cystine, cationic amino acid exchanger |
| $B^0AT$ | Solute carrier family 6, member 14 (SLC4A14) | Brush border | $Na^+$-dependent neutral amino acid transporter |
| CAT1 | Cationic amino acid transporter-1 (SLC7A1) | Basolateral | Transport lysine, arginine and histidine |
| CAT2 | Cationic amino acid transporter-2 (SLC7A2) | Basolateral | Transport lysine, arginine and histidine |
| EAAT3 | Excitatory amino acid transporter 3 (SLC1A1) | Brush border | Transport aspartate, glutamate and cysteine |
| GLUT1 | Glucose transporter-1 (SLC2A1) | Basolateral | Transport glucose, galactose, mannose and glucosamine |
| GLUT2 | Glucose transporter-2 (SLC2A2) | Basolateral | Transport fructose, mannose, galactose, glucose and glucosamine |
| GLUT5 | Glucose transporter-5 (SLC2A5) | Brush border | Transport fructose |
| LAT1 | L type amino acid transporter-1 (SLC7A5) | Basolateral | Transport hydrophobic amino acids |
| LEAP2 | Liver-expressed antimicrobial peptide-2 | Cytosol | Innate immune factor |
| Pept1 | Peptide transporter-1 (SLC15A1) | Brush border | Transport di- and tripeptides |
| rBAT | Solute carrier family 3, member1 (*SLC3A1)* | Brush border | Dimerize with $b^{o,+}AT$ |
| SGLT1 | Sodium glucose transporter-1 (SLC5A1) | Brush border | Transport low concentrations of d-glucose |
| SI | Sucrase isomaltase | Brush border | Hydrolysis of sucrose and isomaltose |
| $y^+LAT1$ | $y^+$ L amino acid transporter-1 (SLC7A7) | Basolateral | $Na^+$-dependent neutral/cationic amino acid exchanger |
| $y^+LAT2$ | $y^+$ L amino acid transporter-2 (SLC7A6) | Basolateral | $Na^+$-dependent neutral/cationic amino acid exchanger |
| ZNT1 | Zinc transporter-1 | Basolateral | Efflux of $Zn^{2+}$ |

**Table I-1. Summary of intestinal genes in these studies.** SLC=Solute carrier.

***Aminopeptidase N (APN).*** The APN/CD13 is a type II metalloprotease that belongs to the M1 family of the Metallopeptidase, clan MA(E). There are two types of APN: the membrane bound aminopeptidase N and the soluble aminopeptidase N, each of which have many isoforms with different functions (Luan and Xu, 2007). APN cleaves neutral amino acids from the N-terminus of oligopeptides (Danziger, 2008). Besides its function as a digestive enzyme, APN is also involved in the trimming of antigens and in the process of antigen presentation (Luan and Xu, 2007).

***Peptide transporter 1 (Pept1).*** The PepT1 (SLC15A1) belongs to the proton-coupled oligopeptide transporter (POT) family (Hu, et al., 2008) and is a low-affinity, high-capacity di- and tri-peptide transporter (Gilbert, et al., 2008b; Hu, et al., 2008; Jappar, et al., 2010). Pept1 is responsible for absorption of most di- and tri- peptides and peptide-like drugs from the small intestinal lumen into the enterocytes (Hu, et al., 2008). Many tissues express PepT1, but the greatest level is found in the small intestine in both pre- and post-hatch chickens. Ceca and large intestine have much reduced expression. Very little expression of PepT1 is detected in other tissue and cell types (Zwarycz and Wong, 2013).

***$b^{o,+}$AT and rBAT transporter complex.*** The $b^{o,+}$AT (SLC7A9) and rBAT (SLC3A1) form a heteromeric amino acid transporter (HAT) by a disulfide bridge (Fotiadis, et al., 2013; Palacin and Kanai, 2004; Verrey, et al., 2004; Wagner, et al., 2001). The $b^{o,+}$AT is the light subunit, which has transporter function. rBAT is the heavy subunit, which is essential for trafficking the complex to the cell membrane (Palacin and Kanai, 2004; Verrey, et al., 2004). Transport activity of this complex is $Na^+$-independent and exchanges extracellular cationic amino acids and cystine for intracellular neutral amino acids (Fotiadis, et al., 2013). $b^{o,+}$AT has high-affinity transport of L-cystine and cationic amino acids and lower affinity transport of neutral amino acids (Verrey, et al., 2004).

***$Na^+$-dependent neutral amino acid transporter ($B^0$AT).*** The $B^0$AT (SLC6A19) transports a broad range of neutral amino acids into the cell (Broer, et al., 2004; Romeo, et al., 2006). Some of these neutral amino acids are used by $b^{o,+}$AT in exchange for cationic amino acids and cysteine (Fotiadis, et al., 2013). Transport of amino acids via $B^0$AT is driven by the membrane potential. The most preferred substrate for $B^0$AT is leucine in a pH-dependent manner, which

strongly increases with alkaline pH (Broer, et al., 2004). $B^0AT$ is highly expressed in the brush border membrane in the small intestine (Broer, et al., 2004; Terada, et al., 2005).

***Excitatory amino acid transporter 3 (EAAT3).*** EAAT3 (SLC1A1), also known as EAAC1, belongs to the $X^-_{AG}$ system, which is a $Na^+$-dependent transporter of anionic amino acids such as aspartate and glutamate and is located at the brush border membrane of enterocytes (Gilbert, et al., 2007; Kanai and Hediger, 1992; Speier, et al., 2012). Glutamate is one of the most abundant amino acids in dietary protein, but the blood concentration is quite low. This is because in the small intestine, glutamate is the energy source of the enterocytes (Iwanaga, et al., 2005) (Fan, et al., 2004), and is also used by the enterocytes to synthetize other amino acids (Blachier, et al., 2009). Fan et al., (2004) showed that EAAT3 is the major L-glutamate transporter. Expression of EAAT3 can be detected along the crypt-villus axis, but there is higher capacity and lower affinity transport activity in crypt than in villus cells (Fan, et al., 2004). In chickens, expression of EAAT3 is greatest in the ileum, which indicates higher uptake of glutamate in the lower part of the small intestine (Gilbert, et al., 2007).

***Alanine, serine, cysteine and threonine transporter (ASCT1).*** The ASCT1 (SLC1A4) is a $Na^+$-dependent neutral amino acid transporter. It was discovered by screening for expressed sequences similar to the sodium-coupled glutamate transporter GLAST1. Unlike the GLAST-related transporter family, ASCT1 does not transport glutamate or aspartate but alanine, serine, cysteine, and threonine (Hofmann, et al., 1994). The activity of ASCT1 requires extracellular $Na^+$, but does not need countertransport of $K^+$ as other GLASTs (Zerangue and Kavanaugh, 1996).

***Cationic amino acid transporters (CAT1/CAT2).*** The CATs (SLC7A) family members are transmembrane glycoprotein- associated amino acid transporters (Verrey, et al., 2004). CAT1 is a high-affinity, low-capacity transporter, while CAT2 is a low-affinity, high-capacity transporter. They are both located at the basolateral membrane of the intestinal enterocyte and are responsible for the efflux of cationic amino acids (Fotiadis, et al., 2013; Verrey, et al., 2004). CATs also play a key role in nitric oxide synthesis by delivering L-arginine for nitric oxide synthase in certain cells (Fotiadis, et al., 2013).

***L-type amino acid transporter 1 (LAT1).*** LAT1 (SLC7A5) was the first cloned light subunit of HATs (Kanai, et al., 1998). It heterodimerizes with the heavy chain 4F2hC protein and mediates

the $Na^+$-independent exchange of large neutral amino acids across the basolateral membrane (Verrey, et al., 2004). Like rBAT, 4F2hC functions to translocate the complex to the cell membrane (Fotiadis, et al., 2013; Verrey, et al., 2004; Wagner, et al., 2001). LAT1 is an obligatory exchanger with a 1:1 ratio (Fotiadis, et al., 2013; Wagner, et al., 2001). The affinity for amino acids is up to 100-fold higher at the extracellular side when compared to the cytosolic side of the transporter. Transport sidedness also affects substrate selectivity, L-leucine, L-isoleucine and L-methionine are better efflux than influx substrates (Fotiadis, et al., 2013).

***$y^+$L amino acid transporters ($y^+$LATs).*** $y^+$LAT1 (SLC7A7) and $y^+$LAT2 (SLC7A6) both heterodimerize with 4F2hC protein, and function as obligatory exchangers of cationic amino acids ($Na^+$-independent) and neutral amino acids ($Na^+$-dependent) (Fotiadis, et al., 2013). They both function in cationic amino acid efflux in the kidney and small intestine at the basolateral membrane (Broer, et al., 2000; Fotiadis, et al., 2013). But $y^+$LAT2 preferentially mediates the efflux of L-arginine in exchange for L-glutamine and $Na^+$. Also $y^+$LAT2 has a broader tissue distribution than $y^+$LAT1 (Broer, et al., 2000).

**Carbohydrate digestion and absorption**

In chicken, the major source of carbohydrates is starch in grains. Digestion of carbohydrates provides an energy source for the body. There are several enzymes in the gastrointestinal tract that degrade starch and glycogen. Sucrase isomaltase secreted by the intestinal cells are responsible for the major part of the final digestion of polysaccharides. Hydrolyzed monosaccharides such as glucose, galactose, mannose and fructose are transported into the enterocyte by the $Na^+$-dependent glucose transporter 1 (SGLT1) and glucose transporter 5 (GLUT5), and exit the cell via glucose transporter 2 (GLUT2) and glucose transporter 1 (GLUT1)(Smith and Morton, 2010). SGLT1, GLUT5 and GLUT2 are the most abundant monosaccharide transporters in the small intestine compared with other hexose carriers (Yoshikawa, et al., 2011).

***Sucrase isomaltase (SI).*** SI is an enzyme complex that is responsible for 80% of the maltase activity in the small intestine (Van Beers et al., 1995). The sucrase subunit hydrolyzes sucrose, but not α(1-6) glucosidic bonds. The isomaltase subunit hydrolyzes α(1-6) glucosidic bonds but not sucrose. Both subunits hydrolyze maltose, maltotriose and hydrophobic aryl- α-glucopyranosides. The complex has no activity towards polysaccharides like starch (Van Beers

et al., 1995). SI is highly expressed in the small intestine, which accounts for 10% of the brush border membrane protein. In chicken, expression of SI has been reported in embryonic and post-hatch intestine (Sklan et al., 2003). Very low expression of SI has been detected in the embryonic yolk sac membrane (Yadgary et al., 2011).

***Sodium-dependent glucose transporter-1 (SGLT1).*** SGLT1 (SLC5A1) is a $Na^+$-dependent glucose cotransporter at the brush border membrane, which was the first cotransporter protein identified using rabbit intestine (Wright and Turk, 2004). SGLT1 is a uniporter, i.e., it pumps one glucose molecule into the cell along with 2 $Na^+$ ions (Hediger and Rhoads, 1994). Expression of SGLT1 can be found at the plasma membrane of cells located at the small intestine, trachea, kidney and heart (Wright and Turk, 2004). SGLT1 is highly expressed in the duodenum, and the expression level decreases in the distal part of the small intestine in mice (Yoshikawa, et al., 2011). In chickens, SGLT1 expression level is higher in the jejunum and ileum than duodenum (Gilbert, et al., 2007).

***Glucose transporter-5 (GLUT5).*** The GLUT5 (SLC2A5) is a $Na^+$-independent high-affinity fructose transporter. It has no glucose transport activity in human and limited glucose transport in rat (Garriga, et al., 2004; Uldry and Thorens, 2004). GLUT5 is located at the brush border membrane of the enterocyte and transports fructose from the intestinal lumen into the cell (Le Gall, et al., 2007). GLUT5 is expressed in many tissues and organs (Yang, et al., 2002). Like SGLT1, GLUT5 has higher expression level in the duodenum in mice (Yoshikawa, et al., 2011), but higher expression in the jejunum and ileum in chickens (Gilbert, et al., 2007).

***Glucose transporter-1 (GLUT1).*** The GLUT1 (SLC2A1) is a $Na^+$-independent transporter for glucose, galactose, mannose and glucosamine (Zhao and Keating, 2007). It was the first glucose transporter to be identified (Boyer, et al., 1996). The expression of GLUT1 in the small intestine is not as abundant as in the stomach and large intestine (Yoshikawa, et al., 2011). GLUT1 normally can only be detected at the basolateral membrane of the enterocytes, but it is present in the brush border membrane in diabetic rats (Boyer, et al., 1996).

***Glucose transporter-2 (GLUT2).*** The GLUT2 (SLC2A2) transporter is located at the basolateral membrane, mediates the $Na^+$-independent, low-affinity transport of glucose, galactose, mannose and fructose, and high-affinity transport of glucosamine (Uldry and Thorens, 2004). GLUT2

translocation to the brush border membrane has also been reported (Mithieux, 2005). In mice, GLUT2 is highly expressed in the proximal half of the small intestine (Yoshikawa, et al., 2011).

### Mineral absorption

Absorption of ion minerals and trace elements occurs in the jejunum and ileum. Calcium, magnesium and phosphate can be absorbed by passive diffusion, but also can be transported across the membrane by active transporters like other ions such as sodium and zinc (Leeson, et al., 2001). The rate of absorption depends on pH, membrane potential, transporters and the presence of other minerals (Leeson, et al., 2001). The body requires minerals in many physiological processes (Smith and Morton, 2010). Calcium is important in bone development and cellular signaling pathways. Magnesium is an important co-factor for many enzymes. Phosphate is also involved in bone formation, acid-base balance and nucleic acid synthesis. Sodium is the key element in maintaining and changing membrane potential, and is also required for many nutrient co-transporters as discussed above. Zinc is a trace mineral, which functions as a cofactor of enzymes, nuclear factors and hormones (Devergnas, et al., 2004).

***Zinc transporter 1 (ZNT1).*** The ZNT proteins are members of the cation diffusion facilitator family. They function in transporting zinc out of the cells or contained in cellular compartments (Tako, et al., 2005). ZNTs are expressed in a tissue-specific manner, ZNT1 is ubiquitously expressed in the body, but is most abundant at the basolateral membrane of enterocytes in the duodenum and jejunum (McMahon and Cousins, 1998). Expression of ZNT1 in the small intestine can be induced by increasing dietary zinc, which was first found in rat (McMahon and Cousins, 1998) and later confirmed in chicken (Tako, et al., 2005).

## Avian coccidiosis and *Eimeria*

Coccidiosis is a major disease of poultry caused by the intestinal protozoa *Eimeria* (Conway and McKenzie, 2007). Lesions in the intestinal mucosa reduce feed efficiency and body weight gain. A damaged intestinal barrier leads to bacterial infection, which can increase mortality in birds. Coccidiosis is responsible for the loss of billions of dollars in the poultry industry worldwide (Dalloul, et al., 2007). The common treatment for coccidiosis is use of anticoccidial drugs, but large-scale and long-term use of these drugs has led to the worldwide development of resistance against most of these drugs. Live attenuated and non-attenuated anticoccidial vaccines have

shown positive results in preventing coccidiosis. Highly efficient and low-cost anticoccidial vaccines could potentially replace anticoccidial drugs in the future (Peek and Landman, 2011).

**Life cycle of *Eimeria***

The life cycle of *Eimeria* takes about 4 to 7d to complete. The bird can pick up oocysts from the environment by swallowing infected litter. The life cycle of *Eimeria* is shown in Figure I-4. An oocyst contains 4 sporocysts, which each contains 2 sporozoites. Oocysts are generally ovoid to ellipsoid in shape, and range from 10-40µm in length by 10-30µm in width. The wall of the oocyst contains peptide, lipid and carbohydrate. The likely physical arrangement of the components places the lipid in a 10 nm thick outer layer, covering a 90 nm thick layer of glycoprotein (Stotish, et al., 1978). After the bird consumes the oocyst, 8 sporozoites will be released into the digestive system. The sporozoites will invade the intestinal epithelial cells, and use the host cell as a nutrient supply for replication. After several generations of asexual multiplication, a sexual stage occurs in which male and female gametes unite and form new oocysts that are protected by a thick wall. These oocysts are shed in the feces, to be picked up by other animals (Allen and Fetterer, 2002).

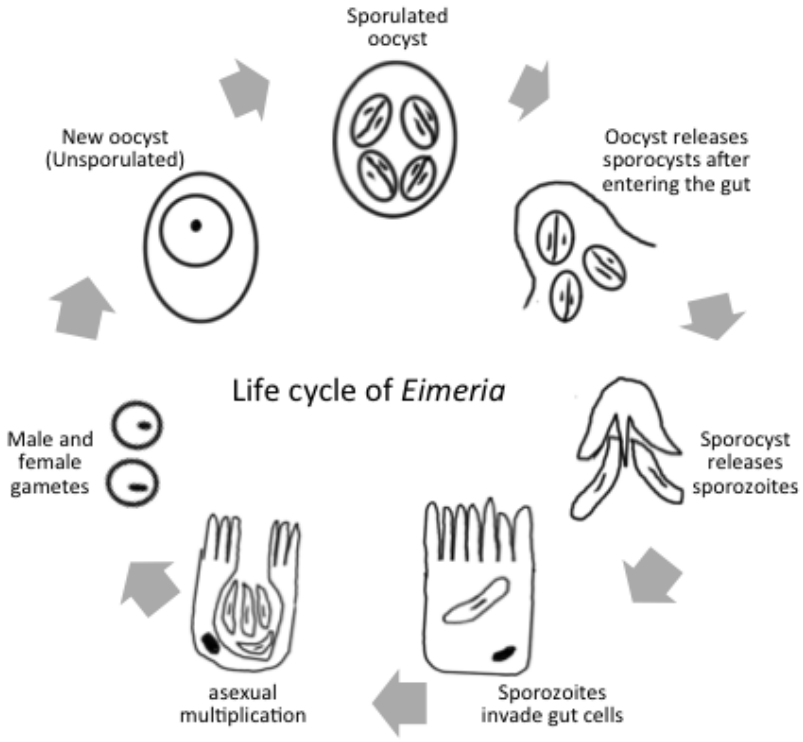


**Figure I-4. Life cycle of *Eimeria*.** (not drawn to scale) (Su, 2013).

### *Eimeria* infection in chicken

*Eimeria* infection is species- and site-specific. The species of *Eimeria* that infect chickens are different from those that infect turkeys. In the U.S., the three species of *Eimeria* that most impact the poultry industry are *E. acervulina*, *E. maxima* and *E. tenella*. *E. acervulina* infects the duodenum, *E. maxima* the jejunum, and *E. tenella* the ceca (Lillehoj and Trout, 1996). Lesions in the intestinal mucosa can be measured on a scale of 0 to 4. A score of 0 shows no lesions, and a score of 4 shows many lesions (Johnson and Reid, 1970). Chickens challenged with *E. maxima* oocysts yield the same lesion score as chickens challenged with a higher dose of *E. acervulina* or *E. tenella* oocysts. A study conducted to compare the relative sensitivities of *E. acervulina*, *E. maxima*, and *E. tenella* oocysts to dessication showed that *E. maxima* oocysts have greater resistance to drying compared to *E. acervulina* and *E. tenella* (Jenkins, et al., 2013).

### Immune response to *Eimeria* challenge in chicken

The mucosal immune system is composed of the mucosal associated lymphoid tissues (MALT) that attacks the pathogen at its site of entry (Yun, et al., 2000a). Gut associated lymphoid tissues (GALT) are the largest component of MALT. Unlike mammals, chickens do not have lymph nodes, they have lymphoid structures such as the bursa of Fabricius, cecal tonsils, Meckel's diverticulum and Peyer's patches (PP) (Lillehoj and Trout, 1996). Microfold cells at the PP present antigens to T lymphocytes at the epithelial layer and antibody-producing B lymphocytes at the lamina propria. Immunoglobulin A (IgA) is produced by B lymphocytes (Lillehoj and Trout, 1996). Following *E. maxima* infection, intestinal IgA level and cytokine interferon-gamma (IFN-gamma) level were increased (Yun, et al., 2000b). Analysis of *E. acervulina*, *E. tenella* and *E. maxima* treated chicken macrophages showed common regulation of interleukins (IL) and chemokines. There was induced expression of IL-1β, IL-6, and IL-18 and repressed expression of IL-16. Expression of macrophage inflammatory protein (MIP)-1β (CCLi1), K203 (CCLi3), and ah221 (CCLi7) are commonly increased but CXCL chemokine K60 (CXCLi1) was found to be induced by macrophage exposure to *E. tenella* only (Dalloul, et al., 2007).

### Liver-expressed antimicrobial peptide-2 (LEAP2)

The chicken LEAP2 gene was first discovered by bioinformatics screening of the chicken genome (Lynn, et al., 2004). Based on the *in silico* sequence, LEAP2 expression was detected in a number of tissues including the small intestine, liver, lung and kidney (Townes, et al., 2004).

Birds orally challenged with *Salmonella enterica* showed upregulation of LEAP2 expression in both small intestine and liver. An *in vitro* assay showed that LEAP2 has antimicrobial activity against *Salmonella* (Townes, et al., 2004). Later, the same research group discovered that LEAP2 could interact with the outer membrane of the bacteria and change its permeability. LEAP2 also has broad-spectrum antimicrobial activity and plays an important role in the chicken innate host defense (Townes, et al., 2009). In contrast, in *E. maxima*-challenged chickens, LEAP2 showed up to 71-fold downregulation in the jejunum, and chickens with greater lesion scores showed greater downregulation of LEAP2. The mechanism behind this expression pattern is to be further investigated, but it is hypothesized that *E. maxima* causes a downregulation of LEAP2 in the intestinal epithelia (Casterlow, et al., 2011).

### Expression of digestive enzymes and nutrient transporters in *E. maxima*- challenged chickens

*Eimeria* infection reduces weight gain, which could be due to changes in expression of nutrient transporters. Analysis of the jejunum of *E. maxima*-challenged broilers showed decreased expression of the brush border membrane amino acid transporters EAAT3 and $b^{o,+}AT$. Expression of the basolateral amino acid transporters LAT1 and ASCT1 was increased, whereas the zinc transporter was decreased (Paris and Wong, 2013). These results suggest that changes in expression of amino acid transporters may cause depletion of the energy source glutamate and some essential amino acids, which may be part of the host defense mechanism for eliminating infected cells and inhibition of pathogen replication.

## Objectives

Changes in expression of digestive enzymes and nutrient transporters in *Eimeria*-challenged chickens have been only studied in the jejunum of *E. maxima*-challenged broilers. In this thesis, the expression of digestive enzymes (APN and SI), a peptide transporter (Pept1), amino acid transporters (ASCT1, $b^{o,+}AT$/rBAT, $B^0AT$, CAT1, CAT2, EAAT3, LAT1, $y^+LAT1$ and $y^+LAT2$), sugar transporters (GLUT1, GLUT2, GLUT5 and SGLT1), a mineral transporter (ZNT1) and an immune factor (LEAP2) was examined in the duodenum, jejunum, ileum and ceca of *Eimeria*-challenged layers and broilers. The objective of the first experiment was to examine the effect of chicken breeds on gene expression following an *E. acervulina*-challenge. The objective of the second experiment was to compare *E. acervulina*-, *E. maxima*- and *E.*

*tenella*-challenged broilers to examine the effect of *Eimeria* species on digestive enzyme and nutrient transporter expression.

# CHAPTER II. EXPRESSION OF DIGESTIVE ENZYMES AND NUTRIENT TRANSPORTERS IN *EIMERIA ACERVULINA*-CHALLENGED LAYERS AND BROILERS

## ABSTRACT

Avian coccidiosis is caused by the intestinal protozoa *Eimeria*. Lesions in the intestinal mucosa cause reduced feed efficiency and body weight gain in *Eimeria*-challenged chickens. The growth reduction may be due to changes in expression of digestive enzymes and nutrient transporters in the intestine. The objective of this thesis was to examine the expression of digestive enzymes: APN and SI, peptide and amino acid transporters: Pept1, ASCT1, $b^{o,+}$AT/rBAT, $B^0$AT, CAT1/2, EAAT3, LAT1 and $y^+$LAT1/2, sugar transporters: GLUT1, GLUT2, GLUT5 and SGLT1, mineral transporter: ZNT1 and an immune factor: LEAP2 in the duodenum, jejunum and ileum of *E. acervulina*-challenged layers and broilers. Layers and broilers showed common downregulation of APN, $b^{o,+}$AT, $B^0$AT, CAT2, EAAT3, GLUT2, rBAT, SI, ZNT1 and LEAP2 in the duodenum. In the jejunum and ileum there were no changes in expression of the genes examined in broilers but there were many changes in layers. These changes in intestinal digestive enzyme and nutrient transporter gene expression may result in a decrease in the efficiency of protein digestion, uptake of essential amino acids and the energy source (glutamate), and disruption of mineral balance. This may ultimately lead to cell death and may be part of the host defense mechanism for eliminating infected cells and inhibition of pathogen replication.

## INTRODUCTION

Avian coccidiosis is characterized by destruction of the mucosa and is caused by the intestinal protozoa *Eimeria* (Conway and McKenzie, 2007). Lesions in the intestinal mucosa reduce feed efficiency and body weight gain, and increase mortality in birds. Coccidiosis is responsible for the loss of billions of dollars in the poultry industry worldwide (Dalloul, et al., 2007). The parasite's site of infection in the small intestine is site specific and *Eimeria acervulina* mainly infects the duodenum (Lillehoj and Trout, 1996). In chicken, the small intestine is where the majority of nutrient absorption occurs (Leeson, et al., 2001). The final digestion of protein and polysaccharides is catalyzed by membrane bound peptidases and glucosidases, respectively. Short peptides, free amino acids and monosaccharides are absorbed by the enterocytes by specific transporters located at the brush border membrane and basolateral membrane (Leeson, et

al., 2001). The growth depression in *Eimeria*-challenged chickens may be due to changes in expression of digestive enzymes and nutrient transporters in the small intestine.

Layer and broiler chickens have been genetically selected for generations to increase egg production or rapid growth, respectively (Koenen, et al., 2002; Yuan, et al., 2009). As a consequence of the selection, these two types of chickens demonstrate striking differences in food intake, body weight gain, body composition and duration of life caused by genetic differences (Koenen, et al., 2002). In general, layers have lower food intake (Hocking, et al., 1997), protein intake (Shariatmadari and Forbes, 1993) and body weight gain (Swennen, et al., 2007) when compared to broilers. Layers and broilers also showed differences in their immunological response to the specific antigen TNP-KLH (trinitrophenyl-conjugated keyhole limpet hemocyanin) (Koenen, et al., 2002). These results demonstrated that broilers display a strong short-term humoral immune response and layers have a long-term humoral response in combination with a strong cellular response. The differences between layers and broilers in innate immune response need to be further analyzed. Upon *Eimeria* challenge, the changes in expression of digestive enzymes and nutrient transporters in the small intestine may also be different between layers and broilers. The objective of this study was to compare changes in nutrient transporter and digestive enzyme gene expression in different sections of the small intestine of layers and broilers following infection with *E. acervulina*.

## MATERIALS AND METHODS

### Chicken and *Eimeria*

This study was 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). Chickens used in this study were Sexsal layer males, which are White rock females crossed with Rhode Island Red males from Moyers hatchery (Quakertown, PA), and Ross Heritage broiler males from Longeneckers Hatchery (Elizabthetown PA). Birds were housed in suspended wire cages (46cm x 30cm = 1380cm$^2$) with 2-3 birds per cage. Birds were fed a standard poultry starter ration (crumbles, 24% protein) and had free access to water. *Eimeria acervulina* was USDA #12 isolate. 1 day old chicks were transported to the USDA-ARS facility (Beltsville, MD) and were orally gavaged with either 1mL *E. acervulina* oocysts at 200,000 oocysts/ bird or not gavaged (control) at 21 d of age.

**Tissue sampling**

Seven days post challenge chickens were euthanized by cervical dislocation and intestinal segments were collected. Duodenum, jejunum and ileum samples were collected from Sexsal layers (n=5) and Ross Heritage broilers (n=6). The contents of the intestine were squeezed out and the tissue segments were immediately stored individually in RNAlater (Invitrogen, Grand Island, NY). The samples were stored at 4 °C for 24 hrs and then were frozen at -70 °C before being shipped to Virginia Tech. Upon arrival each intestinal segment was removed from RNAlater. After homogenizing, a 20-30 mg tissue aliquot was placed in a 2-mL microfuge tube for RNA extraction and the remaining homogenate was placed in a separate 2-mL microfuge tube. Both tubes were frozen on dry ice and stored at -80°C.

**Total RNA extraction**

The 20-30mg of tissue was placed in 500µL Tri Reagent (Molecular Research Center Inc., Cincinnati, OH) and shaked twice at 25Hz/s for 2 min using a TissueLyser II (QIAGEN Inc., Valencia, CA) following the animal tissue protocol. After homogenization100 µL of chloroform were added for phase separation. The RNA pellet was suspended in 0.1% DEPC (Diethylpyrocarbonate, Sigma-Aldrich, St. Louis, MO) treated water depending on the pellet size and incubated for 10 minutes at 56°C. RNA concentration was determined using a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Any sample that had a concentration greater than 2000ng/µL was further diluted and reassayed. RNA quality was assessed by agarose-formaldehyde gel electrophoresis. All extracted RNA samples were stored at -80°C.

**Reverse Transcription**

Total RNA was diluted to 0.1 µg/µL in DEPC water. cDNA was synthesized using the high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Each 20µL reverse transcription reaction contained 2 µL 10X reverse transcription buffer, 2 µL 10X random primers, 1 µL multiscribe reverse transcriptase (50 U/µL), 0.8 µL 25x dNTPs, 9.2 µL DEPC water, and 5 µL of 0.1 µg/µL diluted RNA sample. The RNA and master mix were combined in a 0.5-mL microfuge tube, which was then run in a thermocycler for 10 min at 25 °C followed by 120 min at 37 °C and 5 min at 85 °C. The cDNA was diluted 1:20 with DEPC water and stored at -20°C.

**Quantitative Real-Time PCR**

Quantitative real-time PCR (qPCR) was performed using 96-well plates. Each reaction contained 5 µL diluted cDNA, 20 µL of PCR master mix which contained 12.5 µL 2X SYBR Green Master Mix (Applied Biosystems), 0.5 µL forward primer (5 µM), 0.5 µL reverse primer (5 µM), and 6.5 µL DEPC water. Each reaction was run in duplicate. The plate was sealed with a MicroAmp Optical Adhesive Film (Applied Biosystems) and spun down in a centrifuge to mix reagents and remove bubbles and loaded into an Applied Biosystems 7300 Real-Time PCR instrument (Applied Biosystems). The following real time PCR conditions were used: 95 °C for10 min followed by 40 cycles of 95 °C for 15s and 60 °C for 1 min. Genes analyzed were APN, ASCT1, $b^{o,+}AT$, $B^0AT$, CAT1, CAT2, EAAT3, GLUT1, GLUT2, GLUT5, LAT1, LEAP2, Pept1, rBAT, SGLT1, SI, $y^+LAT1$, $y^+LAT2$ and ZNT1 (Table I-1). The endogenous control was the *chicken beta-actin* gene. All forward and reverse primer sequences are shown in Table II- 1.Primers were designed using the Primer Express software (Applied Biosystems) and synthesized by Eurofins MWG Operon (Huntsville, AL).

| Gene[1] | Forward Primer | Reverse Primer |
|---|---|---|
| Beta-actin | GTCCACCGCAAATGCTTCTAA | TGCGCATTTATGGGTTTTGTT |
| APN | AATACGCGCTCGAGAAAACC | AGCGGGTACGCCGTGTT |
| ASCT1 | TTGGCCGGGAAGGAGAAG | AGACCATAGTTGCCTCATTGAATG |
| $b^{0,+}$AT | CAGTAGTGAATTCTCTGAGTGTGAAGCT | GCAATGATTGCCACAACTACCA |
| $B^0$AT | GGGTTTTGTGTTGGCTTAGGAA | TCCATGGCTCTGGCAGAGAT |
| CAT1 | CAAGAGGAAAACTCCAGTAATTGCA | AAGTCGAAGAGGAAGGCCATAA |
| CAT2 | TGCTCGCGTTCCCAAGA | GGCCCACAGTTCACCAACAG |
| EAAT3 | TGCTGCTTTGGATTCCAGTGT | AGCAATGACTGTAGTGCAGAAGTAATATATG |
| LAT1 | GATTGCAACGGGTGATGTGA | CCCCACACCCACTTTTGTTT |
| LEAP2[2] | CTCAGCCAGGTGTACTGTGCTT | CGTCATCCGCTTCAGTCTCA |
| GLUT1 | TCCTCCTGATCAACCGCAAT | TGTGCCCCGGAGCTTCT |
| GLUT2 | CACACTATGGGCGCATGCT | ATTGTGCCTGGAGGTGTTGGT |
| GLUT5 | TTGCTGGCTTTGGGTTGTG | GGAGGTTGAGGGCCAAAGTC |
| Pept1 | CCCCTGAGGAGGATCACTGTT | CAAAAGAGCAGCAGCAACGA |
| rBAT | CCCGCCGTTCAACAAGAG | AATTAAATCCATCGACTCCTTTGC |
| SGLT1 | ATACCCAAGGTCATAGTCCCAAAC | TGGGTCCCTGAACAAATGAAA |
| SI | CGCAAAAGCACAGGGACAGT | TCGATACGTGGTGTGCTCAGTT |
| $y^+$LAT1 | CAGAAAACCTCAGAGCTCCCTTT | TGAGTACAGAGCCAGCGCAAT |
| $y^+$LAT2 | GCCCTGTCAGTAAATCAGACAAGA | TTCAGTTGCATTGTGTTTTGGTT |
| ZNT1[3] | TCCGGGAGTAATGGAAATCTTC | AATCAGGAACAAACCTATGGGAAA |

**Table II-1. Forward and reverse primers of genes analyzed.**

[1]Primer sequence designed by Gilbert, et al., 2007, unless noted separately.
[2]Primer designed by Casterlow, et al., 2011.
[3]Primer designed by Paris and Wong, 2013.

**Quantitative Real-Time PCR Analysis**

All plates were analyzed individually using the software provided with the 7300

Real-Time PCR instrument and raw Ct data was obtained. Average gene expression relative to the endogenous control for each sample was calculated using the $2^{-\Delta\Delta Ct}$ method described by Livak and Schmittgen (2001). The average ΔCt of the control samples was used to calculate the ΔΔCt value, which was performed separately for each intestinal segment, *Eimeria* treatment and each gene are a group. Data points that exceed ±3 standard deviations from the mean were discarded as outliers.

**Statistical Analysis.**

All data were analyzed by one-way ANOVA using JMP® Statistical Discovery Software from SAS (SAS Institute, Cary, NC). Layers and broilers were analyzed separately. The model included the main effects of treatment, sorted by genes. Significance level was set at $P < 0.05$ when compared to the control.

## RESULTS

***E. acervulina*-challenged layers**

Changes in expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in *E. acervulina*-challenged layers are shown in Table II-1. Expression of amino acid transporters $b^{o,+}AT$, $B^0AT$, rBAT and EAAT3 was decreased to 35%, 19%, 27% and 18% of control, respectively, in the duodenum and to 50%, 46%, 48% and 38% of control, respectively, in the jejunum of *E. acervulina*-challenged layers. CAT2 was decreased to 54%, 61% and 73% of control in the duodenum, jejunum and ileum, respectively, and LAT1 was decreased to 63% of control in the ileum of *E. acervulina*-challenged layers. y+LAT1 was decreased to 68%, 56% and 68% in the duodenum, jejunum and ileum, respectively, and y+LAT 2 was decreased to 70% of control in the jejunum. Peptide transporter Pept1 was decreased to 65% and increased 2-fold in jejunum and ileum, respectively, in *E. acervulina*-challenged layers.

Expression of sugar transporters GLUT1 was decreased to 86% of control in the ileum; GLUT2 was decreased to 40% and 27% of control in the duodenum and jejunum, respectively; SGLT1 was upregulated 1.9 fold in the ileum of *E. acervulina*-challenged layers. Expression of digestive enzyme APN was decreased to 44% of control in the duodenum and increased 1.5-fold in the

ileum; Sucrase isomaltase (SI) was decreased to 55% and 61% of control in the duodenum and jejunum, respectively; zinc transporter (ZNT1) was decreased to 50% and 67% of control in the duodenum and jejunum, respectively, in *E. acervulina*-challenged layers. The antimicrobial peptide LEAP2 was reduced to 15% and 59% of control in the duodenum and jejunum, respectively, and increased 2.4-fold in the ileum of *E. acervulina*-challenged layers.

***E. acervulina*-challenged broilers**

Changes in expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in *E. acervulina*-challenged broilers are shown in Table II-2. Expression of APN, $b^{o,+}AT$, $B^0AT$, rBAT, CAT2 and EAAT3 was decreased to 46%, 24%, 31%, 25% 56% and 25% of control, respectively, in the duodenum. SI, GLUT2 and GLUT5 were decreased to 27%, 11% and 36% of control respectively, in the duodenum. ZNT1 was decreased to 43% and LEAP2 was decreased to 6% of control in the duodenum of *E. acervulina*- challenged broilers. No changes in gene expression were observed in the jejunum and ileum of *E. acervulina*- challenged broilers.

| | | Relative gene expression | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | Group | APN | ASCT1 | $b^{0,+}$AT | $B^0$AT | CAT1 | CAT2 | EAAT3 |
| DU | Cont | 1.08±0.31 | 1.00±0.05 | 1.00±0.02 | 1.08±0.30 | 1.54±0.51 | 1.02±0.12 | 1.08±0.31 |
| | *E.ace* | 0.47±0.04 | 0.93±0.06 | 0.35±0.05 | 0.20±0.03 | 2.10±0.16 | 0.55±0.03 | 0.19±0.01 |
| | P-val | 0.04* | 0.71 | <.0001* | 0.006* | 0.27 | 0.004* | 0.006* |
| JE | Cont | 1.02±0.10 | 1.02±0.10 | 1.03±0.13 | 1.05±0.14 | 1.54±0.39 | 1.01±0.07 | 1.01±0.08 |
| | *E.ace* | 0.78±0.05 | 0.82±0.06 | 0.52±0.06 | 0.48±0.05 | 2.10±0.22 | 0.62±0.08 | 0.38±0.03 |
| | P-val | 0.06 | 0.11 | 0.007* | 0.006* | 0.25 | 0.006* | <.0001* |
| IL | Cont | 1.07±0.17 | 1.02±0.12 | 1.17±0.29 | 1.14±0.26 | 1.48±0.36 | 1.01±0.08 | 1.25±0.33 |
| | *E.ace* | 1.62±0.05 | 0.80±0.07 | 1.42±0.20 | 1.84±0.27 | 1.81±0.10 | 0.74±0.03 | 1.44±0.14 |
| | P-val | 0.02* | 0.14 | 0.50 | 0.10 | 0.41 | 0.01* | 0.62 |

| | | Relative gene expression | | | | | |
|---|---|---|---|---|---|---|---|
| Tissue | Group | GLUT1 | GLUT2 | GLUT5 | LAT1 | LEAP2 | PEPT1 |
| DU | Cont | 1.00±0.03 | 1.05±0.21 | 1.04±0.10 | 1.00±0.07 | 1.14±0.36 | 1.08±0.31 |
| | *E.ace* | 0.93±0.08 | 0.42±0.08 | 0.82±0.20 | 1.31±0.15 | 0.17±0.33 | 0.80±0.23 |
| | P-val | 0.31 | 0.007* | 0.37 | 0.13 | 0.01* | 0.39 |
| JE | Cont | 1.00±0.06 | 1.05±0.15 | 1.03±0.12 | 1.03±0.12 | 1.04±0.13 | 1.02±0.11 |
| | *E.ace* | 0.96±0.03 | 0.28±0.06 | 0.80±0.06 | 1.18±0.05 | 0.61±0.07 | 0.66±0.13 |
| | P-val | 0.46 | 0.001* | 0.11 | 0.27 | 0.02* | 0.07* |
| IL | Cont | 1.00±0.03 | 1.05±0.21 | 1.04±0.10 | 1.03±0.13 | 1.19±0.30 | 1.12±0.25 |
| | *E.ace* | 0.93±0.08 | 0.42±0.08 | 0.82±0.20 | 0.65±0.02 | 2.87±0.29 | 2.22±0.29 |
| | P-val | 0.01* | 0.35 | 0.66 | 0.02* | 0.004* | 0.02* |

| | | Relative gene expression | | | | | |
|---|---|---|---|---|---|---|---|
| Tissue | Group | rBAT | SGLT1 | SI | $y^+$LAT1 | $y^+$LAT2 | ZNT1 |
| DU | Cont | 1.03±0.17 | 1.05±0.18 | 1.02±0.11 | 1.00±0.06 | 1.50±0.48 | 1.03±0.17 |
| | *E.ace* | 0.28±0.04 | 1.16±0.16 | 0.56±0.16 | 0.68±0.04 | 1.07±0.07 | 0.51±0.07 |
| | P-val | 0.0006* | 0.59 | 0.03* | 0.001* | 0.29 | 0.009* |
| JE | Cont | 1.00±0.04 | 1.01±0.08 | 1.00±0.05 | 1.01±0.09 | 1.00±0.06 | 1.02±0.10 |
| | *E.ace* | 0.48±0.03 | 1.14±0.13 | 0.61±0.07 | 0.57±0.05 | 0.70±0.03 | 0.68±0.10 |
| | P-val | <.0001* | 0.42 | 0.001* | 0.002* | 0.002* | 0.05* |
| IL | Cont | 1.18±0.28 | 1.07±0.16 | 1.07±0.18 | 1.00±0.07 | 1.10±0.20 | 1.06±0.18 |
| | *E.ace* | 1.44±0.19 | 2.04±0.20 | 1.55±0.32 | 0.68±0.03 | 1.45±0.13 | 1.36±0.13 |
| | P-val | 0.46 | 0.006* | 0.23 | 0.003* | 0.18 | 0.23 |

**Table II-2. Expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in *E. acervulina*-challenged layers.** DU=duodenum, JE=jejunum, IL=ileum, Cont=control chicks, *E.ace=E.acervulina*, P-val=P-value. Relative gene expression was determined using the $2^{-\Delta\,\Delta Ct}$ method. APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}$AT and $B^0$AT= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT1 and CAT2= Cationic amino acid transporter-

1 and -2; EAAT3= Excitatory amino acid transporter 3; GLUT1, GLUT2 and GLUT5= Glucose transporter-1, -2 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}$AT; SGLT1= Sodium glucose transporter-1; SI= Sucrase isomaltase; $y^+$LAT1 and $y^+$LAT2= $y^+$ L amino acid transporter-1 and -2, respectively; ZNT1= Zinc transporter-1. * Indicates statistical significance from control at $p<0.05$.

| | | Relative gene expression | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | Group | APN | ASCT1 | $b^{0,+}$AT | $B^0$AT | CAT1 | CAT2 | EAAT3 |
| DU | Cont | 1.08±0.16 | 1.04±0.12 | 1.10±0.20 | 1.18±0.25 | 2.89±1.67 | 1.11±0.16 | 1.13±0.23 |
| | *E.ace* | 0.50±0.09 | 1.28±0.23 | 0.26±0.03 | 0.37±0.04 | 11.45±3.37 | 0.62±0.06 | 0.28±0.05 |
| | P-val | 0.005* | 0.83 | 0.0001* | 0.01* | 0.17 | 0.02* | 0.002* |
| JE | Cont | 1.14±0.23 | 1.05±0.16 | 1.30±0.46 | 1.47±0.64 | 3.39±1.93 | 1.03±0.11 | 1.31±0.41 |
| | *E.ace* | 0.89±0.16 | 1.07±0.10 | 0.87±0.26 | 0.88±0.26 | 10.50±2.90 | 1.39±0.44 | 0.74±0.11 |
| | P-val | 0.50 | 1.00 | 0.81 | 0.65 | 0.61 | 0.58 | 0.56 |
| IL | Cont | 1.04±0.13 | 1.05±0.14 | 1.11±0.23 | 1.07±0.19 | 3.99±2.73 | 1.09±0.20 | 1.10±0.22 |
| | *E.ace* | 0.88±0.06 | 1.14±0.13 | 0.87±0.13 | 1.10±0.19 | 9.58±2.70 | 1.11±0.17 | 1.11±0.17 |
| | P-val | 0.37 | 0.99 | 0.49 | 1.00 | 0.49 | 1.00 | 1.00 |

| | | Relative gene expression | | | | | |
|---|---|---|---|---|---|---|---|
| Tissue | Group | GLUT1 | GLUT2 | GLUT5 | LAT1 | LEAP2 | Pept1 |
| DU | Cont | 1.03±0.11 | 1.19±0.25 | 1.10±0.21 | 1.04±0.12 | 1.20±0.24 | 1.19±0.26 |
| | *E.ace* | 1.58±0.26 | 0.13±0.03 | 0.40±0.14 | 1.40±0.21 | 0.07±0.02 | 0.82±0.20 |
| | P-val | 0.15 | 0.0004* | 0.007* | 0.78 | 0.002* | 0.72 |
| JE | Cont | 1.02±0.09 | 1.11±0.18 | 1.12±0.20 | 1.06±0.19 | 1.14±0.18 | 1.12±0.25 |
| | *E.ace* | 1.16±0.09 | 0.39±0.12 | 1.23±0.39 | 1.37±0.18 | 0.94±0.24 | 0.90±0.21 |
| | P-val | 0.90 | 0.10 | 0.98 | 0.94 | 0.89 | 0.81 |
| IL | Cont | 1.03±0.10 | 4.06±3.14 | 1.04±0.14 | 1.08±0.18 | 6.03±4.57 | 1.10±0.22 |
| | *E.ace* | 1.00±0.08 | 1.35±0.50 | 0.97±0.23 | 1.14±0.21 | 2.64±0.68 | 1.18±0.34 |
| | P-val | 1.00 | 0.56 | 1.00 | 1.00 | 0.65 | 0.99 |

| | | Relative gene expression | | | | | |
|---|---|---|---|---|---|---|---|
| Tissue | Group | rBAT | SGLT1 | SI | $y^+$LAT1 | $y^+$LAT2 | ZNT1 |
| DU | Cont | 1.12±0.22 | 1.05±0.15 | 1.13±0.21 | 1.08±0.17 | 1.04±0.12 | 1.10±0.20 |
| | *E.ace* | 0.28±0.06 | 0.72±0.07 | 0.30±0.10 | 0.74±0.17 | 0.78±0.09 | 0.47±0.06 |
| | P-val | 0.0004* | 0.56 | 0.004* | 0.24 | 0.22 | 0.008* |
| JE | Cont | 1.13±0.24 | 1.02±0.09 | 1.02±0.09 | 1.04±0.12 | 1.03±0.10 | 1.04±0.13 |
| | *E.ace* | 0.93±0.16 | 1.05±0.11 | 0.99±0.10 | 1.39±0.73 | 0.98±0.10 | 0.95±0.13 |
| | P-val | 0.88 | 1.00 | 0.99 | 0.85 | 0.99 | 0.95 |
| IL | Cont | 1.03±0.11 | 1.04±0.13 | 1.15±0.28 | 1.04±0.12 | 1.01±0.05 | 1.16±0.30 |
| | *E.ace* | 0.89±0.11 | 0.83±0.11 | 1.38±0.14 | 0.83±0.14 | 0.96±0.08 | 1.04±0.12 |
| | P-val | 0.59 | 0.56 | 0.77 | 0.45 | 0.94 | 0.94 |

**Table II-3. Expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in *E. acervulina*-challenged broilers.** DU=duodenum, JE=jejunum, IL=ileum, Cont=control chicks, *E.ace=E.acervulina*, P-val=P-value. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method. APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}$AT and $B^0$AT= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT1 and CAT2= Cationic amino acid transporter-1 and -2; EAAT3= Excitatory amino acid transporter 3; GLUT1, GLUT2 and GLUT5= Glucose

transporter-1, -2 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}$AT; SGLT1= Sodium glucose transporter-1; SI= Sucrase isomaltase; $y^+$LAT1 and $y^+$LAT2= $y^+$ L amino acid transporter-1 and -2, respectively; ZNT1= Zinc transporter-1. * Indicates statistical significance from control at $p<0.05$.

## DISCUSSION

In chicken, the duodenum is important for digestive enzyme secretion and some absorption of nutrients. Most nutrient absorption occurs in the jejunum and ileum (Leeson, et al., 2001). The difference in food intake and body weight gain between layers and broilers may be due to different expression profiles of digestive enzymes and nutrient transporters in jejunum and ileum. It is not surprising that layers and broilers shared many similarities in the duodenum in response to *E. acervulina*-challenge: down regulation of APN, $b^{o,+}$AT/rBAT, $B^0$AT, CAT2, EAAT3, GLUT2, LEAP2, SI and ZNT1 (Figure II-1) but no changes in gene expression was observed in the jejunum and ileum section in broilers (Figure II-2). This difference may result from the divergent selection of chickens. Layers were selected for egg laying and broilers were selected for rapid growth. At 21 day of age, a broiler chicken is about twice the body weight of a layer chicken. The difference in body weight gain in layers and broilers may result from physiological difference at the molecular level in the intestine. In this experiment, there were striking differences in changes of gene expression in the jejunum and ileum of *E. acervulina*-challenged layers and broilers.

Brush border membrane transporters like $b^{o,+}$AT/rBAT, $B^0$AT and EAAT3, which regulate free amino acid uptake from the intestinal lumen to the epithelial cells, are downregulated in the duodenum of *E. acervulina*-challenged layers and broilers. This would result in reduced influx of essential amino acids to infected cells. Especially decreased expression of EAAT3 would result in a depletion of the energy source (glutamate) to the intestinal cells. $b^{o,+}$AT/rBAT and EAAT3 were also down regulated in *E. maxima*- challenged broilers (Paris and Wong, 2013), which may indicate a common mechanism of intestine cells responding to *Eimeria* challenge. Both ASCT1 and LAT1 were upregulated in the jejunum of *E. maxima*- challenged broilers (Paris and Wong, 2013), which indicated increased efflux of amino acid from the enterocyte. The upregulation of ASCT1 and LAT1 was not found in *E. acervulina*-challenged layers and broilers.

Brush border membrane cationic amino acid transporter $b^{o,+}$AT/rBAT and basolateral membrane cationic amino acid transporter CAT2 were downregulated in the duodenum of *E. acervulina*-challenged layers and broilers. Decreased expression of $b^{o,+}$AT/rBAT would lead to decreased cationic amino acid influx into the enterocyte. Downregulation of CAT2 may result from decreased substrate to this transporter.

Digestive enzyme SI at the brush border membrane hydrolyzes sucrose and isomaltose to monosaccharides. Decreased expression of SI can lead to less efficient digestion and absorption of polysaccharides. Downregulation of sugar transporter GLUT2 at the basolateral membrane may result from decreased expression of SI.

ZNT1 functions in efflux of $Zn^{2+}$ at the basolateral membrane of enterocytes. Expression of ZNT1 is important to protect the cell against zinc toxicity (Nolte, et al., 2004). Zinc, as an antioxidant, can also protect the host against *E. acervulina*-induced oxidative damage (Georgieva, et al., 2011). Decreased expression of ZNT1 can either promote zinc toxicity and programed cell death and/or reduce the damage caused by *Eimeria*.

LEAP-2 expression is proposed to be necessary for resistance to *E. maxima* infection and that upon entering cells, *E. maxima* leads to the downregulation of LEAP-2, resulting in more severe infection (Casterlow, et al., 2011). In both layers and broilers, LEAP-2 expression is decreased in the primary target site of *E. acervulina*: duodenum. Layers also showed downregulation of LEAP2 in the jejunum. There was an increase of LEAP-2 in the ileum in layers, which could be compensating for the low LEAP-2 level in the upper part of the intestine.

In summary, *E. acervulina*-challenged layers and broilers showed many similarities in downregulation of digestive enzymes and nutrient transporters in the duodenum, and no change in the jejunum and ileum in broilers compared to layers.

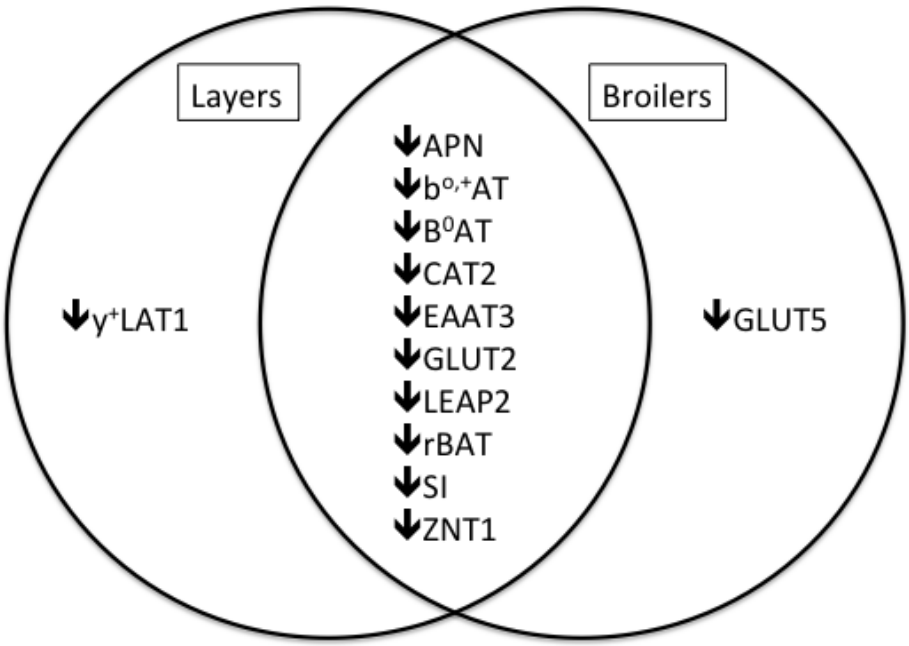


**Figure II-1. Summary of gene expression changes in duodenum of *Eimeria acervulina*-challenged layers and broilers.** APN= Aminopeptidase N; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT2= Cationic amino acid transporter-2; EAAT3= Excitatory amino acid transporter 3; GLUT2 and GLUT5= Glucose transporter-2 and -5, respectively; LEAP2= Liver-expressed antimicrobial peptide-2; rBAT= protein related to $b^{0,+}AT$; SI= Sucrase isomaltase; $y^+LAT1$= $y^+$ L amino acid transporter-1; ZNT1= Zinc transporter-1; ↓=downregulation.

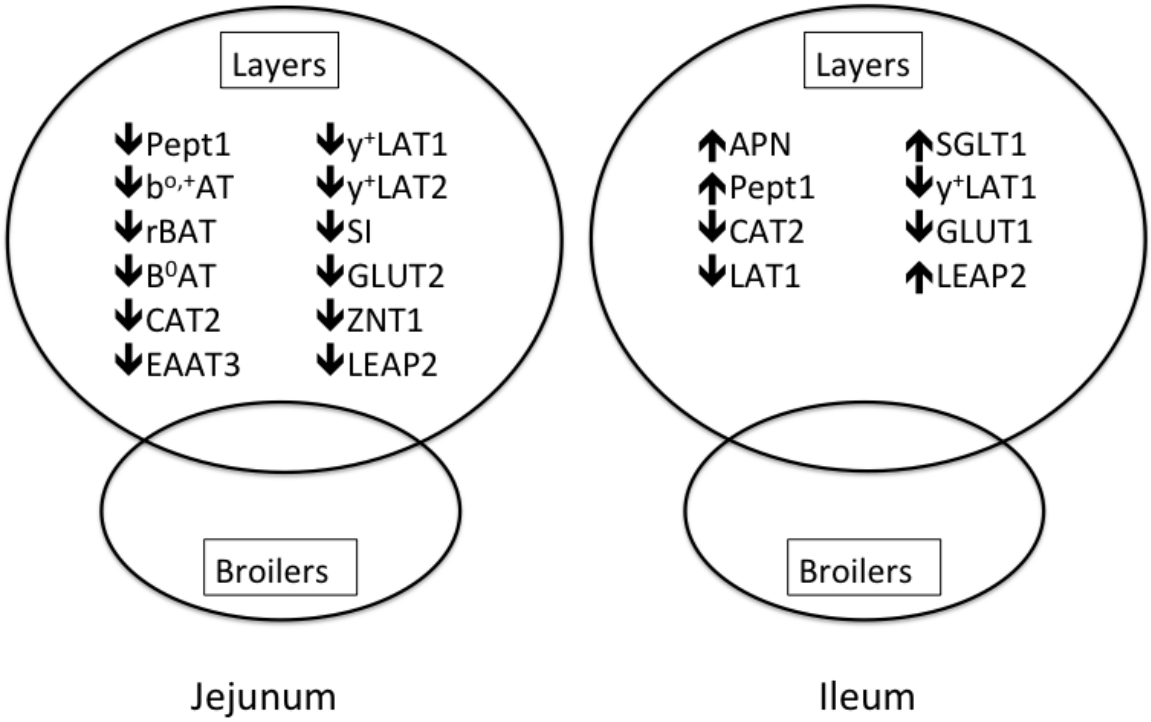


**Figure II-2. Summary of gene expression changes in jejunum and ileum of *Eimeria acervulina*-challenged layers and broilers.** APN= Aminopeptidase N; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT2= Cationic amino acid transporter-2; EAAT3= Excitatory amino acid transporter 3; GLUT1 and GLUT2= Glucose transporter-1 and -2, respectively; LAT1=L type amino acid transorter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}AT$; SGLT1= Sodium glucose transporter-1; SI= Sucrase isomaltase; $y^+LAT1$ and $y^+LAT2$= $y^+$ L amino acid transporter-1 and -2; ZNT1= Zinc transporter-1; ↓=downregulation; ↑=upregulation.

## LITERATURE CITED


Casterlow, S., H. Li, E. R. Gilbert, R. A. Dalloul, A. P. McElroy, D. A. Emmerson, and E. A. Wong. 2011. An antimicrobial peptide is downregulated in the small intestine of Eimeria maxima-infected chickens. Poult Sci 90:1212-1219. doi 10.3382/ps.2010-01110

Conway, D. P., and M. E. McKenzie. 2007. Poultry coccidiosis : diagnostic and testing procedures. 3rd ed. Blackwell Pub., Ames, Iowa.

Dalloul, R. A., T. W. Bliss, Y. H. Hong, I. Ben-Chouikha, D. W. Park, C. L. Keeler, and H. S. Lillehoj. 2007. Unique responses of the avian macrophage to different species of Eimeria. Mol Immunol 44:558-566. doi 10.1016/j.molimm.2006.02.004

Georgieva, N. V., M. Gabrashanska, V. Koinarski, and Z. Yaneva. 2011. Zinc Supplementation against Eimeria acervulina-Induced Oxidative Damage in Broiler Chickens. Vet Med Int 2011:647124. doi 10.4061/2011/647124

Hocking, P. M., B. O. Hughes, and S. Keer-Keer. 1997. Comparison of food intake, rate of consumption, pecking activity and behaviour in layer and broiler breeder males. Br Poult Sci 38:237-240. doi 10.1080/00071669708417978

Koenen, M. E., A. G. Boonstra-Blom, and S. H. Jeurissen. 2002. Immunological differences between layer- and broiler-type chickens. Vet Immunol Immunopathol 89:47-56.

Leeson, S., M. L. Scott, and J. D. Summers. 2001. Nutrition of the chicken. 4th ed. University Books, Guelph, Ontario.

Lillehoj, H. S., and J. M. Trout. 1996. Avian gut-associated lymphoid tissues and intestinal immune responses to Eimeria parasites. Clin Microbiol Rev 9:349-360.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408. doi 10.1006/meth.2001.1262

Nolte, C., A. Gore, I. Sekler, W. Kresse, M. Hershfinkel, A. Hoffmann, H. Kettenmann, and A. Moran. 2004. ZnT-1 expression in astroglial cells protects against zinc toxicity and slows the accumulation of intracellular zinc. Glia 48:145-155. doi 10.1002/glia.20065

Paris, N. E., and E. A. Wong. 2013. Expression of digestive enzymes and nutrient transporters in the intestine of Eimeria maxima-infected chickens. Poult Sci 92:1331-1335. doi 10.3382/ps.2012-02966

Shariatmadari, F., and J. M. Forbes. 1993. Growth and food intake responses to diets of different protein contents and a choice between diets containing two concentrations of protein in broiler and layer strains of chicken. Br Poult Sci 34:959-970. doi 10.1080/00071669308417656

Swennen, Q., P. J. Verhulst, A. Collin, A. Bordas, K. Verbeke, G. Vansant, E. Decuypere, and J. Buyse. 2007. Further investigations on the role of diet-induced thermogenesis in the regulation of feed intake in chickens: comparison of adult cockerels of lines selected for high or low residual feed intake. Poult Sci 86:1960-1971.

Yuan, L., Y. Ni, S. Barth, Y. Wang, R. Grossmann, and R. Zhao. 2009. Layer and broiler chicks exhibit similar hypothalamic expression of orexigenic neuropeptides but distinct expression of genes related to energy homeostasis and obesity. Brain Res 1273:18-28. doi 10.1016/j.brainres.2009.03.052

# CHAPTER III. EXPRESSION OF DIGESTIVE ENZYMES AND NUTRIENT TRANSPORTERS IN *EIMERIA* CHALLENGED BROILERS


## ABSTRACT

Avian coccidiosis is caused by the intestinal protozoa *Eimeria*. The parasite's site of infection in the intestine is site specific. *Eimeria acervulina* infects the duodenum, *E. maxima* the jejunum, and *E. tenella* the ceca. Lesions in the intestinal mucosa cause reduced feed efficiency and body weight gain in *Eimeria*-challenged chickens. The growth reduction may be due to changes in expression of digestive enzymes and nutrient transporters in the intestine. The objective of this thesis was to examine the expression of digestive enzymes: APN and SI, peptide and amino acid transporters: Pept1, ASCT1, $b^{o,+}$AT/rBAT, $B^0$AT, CAT1/2, EAAT3, LAT1 and $y^+$LAT1/2, sugar transporters: GLUT1, GLUT2, GLUT5 and SGLT1, mineral transporter: ZNT1 and an immune factor: LEAP2 in the duodenum, jejunum, ileum and ceca of different *Eimeria*-challenged broilers. *E. acervulina*-challenged duodenum, *E. maxima*-challenged jejunum and *E. tenella*-challenged ceca samples showed common downregulation of APN, GLUT5 and ZNT1. In *E. acervulina*- and *E. maxima*-challenged chickens there was downregulation of LEAP2, but not in *E. tenella*-challenged chickens. CAT2, EAAT3, rBAT and SI were downregulated in *E. acervulina*- and *E. tenella*-challenged chickens, but not in *E. maxima*-challenged chickens. There was upregulation of LAT1 in *E. maxima*- and *E. tenella*-challenged chickens, but not in *E. acervulina*--challenged chickens. These results demonstrate that there are common and species-specific changes in intestinal gene expression in response to challenge by different *Eimeria* species in chicken. These changes in intestinal digestive enzyme, nutrient transporter gene expression may result in a decrease in the efficiency of protein digestion, uptake of essential amino acids and the energy source (glutamate), and disruption of mineral balance. This may ultimately lead to cell death and may be part of the host defense mechanism for eliminating infected cells and inhibition of pathogen replication.


## INTRODUCTION

Avian coccidiosis is a major disease of poultry caused by the intestinal protozoa *Eimeria* (Conway and McKenzie, 2007). Lesions in the intestinal mucosa reduce feed efficiency and body weight gain. A damaged intestinal barrier leads to bacterial infection, which can increase

mortality in birds. Coccidiosis is responsible for the loss of billions of dollars in the poultry industry worldwide (Dalloul, et al., 2007). In the U.S., the three species of *Eimeria* that most impact the poultry industry are *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella*. *Eimeria* infection is site specific, *E. acervulina* infects the duodenum, *E. maxima* the jejunum, and *E. tenella* the ceca (Lillehoj and Trout, 1996). Analysis of immune factor expression in *E. acervulina*, *E. maxima*, and *E. tenella* oocysts challenged chicken macrophages showed common and different responses to *Eimeria* challenge. Many interleukins and chemokines were upregulated, but one chemokine K60 (CXCLi1) was only found increased in *E. tenella* oocysts challenged macrophages (Dalloul et al., 2007). Liver-expressed antimicrobial peptide-2 (LEAP2) is an antimicrobial peptide that disrupts the membrane of bacteria, and is upregulated in *Salmonella*-challenged chickens (Townes, et al., 2004). In contrast, LEAP2 was found to be downregulated in the jejunum of *E. maxima*-challenged broilers (Casterlow, et al., 2011).

The small intestine is the primary site for nutrient absorption in chickens (Leeson, et al., 2001). The final digestion of proteins and polysaccharides is catalyzed by membrane bound peptidases and glucosidases, respectively. Short peptides, free amino acids and monosaccharides are transported by the intestinal enterocytes by specific transporters located at the brush border membrane and basolateral membrane (Leeson, et al., 2001). Because *Eimeria*-challenged chickens showed reduced feed efficiency and body weight gain, it is likely due to changes in expression of digestive enzymes and nutrient transporters in the intestine. Paris and Wong (2013) reported decreased expression of the brush border membrane amino acid transporters EAAT3 and $b^{o,+}AT$, increased expression of the basolateral amino acid transporters LAT1 and ASCT1 and decreased expression of the zinc transporter ZNT1 in the jejunum of *E. maxima*-challenged broilers. The objective of this study was to compare changes in nutrient transporter and digestive enzyme gene expression in different sections of the small intestine following infection with *E. acervulina*, *E. maxima*, and *E. tenella*.

## MATERIALS AND METHODS

### Chicken and *Eimeria*

This study was 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). Chickens used in this study were Ross Heritage broiler males from

Longeneckers Hatchery (Elizabthetown PA). Birds were housed in suspended wire cages (46cm x 30cm = 1380cm$^2$) with 2-3 birds per cage. Birds were fed a standard poultry starter ration (crumbles, 24% protein) and had free access to water.

*Eimeria* are all USDA strains: *E. acervulina* (USDA #12 isolate), *E. maxima* (Tysons isolate) and *E. tenella* (Wampler isolate). 1 day old chicks were transported to the USDA-ARS facility (Beltsville, MD) and were orally gavaged with either 1mL *Eimeria* oocysts or not gavaged at 21 d of age. The 4 treatments in this study are *E. acervulina* (200,000 oocysts/ bird), *E. maxima* (10,000 oocysts/bird), *E. tenella* (100,000 oocysts/bird) and control (not gavaged). Initial body weight of the chickens was obtained on 21d.

**Tissue sampling**

Seven days post challenge chickens were weighted and euthanized by cervical dislocation and intestinal segments: duodenum, jejunum, ileum and ceca were collected (n=6). The contents of the intestine were squeezed out and the tissue segments were immediately stored individually in RNAlater (Invitrogen, Grand Island, NY). The samples were stored at 4 °C for 24 hrs and then were frozen at -70 °C before being shipped to Virginia Tech. Upon arrival each intestinal segment was removed from RNAlater. After homogenizing, a 20-30 mg tissue aliquot was placed in a 2-mL microfuge tube for RNA extraction and the remaining homogenate was placed in a separate 2-mL microfuge tube. Both tubes were frozen on dry ice and stored at -80°C.

**Total RNA extraction**

The 20-30 mg of homogenized tissue was placed in 500µL Tri Reagent (Molecular Research Center Inc., Cincinnati, OH) and shaken twice at 25Hz/s for 2 min using a TissueLyser II (QIAGEN Inc., Valencia, CA) following the animal tissue protocol. After homogenization100 µL of chloroform were added for phase separation. The RNA pellet was suspended in 0.1% DEPC (Diethylpyrocarbonate, Sigma-Aldrich, St. Louis, MO) treated water depending on the pellet size and incubated for 10 minutes at 56°C. RNA concentration was determined using a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Any sample that had a concentration greater than 2000ng/µL was further diluted and reassayed. RNA quality was assessed by agarose-formaldehyde gel electrophoresis. All extracted RNA samples were stored at -80°C.

**Reverse Transcription**

Total RNA was diluted to 0.1 µg/µL in DEPC water. cDNA was synthesized using the high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Each 20µL reverse transcription reaction contained 2 µL 10X reverse transcription buffer, 2 µL 10X random primers, 1 µL multiscribe reverse transcriptase (50 U/µL), 0.8 µL 25x dNTPs, 9.2 µL DEPC water, and 5 µL of 0.1 µg/µL diluted RNA sample. The RNA and master mix were combined in a 0.5-mL microfuge tube, which was then run in a thermocycler for 10 min at 25 °C followed by 120 min at 37 °C and 5 min at 85 °C. The cDNA was diluted 1:20 with DEPC water and stored at -20°C.

**Quantitative Real-Time PCR**

Quantitative real-time PCR (qPCR) was performed using 96-well plates. Each reaction contained 5 µL diluted cDNA, 20 µL of PCR master mix which contained 12.5 µL 2X SYBR Green Master Mix (Applied Biosystems), 0.5 µL forward primer (5 µM), 0.5 µL reverse primer (5 µM), and 6.5 µL DEPC water. Each reaction was run in duplicate. The plate was sealed with a MicroAmp Optical Adhesive Film (Applied Biosystems) and spun down in a centrifuge to mix reagents and remove bubbles and loaded into an Applied Biosystems 7300 Real-Time PCR instrument (Applied Biosystems). The following real time PCR conditions were used: 95 °C for10 min followed by 40 cycles of 95 °C for 15s and 60 °C for 1 min. Genes analyzed were APN, ASCT1, $b^{o,+}$AT, $B^0$AT, CAT1, CAT2, EAAT3, GLUT1, GLUT2, GLUT5, LAT1, LEAP2, Pept1, rBAT, SGLT1, SI, $y^+$LAT1, $y^+$LAT2 and ZNT1 (Table I-1). The endogenous control was the chicken beta-actin gene. All forward and reverse primer sequences are shown in Table II- 1.Primers were designed using the Primer Express software (Applied Biosystems) and synthesized by Eurofins MWG Operon (Huntsville, AL).

**Quantitative Real-Time PCR Analysis**

All plates were analyzed individually using the software provided with the 7300

Real-Time PCR instrument and raw Ct data was obtained. Average gene expression relative to the endogenous control for each sample was calculated using the $2^{-\Delta\Delta Ct}$ method described by Livak and Schmittgen (2001). For gene expression changes affected by different *Eimeria* challenge, the average ΔCt of the 6 control samples was used to calculate the ΔΔCt value, which

was performed separately for each intestinal segment, *Eimeria* treatment and each gene are a group. For comparison of gene expression in different segments of the intestine in the control group, the average ΔCt of the 6 duodenum samples was used to calculate the ΔΔCt value. Data points that exceed ±3 standard deviations from the mean were discarded as outliers.

**Statistical Analysis.**

All data were analyzed by ANOVA using JMP® Statistical Discovery Software from SAS (SAS Institute, Cary, NC). Tukey's test was used for pairwise comparisons of body weight gain between different treatment groups. For gene expression of each *Eimeria* challenge, the model included the main effects of treatment, sorted by genes. Significant effects ($P < 0.05$) were further evaluated with Dunnett's test for comparisons with the control. For gene expression of each intestinal segment in control birds, the model included the main effects of intestinal segment, forted by genes. Significant effects ($P < 0.05$) were further evaluated with Tukey's test for pairwise comparisions.

## RESULTS

**Body weight gain for *Eimeria* challenged broilers**

During the 7 d challenge, control chickens gained 499±37 g (mean±SE), whereas *E. acervulina*-challenged chickens gained 291±95 g, *E. maxima*-challenged chickens gained 349±23 g and *E. tenella*-challenged chickens gained 460±31 g. Body weight gain was numerically decreased in *E. acervulina-, E. maxima-* and *E. tenella*-challenged broilers by 42%, 30% and 8% of control, respectively, but not significantly different at $P<0.05$ (Table III-1).

| Treatment group | Weight gain, g (mean±SE) | P-value | Weight gain depression (%) |
|---|---|---|---|
| Control | 499±37[a] | | |
| *E. acervulina* | 291±95[a] | 0.06[1] | 42 |
| *E. maxima* | 349±23[a] | 0.24 | 30 |
| *E. tenella* | 460±31[a] | 0.96 | 8 |

**Table III-1. Body weight gain for *Eimeria* challenged broilers.** [a]Means indicated with same superscript are not significantly different at $P<0.05$. [1]P-value listed in this table were from comparison with control.

Different species of *Eimeria* preferentially infect specific regions of the intestine. *Eimeria acervulina* infects the duodenum, *E. maxima* the jejunum, and *E. tenella* the ceca. Changes in expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in *E, acervulina*, *E. maxima*, and *E. tenella* challenged broilers are shown for duodenum (Table III-2), jejunum (Table III-3), ileum (Table III-4), and ceca (Table III-5).

***E. acervulina*-challenged broilers**

Expression of the amino acid transportes APN, $b^{o,+}AT$, $B^0AT$, rBAT, CAT2 and EAAT3 was decreased to 46%, 24%, 31%, 25% 56% and 25% of control, respectively, in the duodenum (Table III-2) and rBAT was decreased to 50% of control in the ceca (Table III-5) of *E. acervulina*- challenged broilers.

The glucose transporter GLUT2 was decreased to 11% and 39% of control in the duodenum (Table III-2) and ceca (Table III-5), respectively, and GLUT5 was decreased to 36% and 68% of control in the duodenum (Table III-2) and ceca (Table III-5), respectively, in *E. acervulina*-challenged broilers. Expression of SI and ZNT1 was decreased to 27% and 43% of control, respectively, in the duodenum (Table III-2). APN and ZNT1was decreased to 33% and 32% of control, respectively, in the ceca (Table III-5). LEAP2 was decreased to 6% of control in the duodenum (Table III-2) of *E. acervulina*- challenged broilers. No change in gene expression was observed in the jejunum and ileum.

***E. maxima*-challenged broilers**

Changes in expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in *E. maxima*-challenged broilers are summarized in Tables III-2 to III-5. APN was decreased to 51% and 18% in jejunum (Table III-3) and ceca (Table III-5). ASCT1 was increased 2.4-, 3.2- and 2.5 fold in duodenum (Table III-2), jejunum (Table III-3) and ileum (Table III-4) of *E. maxima*-challenged broilers. Expression of $b^{o,+}AT$ was decreased to 54%, 32% and 6% and in the duodenum (Table III-2), ileum (Table III-4) and ceca (Table III-5), respectively. $B^0AT$ was decreased to 0.2% of control and EAAT3 was decreased to 1.5% of control in ceca (Table III-5). CAT2 was decreased to 41% and 51% in duodenum (Table III-2) and ileum (Table III-4), and increased 1.8-fold in the ceca (Table III-5) in *E. maxima*-challenged broilers. rBAT was decreased to 48%, 45% and 0.9% of control in duodenum (Table III-2), ileum (Table III-4) and ceca (Table III-5), respectively. LAT1 was increased 3.2-, 5.3- and 4.2-fold in duodenum (Table III-2), jejunum (Table III-3) and ileum (Table III-4), respectively in *E. maxima*-challenged broilers. $y^+$LAT1was decreased to 60% of control in the ileum (Table III-4); Pept1 was decreased to 0.2% of control in the ceca (Table III-5) in *E. maxima*-challenged broilers.

SI was decreased to 1% of control in ceca (Table III-2). Sugar transporter GLUT1 was increased 1.9-, 2.1-, 1.5- and 1.6-fold in duodenum (Table III-2), jejunum (Table III-3), ileum (Table III-4) and ceca (Table III-5), respectively. GLUT2 was downregulated to 29% and 8% in duodenum (Table III-2) and ceca (Table III-5) in *E. maxima*-challenged broilers. GLUT5 was decreased to 50%, 24% and 5% of control in duodenum (Table III-2), jejunum (Table III-3) and ceca (Table III-5), respectively.

ZNT1 was decreased to 36% of control in jejunum (Table III-3) and increased to1.7-fold in ceca (Table III-5). LEAP2 was decreased to 17%, 10% and 11% of control in duodenum (Table III-2), jejunum (Table III-3) and ceca (Table III-5) in *E. maxima*-challenged broilers.

***E. tenella*-challenged broilers**

Changes in expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in *E. tenella*-challenged broilers are summarized in Tables III-2 to III-5. In the ceca of *E. tenella*-challenged broilers, APN, Pept1, CAT2, EAAT3, rBAT, SI, GLUT5 and ZNT1 was downregulated to 9%, 31%, 18%, 29%, 33%, 17%, 40% and 13% of control (Table III-5). While LAT1 was up regulated 2.7-fold (Table III-5). LEAP2 was up regulated 2-fold in jejunum (Table

III-3) of *E. tenella*-challenged broilers. No gene expression changes were observed in the duodenum and ileum of *E. tenella*-challenged broilers.

Summaries of gene expression changes to different *Eimeria* species are shown for duodenum (Figure III-1), jejunum (Figure III-2), ileum (Figure III-3) and ceca (Figure III-4), which illustrate common and species-specific changes in response to *Eimeria* challenge. In the duodenum, there was downregulation of $b^{o,+}$AT, rBAT, CAT2, GLUT2 GLUT5 and LEAP2 in *E.acervulina*- and *E. maxima*-challenged broilers (Figure III-1). *E.acervulina*- challenged birds also showed decreased expression of APN, $B^0$AT, EAAT3, SI and ZNT1. *E. maxima*-challenged broilers showed upregulation of ASCT1, GLUT1 and LAT1. No gene expression changes were observied in the *E. tenella*-challenged duodenum samples. In the jejunum, *E. maxima*-challenged broilers showed upregulation of ASCT1, GLUT1 and LAT1, and there were also downregulation of APN, GLUT5 LEAP2 and ZNT1 (Figure III-2). *E. tenella*-challenged jejunum samples had increased expression of LEAP2. No gene expression changes were observed in the *E.acervulina*-challenged jejunum samples. Only *E. maxima*-challenged broilers showed changes in gene expression in the ileum (Figure III-3). ASCT1, GLUT1 and LAT1 were upregulated and $b^{o,+}$AT/rBAT, CAT2 and $y^+$LAT1 was downregulated. Upregulation of ASCTI, GLUT1 and LAT1 in *E. maxima*-challenged broilers was common for duodenum, jejunum and ileum. Since there was no change of gene expression on digestive enzyme and nutrient transporter in the small intestine of *E. tenella*-challenged birds, it is not surprising that *E. tenella*-challenged birds did not show body weight gain depression. Even though *E. tenella* only infects the ceca, there were many common and unique changes in different species of *Eimeria* challenged ceca samples (Figure III-4). APN, GLUT5, and rBAT were commonly downregulated in all three *Eimeria* species. *E.acervulina*- and *E. maxima*-challenged ceca samples showed common downregulation of GLUT2. *E.acervulina*- and *E. tenella*-challenged samples both had decreased expression of ZNT1. *E. maxima*- and *E. tenella*-challenged ceca samples showed down regulation of EAAT3, Pept1 and SI. *E.acervulina*- and *E. maxima* have specific infection sites in the small intestine, the changes in gene expression cased by *E.acervulina*- and *E. maxima* infection in the ceca may be due to the structural and functional difference between the small intestine and the ceca.

| | Relative gene expression | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | APN | ASCT1 | $b^{0,+}AT$ | $B^0AT$ | CAT1 | CAT2 | EAAT3 | GLUT1 | GLUT2 | GLUT5 |
| Cont | 1.08±0.16 | 1.04±0.12 | 1.10±0.20 | 1.18±0.25 | 2.89±1.67 | 1.11±0.16 | 1.13±0.23 | 1.03±0.11 | 1.19±0.25 | 1.10±0.21 |
| *E.ace* | 0.50±0.09 | 1.28±0.23 | 0.26±0.03 | 0.37±0.04 | 11.45±3.37 | 0.62±0.06 | 0.28±0.05 | 1.58±0.26 | 0.13±0.03 | 0.40±0.14 |
| P-val | 0.005* | 0.83 | 0.0001* | 0.01* | 0.17 | 0.02* | 0.002* | 0.15 | 0.0004* | 0.007* |
| *E.max* | 0.86±0.88 | 2.50±0.43 | 0.59±0.09 | 1.11±0.22 | 10.83±4.69 | 0.45±0.06 | 0.84±0.11 | 1.99±0.25 | 0.35±0.10 | 0.55±0.14 |
| P-val | 0.44 | 0.001* | 0.01* | 0.98 | 0.22 | 0.001* | 0.37 | 0.008* | 0.004* | 0.04* |
| *E.ten* | 1.05±0.10 | 0.94±0.06 | 1.05±0.08 | 1.24±0.16 | 4.89±2.08 | 0.92±0.09 | 1.13±1.47 | 1.21±0.12 | 1.16±0.17 | 0.89±0.06 |
| P-val | 1.00 | 0.98 | 0.98 | 0.99 | 0.94 | 0.66 | 1.00 | 0.86 | 1.00 | 0.59 |

| | Relative gene expression | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | LAT1 | LEAP2 | Pept1 | rBAT | SGLT1 | SI | $y^+LAT1$ | $y^+LAT2$ | ZNT1 |
| Cont | 1.04±0.12 | 1.20±0.24 | 1.19±0.26 | 1.12±0.22 | 1.05±0.15 | 1.13±0.21 | 1.08±0.17 | 1.04±0.12 | 1.10±0.20 |
| *E.ace* | 1.40±0.21 | 0.07±0.02 | 0.82±0.20 | 0.28±0.06 | 0.72±0.07 | 0.30±0.10 | 0.74±0.17 | 0.78±0.09 | 0.47±0.06 |
| P-val | 0.78 | 0.002* | 0.72 | 0.0004* | 0.56 | 0.004* | 0.24 | 0.22 | 0.008* |
| *E.max* | 3.28±0.59 | 0.20±0.10 | 1.11±0.40 | 0.54±0.07 | 1.76±0.37 | 0.97±0.19 | 0.67±0.11 | 1.07±0.10 | 0.90±0.12 |
| P-val | 0.0003* | 0.007* | 1.00 | 0.01* | 0.08 | 0.82 | 0.12 | 0.99 | 0.60 |
| *E.ten* | 1.06±0.11 | 1.45±0.32 | 0.76±0.30 | 1.03±0.10 | 0.85±0.15 | 1.11±0.11 | 0.95±0.08 | 1.07±0.10 | 1.03±0.11 |
| P-val | 1.00 | 0.73 | 0.62 | 0.93 | 0.84 | 1.00 | 0.86 | 0.99 | 0.96 |

**Table III-2. Expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in duodenum of *Eimeria*-challenged broilers.** *E.ace=E.acervulina*; *E.max= E. maxima; E. ten= E. tenella;* Cont=control chicks; P-val=P-value. Relative gene expression was determined using the $2^{-\Delta\,\Delta Ct}$ method. APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT1 and CAT2= Cationic amino acid transporter-1 and -2; EAAT3= Excitatory amino acid transporter 3; GLUT1, GLUT2 and GLUT5= Glucose transporter-1, -2 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}AT$; SGLT1= Sodium glucose transporter-1; SI= Sucrase isomaltase; $y^+LAT1$ and $y^+LAT2$= $y^+$ L amino acid transporter-1 and -2, respectively; ZNT1= Zinc transporter-1. * Indicates statistical significance from control at $p<0.05$.

| | Relative gene expression | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | APN | ASCT1 | $b^{0,+}AT$ | $B^0AT$ | CAT1 | CAT2 | EAAT3 | GLUT1 | GLUT2 | GLUT5 |
| Cont | 1.14±0.23 | 1.05±0.16 | 1.30±0.46 | 1.47±0.64 | 3.39±1.93 | 1.03±0.11 | 1.31±0.41 | 1.02±0.09 | 1.11±0.18 | 1.12±0.20 |
| *E.ace* | 0.89±0.16 | 1.07±0.10 | 0.87±0.26 | 0.88±0.26 | 10.50±2.90 | 1.39±0.44 | 0.74±0.11 | 1.16±0.09 | 0.39±0.12 | 1.22±0.39 |
| P-val | 0.50 | 1.00 | 0.81 | 0.65 | 0.61 | 0.58 | 0.56 | 0.90 | 0.10 | 0.98 |
| *E.max* | 0.58±0.02 | 3.31±0.69 | 0.31±0.03 | 0.69±0.06 | 19.63±8.13 | 0.40±0.05 | 0.57±0.04 | 2.11±0.31 | 0.31±0.09 | 0.20±0.04 |
| P-val | 0.04* | 0.002* | 0.26 | 0.45 | 0.07 | 0.17 | 0.37 | 0.001* | 0.06 | 0.04* |
| *E.ten* | 1.04±0.12 | 1.13±0.38 | 1.51±0.67 | 1.26±0.50 | 6.99±4.02 | 0.84±0.14 | 1.50±0.60 | 0.91±0.13 | 1.50±0.40 | 1.02±0.22 |
| P-val | 0.93 | 0.94 | 0.97 | 0.97 | 0.92 | 0.89 | 0.97 | 0.95 | 0.50 | 0.98 |

| | Relative gene expression | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | LAT1 | LEAP2 | Pept1 | rBAT | SGLT1 | SI | $y^+LAT1$ | $y^+LAT2$ | ZNT1 |
| Cont | 1.06±0.19 | 1.14±0.18 | 1.12±0.25 | 1.13±0.24 | 1.02±0.09 | 1.02±0.09 | 1.04±0.12 | 1.03±0.10 | 1.04±0.13 |
| *E.ace* | 1.37±0.18 | 0.94±0.24 | 0.90±0.21 | 0.93±0.16 | 1.05±0.11 | 0.99±0.10 | 1.39±0.73 | 0.98±0.10 | 0.95±0.13 |
| P-val | 0.94 | 0.89 | 0.81 | 0.88 | 1.00 | 0.99 | 0.85 | 0.99 | 0.95 |
| *E.max* | 5.57±0.92 | 0.11±0.03 | 0.61±0.16 | 0.32±0.03 | 1.05±0.19 | 0.72±0.08 | 0.71±0.10 | 0.68±0.05 | 0.37±0.05 |
| P-val | <.001* | 0.02* | 0.25 | 0.06 | 1.00 | 0.14 | 0.87 | 0.38 | 0.02* |
| *E.ten* | 1.02±0.20 | 2.30±0.39 | 0.55±0.23 | 1.33±0.37 | 1.25±0.48 | 1.11±0.14 | 0.96±0.13 | 1.25±0.31 | 1.07±0.24 |
| P-val | 1.00 | 0.01* | 0.18 | 0.89 | 0.87 | 0.88 | 1.00 | 0.68 | 1.00 |

**Table III-3. Expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in jejunum of *Eimeria*-challenged broilers.** *E.ace=E.acervulina*; *E.max= E. maxima; E. ten= E. tenella;* Cont=control chicks; P-val=P-value. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method. APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT1 and CAT2= Cationic amino acid transporter-1 and -2; EAAT3= Excitatory amino acid transporter 3; GLUT1, GLUT2 and GLUT5= Glucose transporter-1, -2 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}AT$; SGLT1= Sodium glucose transporter-1; SI= Sucrase isomaltase; $y^+LAT1$ and $y^+LAT2$= $y^+$ L amino acid transporter-1 and -2, respectively; ZNT1= Zinc transporter-1. * Indicates statistical significance from control at $p<0.05$.

| | Relative gene expression | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | APN | ASCT1 | $b^{0,+}AT$ | $B^0AT$ | CAT1 | CAT2 | EAAT3 | GLUT1 | GLUT2 | GLUT5 |
| Cont | 1.04±0.13 | 1.05±0.14 | 1.11±0.23 | 1.07±0.19 | 3.99±2.73 | 1.09±0.20 | 1.10±0.22 | 1.03±0.10 | 4.06±3.14 | 1.04±0.14 |
| *E.ace* | 0.88±0.06 | 1.14±0.13 | 0.87±0.13 | 1.10±0.19 | 9.58±2.70 | 1.11±0.17 | 1.11±0.17 | 1.00±0.08 | 1.35±0.50 | 0.97±0.23 |
| P-val | 0.37 | 0.99 | 0.49 | 1.00 | 0.49 | 1.00 | 1.00 | 1.00 | 0.56 | 1.00 |
| *E.max* | 0.77±0.08 | 2.61±0.11 | 0.36±0.01 | 0.89±0.004 | 12.70±2.80 | 0.55±0.42 | 0.76±0.002 | 1.51±0.21 | 1.74±0.01 | 0.87±0.01 |
| P-val | 0.07 | 0.002* | 0.004* | 0.74 | 0.18 | 0.05* | 0.37 | 0.02* | 0.67 | 0.95 |
| *E.ten* | 1.13±0.08 | 0.86±0.09 | 1.29±0.11 | 1.20±0.16 | 4.19±1.79 | 0.97±0.11 | 1.50±0.17 | 0.83±0.12 | 2.52±1.24 | 1.59±0.43 |
| P-val | 0.80 | 0.93 | 0.76 | 0.89 | 1.00 | 0.90 | 0.25 | 0.05 | 0.86 | 0.40 |

| | Relative gene expression | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | LAT1 | LEAP2 | Pept1 | rBAT | SGLT1 | SI | $y^+LAT1$ | $y^+LAT2$ | ZNT1 |
| Cont | 1.08±0.18 | 6.03±4.57 | 1.10±0.22 | 1.03±0.11 | 1.04±0.13 | 1.15±0.28 | 1.04±0.12 | 1.01±0.05 | 1.16±0.30 |
| *E.ace* | 1.14±0.21 | 2.64±0.68 | 1.18±0.34 | 0.89±0.11 | 0.83±0.11 | 1.38±0.14 | 0.83±0.14 | 0.96±0.08 | 1.04±0.12 |
| P-val | 1.00 | 0.65 | 0.99 | 0.59 | 0.56 | 0.77 | 0.45 | 0.94 | 0.94 |
| *E.max* | 4.50±0.11 | 1.70±0.01 | 0.95±0.001 | 0.46±0.001 | 0.98±0.004 | 1.55±0.001 | 0.62±0.14 | 0.82±0.03 | 0.81±0.19 |
| P-val | 0.001* | 0.47 | 0.97 | 0.001* | 0.98 | 0.41 | 0.04* | 0.23 | 0.61 |
| *E.ten* | 0.80±0.10 | 3.69±1.26 | 0.68±0.26 | 1.29±0.08 | 0.83±0.12 | 1.36±0.25 | 0.78±0.09 | 0.96±0.07 | 0.87±0.10 |
| P-val | 0.97 | 0.88 | 0.60 | 0.14 | 0.57 | 0.83 | 0.26 | 0.92 | 0.46 |

**Table III-4. Expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in ileum of *Eimeria*-challenged broilers.** *E.ace=E.acervulina*; *E.max= E. maxima; E. ten= E. tenella;* Cont=control chicks; P-val=P-value. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method. APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT1 and CAT2= Cationic amino acid transporter-1 and -2; EAAT3= Excitatory amino acid transporter 3; GLUT1, GLUT2 and GLUT5= Glucose transporter-1, -2 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}AT$; SGLT1= Sodium glucose transporter-1; SI= Sucrase isomaltase; $y^+LAT1$ and $y^+LAT2$= $y^+$ L amino acid transporter-1 and -2, respectively; ZNT1= Zinc transporter-1.
* Indicates statistical significance from control at $p<0.05$.

| | Relative gene expression | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | APN | ASCT1 | $b^{0,+}AT$ | $B^0AT$ | CAT1 | CAT2 | EAAT3 | GLUT1 | GLUT2 | GLUT5 |
| Cont | 1.31±0.33 | 1.00±0.04 | 1.25±0.22 | 1.14±0.28 | 3.87±2.23 | 1.06±0.15 | 1.26±0.47 | 1.01±0.07 | 1.19±0.29 | 1.02±0.10 |
| *E.ace* | 0.43±0.20 | 0.91±0.15 | 1.76±0.26 | 1.46±0.31 | 8.67±3.58 | 0.74±0.10 | 0.55±0.08 | 0.62±0.09 | 0.46±0.08 | 0.69±0.10 |
| P-val | 0.01* | 0.97 | 0.16 | 0.64 | 0.49 | 0.64 | 0.11 | 0.11 | 0.01* | 0.01* |
| *E.max* | 0.23±0.20 | 1.00±0.15 | 0.07±0.26 | 0.002±0.31 | 7.47±3.58 | 1.93±0.10 | 0.02±0.08 | 1.66±0.09 | 0.10±0.08 | 0.05±0.10 |
| P-val | 0.003* | 1.00 | 0.0007* | 0.006* | 0.69 | 0.04* | 0.004* | 0.005* | 0.001* | <.0001* |
| *E.ten* | 0.12±0.02 | 1.21±0.28 | 1.57±0.16 | 0.86±0.19 | 5.31±2.41 | 0.19±0.06 | 0.37±0.05 | 0.90±0.09 | 0.80±0.14 | 0.41±0.05 |
| P-val | 0.001* | 0.75 | 0.50 | 0.72 | 0.97 | 0.04* | 0.04* | 0.87 | 0.26 | <.0001* |

| | Relative gene expression | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | LAT1 | LEAP2 | Pept1 | rBAT | SGLT1 | SI | $y^+LAT1$ | $y^+LAT2$ | ZNT1 |
| Cont | 1.01±0.07 | 2.45±0.98 | 1.43±0.48 | 1.17±0.23 | 1.09±0.21 | 1.09±0.19 | 1.03±0.11 | 1.01±0.08 | 1.04±0.14 |
| *E.ace* | 1.40±0.40 | 1.09±0.49 | 1.43±0.24 | 0.58±0.08 | 0.75±0.13 | 0.92±0.29 | 0.92±0.19 | 0.93±0.18 | 0.33±0.12 |
| P-val | 0.86 | 0.23 | 1.00 | 0.01* | 0.94 | 0.83 | 0.92 | 1.00 | 0.004* |
| *E.max* | 1.27±0.40 | 0.28±0.49 | 0.003±0.24 | 0.01±0.08 | 0.02±0.13 | 0.01±0.29 | 1.07±0.19 | 0.35±0.18 | 1.70±0.12 |
| P-val | 0.95 | 0.02* | 0.004* | <.0001* | 0.38 | 0.001* | 0.99 | 0.34 | 0.006* |
| *E.ten* | 2.77±0.74 | 0.54±0.15 | 0.44±0.13 | 0.39±0.11 | 2.77±1.05 | 0.19±0.06 | 0.51±0.15 | 1.60±0.61 | 0.13±0.05 |
| P-val | 0.02* | 0.06 | 0.05* | 0.001* | 0.10 | 0.004* | 0.06 | 0.44 | 0.0003* |

**Table III-5. Expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in ceca of *Eimeria*-challenged broilers.** *E.ace=E.acervulina*; *E.max= E. maxima; E. ten= E. tenella;* Cont=control chicks; P-val=P-value. Relative gene expression was determined using the $2^{-\Delta\,\Delta Ct}$ method. APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT1 and CAT2= Cationic amino acid transporter-1 and -2; EAAT3= Excitatory amino acid transporter 3; GLUT1, GLUT2 and GLUT5= Glucose transporter-1, -2 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}AT$; SGLT1= Sodium glucose transporter-1; SI= Sucrase isomaltase; $y^+LAT1$ and $y^+LAT2$= $y^+$ L amino acid transporter-1 and -2, respectively; ZNT1= Zinc transporter-1. * Indicates statistical significance from control at $p<0.05$.

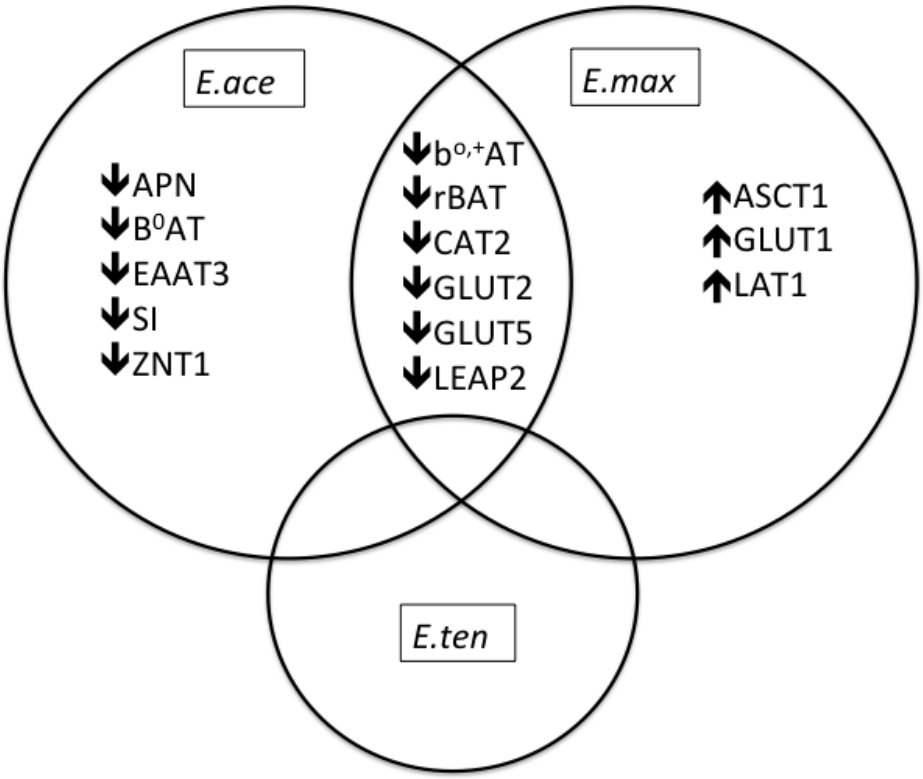


**Figure III-1. Summary of gene expression changes to different *Eimeria* in the duodenum.** *E.ace=E.acervulina*;*E.max= E. maxima; E. ten= E. tenella*; APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT2= Cationic amino acid transporter-2; EAAT3= Excitatory amino acid transporter 3; GLUT2 and GLUT5= Glucose transporter-2 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; rBAT= protein related to $b^{0,+}AT$; SI= Sucrase isomaltase; ZNT1= Zinc transporter-1; ↓=downregulation; ↑=upregulation.

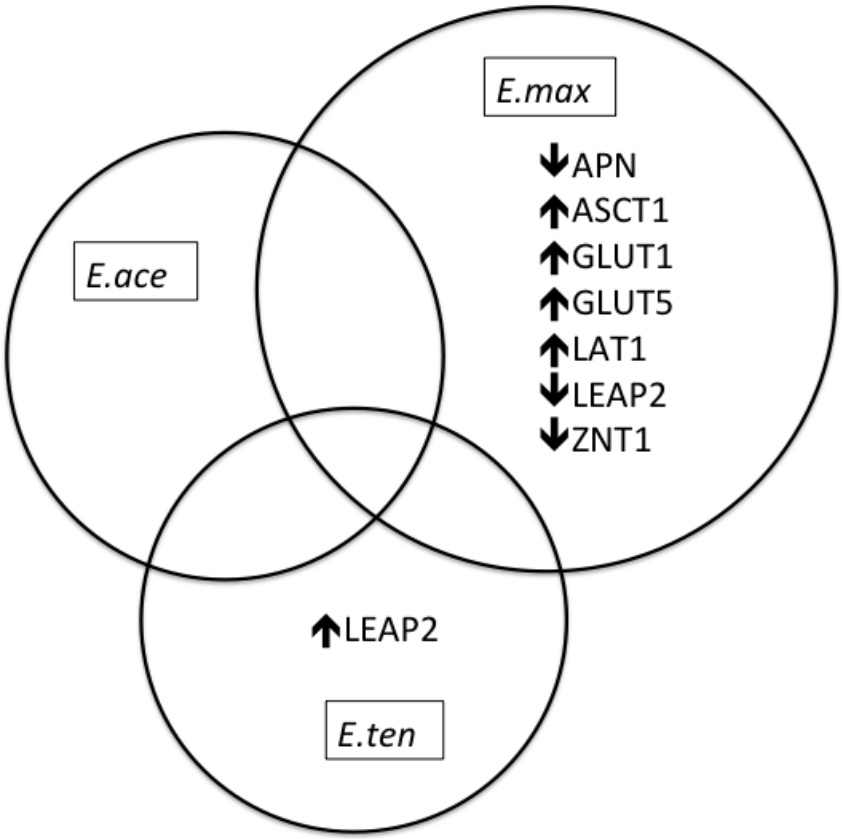


**Figure III-2. Summary of gene expression changes to different *Eimeria* in the jejunum.** *E.ace=E.acervulina*; *E.max= E. maxima; E. ten= E. tenella*; APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; GLUT1 and GLUT5= Glucose transporter-1 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; ZNT1= Zinc transporter-1; ↓=downregulation; ↑=upregulation.

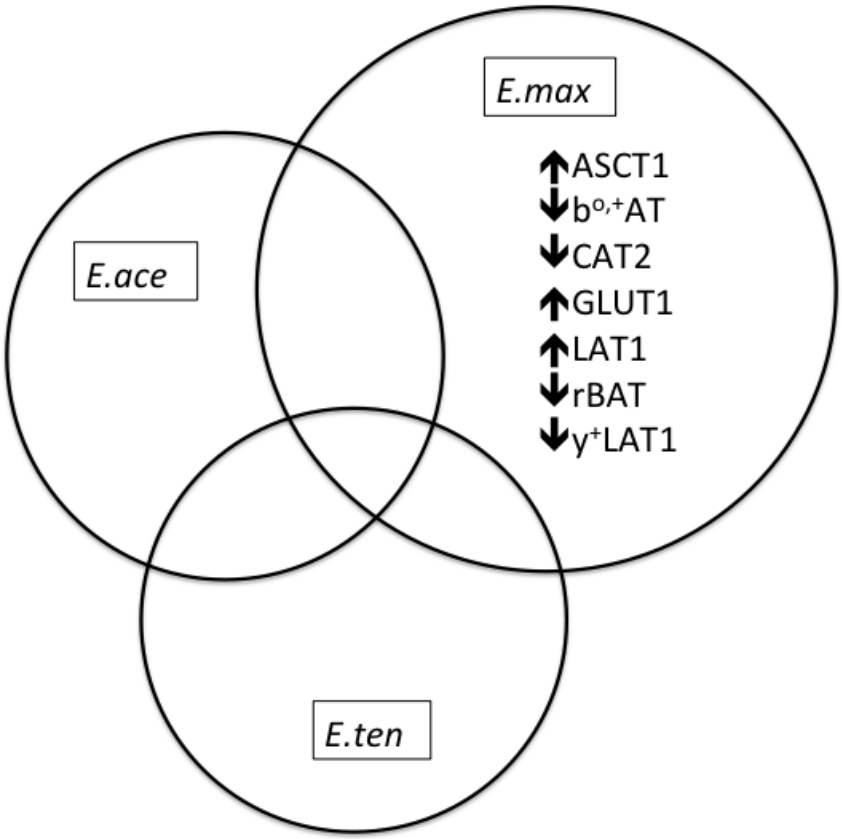


**Figure III-3. Summary of gene expression changes to different *Eimeria* in the ileum.**
*E.ace=E.acervulina*; *E.max= E. maxima; E. ten= E. tenella*; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}AT$ = $Na^{+}$-independent neutral amino acid transporter; GLUT1 = Glucose transporter-1; LAT1=L type amino acid transporter-1; rBAT= protein related to $b^{0,+}AT$; $y^{+}LAT1$= $y^{+}$ L amino acid transporter-1; ↓=downregulation; ↑=upregulation.

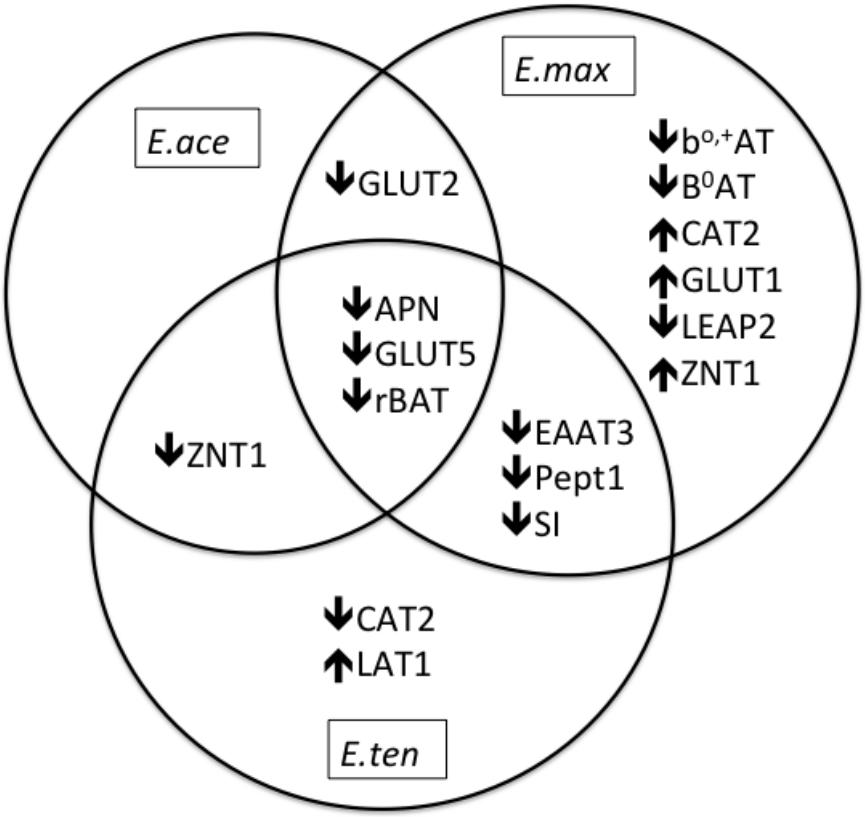


**Figure III-4. Summary of gene expression changes to different *Eimeria* in the ceca.** *E.ace=E.acervulina*; *E.max= E. maxima; E. ten= E. tenella*; APN= Aminopeptidase N; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT2= Cationic amino acid transporter-2; EAAT3= Excitatory amino acid transporter 3; GLUT1, GLUT2 and GLUT5= Glucose transporter-1, -2 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}AT$; SI= Sucrase isomaltase; ZNT1= Zinc transporter-1; ↓=downregulation; ↑=upregulation.

**Relative gene expression in different intestinal segment**

Expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in different intestinal segment in control birds is summarized in Table III-6. Relative expression level was standardized to duodenum samples. The main purpose of this part of the study is to examine the difference in gene expression between the small intestine and the ceca. Many genes were down regulated in the ceca when compared to the small intestinal samples. Expression level of $B^0AT$, LEAP2, Pept1 and SI in the ceca was expressed at less than 1% of duodenum, jejunum and ileum samples. $b^{0,+}AT$, EAAT3, GLUT2, GLUT5 rBAT and SGLT1 was expressed between 1 and 6% of the small intestinal samples. APN and $y^+LAT2$ expression was expressed at about 40% in the ceca when compared to the small intestinal samples. There was an approximately 3-fold upregulation of CAT2 and GLUT1 in the ceca relative to the small intestine. Genes such as ASCT1, CAT1, LAT1, $y^+LAT1$ and ZNT1 showed similar expression levels in duodenum, jejunum, ileum and ceca in the control group chickens.

| | Relative gene expression | | | | | | |
|---|---|---|---|---|---|---|---|
| Tissue | APN | ASCT1 | $b^{0,+}AT$ | $B^0AT$ | CAT1 | CAT2 | EAAT3 |
| DU | 1.08±0.16$^{ab}$ | 1.04±0.12$^{a}$ | 1.10±0.20$^{a}$ | 1.18±0.25$^{ab}$ | 2.89±1.67$^{a}$ | 1.05±0.16$^{b}$ | 1.13±0.23$^{ab}$ |
| JE | 1.48±0.29$^{a}$ | 1.05±0.16$^{a}$ | 0.88±0.31$^{ab}$ | 1.60±0.70$^{a}$ | 2.38±1.36$^{a}$ | 0.86±0.09$^{b}$ | 2.44±0.77$^{a}$ |
| IL | 1.64±0.21$^{a}$ | 1.03±0.13$^{a}$ | 1.05±0.22$^{a}$ | 1.86±0.33$^{a}$ | 3.57±2.45$^{a}$ | 1.11±0.21$^{b}$ | 2.36±0.48$^{a}$ |
| CE | 0.47±0.12$^{b}$ | 1.02±0.04$^{a}$ | 0.04±0.01$^{b}$ | 0.002±0.0005$^{b}$ | 4.33±2.50$^{a}$ | 3.55±0.49$^{a}$ | 0.05±0.02$^{b}$ |

| | Relative gene expression | | | | | |
|---|---|---|---|---|---|---|
| Tissue | GLUT1 | GLUT2 | GLUT5 | LAT1 | LEAP2 | Pept1 |
| DU | 1.03±0.11$^{b}$ | 1.19±0.25$^{ab}$ | 1.10±0.21$^{a}$ | 1.04±0.12$^{a}$ | 1.20±0.24$^{a}$ | 1.19±0.26$^{a}$ |
| JE | 1.21±0.11$^{b}$ | 1.41±0.22$^{a}$ | 1.39±0.24$^{a}$ | 0.77±0.14$^{a}$ | 0.59±0.10$^{ab}$ | 0.84±0.18$^{a}$ |
| IL | 1.57±0.15$^{b}$ | 0.41±0.31$^{bc}$ | 0.96±0.13$^{a}$ | 0.82±0.14$^{a}$ | 0.43±0.33$^{ab}$ | 0.76±0.15$^{a}$ |
| CE | 3.18±0.23$^{a}$ | 0.02±0.005$^{c}$ | 0.06±0.01$^{b}$ | 1.12±0.08$^{a}$ | 0.003±0.001$^{b}$ | 0.003±0.001$^{b}$ |

| | Relative gene expression | | | | | |
|---|---|---|---|---|---|---|
| Tissue | rBAT | SGLT1 | SI | $y^+LAT1$ | $y^+LAT2$ | ZNT1 |
| DU | 1.12±0.22$^{a}$ | 1.05±0.15$^{b}$ | 1.13±0.21$^{b}$ | 1.08±0.17$^{a}$ | 1.04±0.12$^{a}$ | 1.10±0.20$^{a}$ |
| JE | 0.99±0.19$^{a}$ | 1.62±0.14$^{ab}$ | 1.97±0.16$^{a}$ | 1.08±0.57$^{a}$ | 0.98±0.10$^{a}$ | 0.69±0.09$^{a}$ |
| IL | 0.80±0.09$^{a}$ | 1.96±0.24$^{a}$ | 1.13±0.27$^{b}$ | 0.89±0.11$^{a}$ | 0.94±0.05$^{a}$ | 0.59±0.15$^{a}$ |
| CE | 0.02±0.003$^{b}$ | 0.03±0.01$^{c}$ | 0.005±0.001$^{c}$ | 1.30±0.13$^{a}$ | 0.36±0.03$^{b}$ | 0.87±0.11$^{a}$ |

**Table III-6. Expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in different intestinal segments in control group chickens.** DU=duodenum; JE=jejunum; IL=ileum. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method. APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT1 and CAT2= Cationic amino acid transporter-1 and -2; EAAT3= Excitatory amino acid transporter 3; GLUT1, GLUT2 and GLUT5= Glucose transporter-1, -2 and -5, respectively; LAT1=L type amino acid transporter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}AT$; SGLT1= Sodium glucose transporter-1; SI= Sucrase isomaltase; $y^+LAT1$ and $y^+LAT2$= $y^+$ L amino acid transporter-1 and -2, respectively; ZNT1= Zinc transporter-1. $^{a,b,c}$ Means not indicated with same superscript are significantly different at $p<0.05$.

## DISCUSSION

Although *Eimeria* challenged broilers did not show a significant difference in body weight gain when compared to the control birds, *E. acervulina*- and *E. maxima*-challenged broilers showed numerically 42% and 30% of weight gain depression, respectively. Since this study was designed to have about 20% of weight gain depression, it was very likely that the chickens did have true weight gain depression, but due to the biological variance, the depression was not statistically significant. From the result we see there was no significant difference in expression of digestive enzyme and nutrient transporter expression change in the small intestine of *E. tenella*-challenged chickens, this may explain why *E. tenella*-challenged birds did not show the expected body weight gain depression.

The expression level of digestive enzymes and nutrient transporters in different segmens of the intestine indicated that most of the genes examined in this study have lower expression level in the ceca when compared to the small intestine. In the most extreme case: the expression of $B^0AT$, the neutral amino acid transporter located at the brush border membrane, in the ceca was less than 0.2% of the amount in the small intestine. Most of the genes analyzed did not show a significant difference in expression level between different segments of the small intestine, but there were many differences between the small intestine and the ceca, this result indicates a functional difference between the small intestine and the ceca for nutrient digestion and absorption.

Changes in expression of digestive enzymes and nutrient transporters throughout development or in response to different diets have been extensively studied in chicken (Gilbert, et al., 2008a). The genes examined in *Eimeria*-challenged chickens are linked to immune factors expressed by the chicken or the *Eimeria* transcriptome. Recently, a microarray study reported the transcriptome for chicken cecal epithelial cells upon *E. tenella*-challenge (Guo, et al., 2013). These results indicated most upregulated genes are involved in the immune response, cell differentiation, apoptosis and signaling pathways. The downregulated genes are generally metabolic enzymes, membrane components, and some transporters. A specific study on transcription of digestive enzymes and nutrient transporters in *Eimeria*-challenged chickens is limited to jejunum sample of *E. maxima*-challenged broilers (Paris and Wong, 2013). This study

investigated the expression of digestive enzymes and nutrient transporters in the intestine of *E. acervulina*-, *E. maxima*- and *E. tenella*-challenged broilers.

Three genes (APN, GLUT5 and ZNT1) were down regulated in all three species of *Eimeria* at their respective target sites (Figure III-5). APN represents 8% of total protein at the brush border membrane (Semenza, 1986). GLUT5 and ZNT1 are also most abundantly expressed in the intestine compared to other tissues and organs (Davidson, et al., 1992; Yu, et al., 2007). APN is involved in the final digestion of protein where it releases free amino acids from the N-terminus of the peptide. Decreased expression of APN can result in reduced efficiency of amino acid absorption. GLUT5 is the high affinity fructose transporter in the small intestine, which is responsible for fructose uptake at the enterocytes. Reduced expression of APN and GLUT5 can contribute to the depression of nutrient supply for the enterocytes and further result in reduced body weight gain of the animal. ZNT1 functions in efflux of $Zn^{2+}$ at the basolateral membrane of enterocytes. Expression of ZNT1 is important to protect the cell against zinc toxicity (Nolte, et al., 2004). Zinc, as an antioxidant, can also protect the host against *E. acervulina*-induced oxidative damage (Georgieva, et al., 2011). Decreased expression of ZNT1 can either promote zinc toxicity and cell death and/or reduce the damage caused by *Eimeria*. There is evidence that these three genes can be upregulated by increasing feeding of their respective substrates (Christel, et al., 2007; Monteiro, et al., 1999; Tako, et al., 2005). But the cause and effect between downregulation of these three genes and decrease in nutrient intake in *Eimeria*-challenged chickens are yet to be investigated.

*E. acervulina*-challenged duodenum samples and *E. tenella*-challenged ceca samples showed the same common downregulation patterns for CAT2, EAAT3, rBAT and SI. Among those, EAAT3 plays an important role in enterocyte metabolism as it transports glutamate, the energy source of intestinal epithelial cells. Downregulation of EAAT3 also was observed by Paris and Wong (2013) in *E. maxima*-challenged jejunum samples but not in the jejunum samples in this study. This could be due to the difference in broilers used, Aviagen line A for Paris and Wong (2013) and Ross Heritage line for this study. Aviagen line A is a Ross line that was developed by feeding a corn-soy-based diet with lower relative amino acid concentrations (Gilbert, et al., 2007). The Ross Heritage line in this study was not been heavily selected. Decreased influx of glutamate can result in energy depletion, which can lead to accelerated programed cell death. This may be part of the host defense mechanism for eliminating coccidial infection.

Downregulation of rBAT can result in reduced $b^{0,+}AT$ presence at the brush border membrane, which may lead to reduced neutral amino acids transported into the cell. Decreased SI expression can affect the digestion of polysaccharides. Downregulation of CAT2 can lead to less cationic amino acid transport out of the cell, which may disrupt the electrical balance in the cell.

*E. maxima*-challenged jejunum samples and *E. tenella*-challenged ceca samples showed the same upregulation of LAT1. LAT1 transports large neutral amino acids like phenylalanine, which can be hydrolyzed into tyrosine (Ory and Lyman, 1955). Tyrosine is abundant in *E. maxima* oocycts walls (Belli, et al., 2009) and increased efflux of phenylalanine may cause a defect of oocyst wall formation. This may be a common mechanism for chickens to fight against *E. maxima* and *E. tenella* challenge. *E. maxima*-challenged chickens also showed increase expression of ASCT1. Even more robust increase of ASCT1 and LAT1 expression is observed in different strains of *E. maxima* infected broilers (Paris and Wong, 2013). Thus, increasing expression of basolateral amino acid transporters may be a common mechanism for *E. maxima* inhibition.

LEAP2 is an antimicrobial peptide that changes the permeability of parasite membranes. Intestinal LEAP2 level is upregulated in *Salmonella enterica*-infected chickens (Townes, et al., 2004). Both *E. acervulina*-challenged duodenum samples and *E. maxima*-challenged jejunum samples showed down regulation of LEAP2. The mechanism behind this downregulation is not yet clear, but it is proposed that upon entering the host cell *Eimeria* turns down the expression of LEAP2 (Casterlow, et al., 2011). In this study, in the jejunum of *E. tenella*-challenged broilers LEAP2 was up regulated and no significant change was observed in the ceca. In chickens challenged with 50,000 and 500,000 *Eimeria praecox* oocysts, LEAP2 is downregulated in both duodenum and jejunum at day 4 and day 5 after challenge (Sumners, et al., 2011). But at day 7 after challenge, no significant LEAP2 expression change is observed. In this study, there was no significant LEAP2 expression downregulation in the ceca of *E. tenella*-challenged broilers, LEAP2 change may be significant at a different time point.

In summary, many genes examined in this study showed common regulation in two or three species of *Eimeria* at their respective target site. Downregulation of APN, GLUT5 and ZNT1 may result in peptide and fructose depletion and zinc balance disruption in the infected cell. This may result in cell death and inhibits parasite replication.

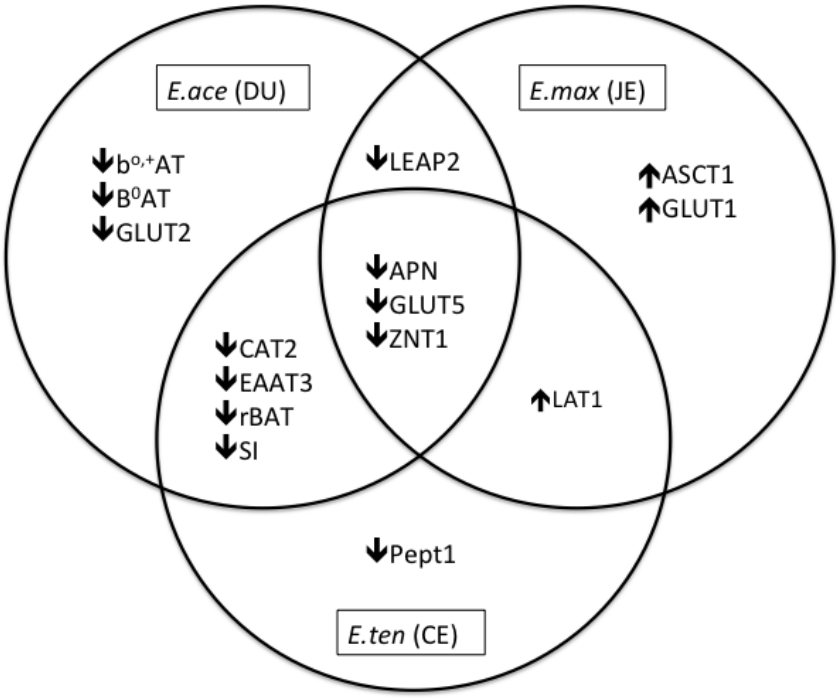


**Figure III-5. Summary of gene expression changes to different *Eimeria* in their respective target tissue.** *E.ace=E.acervulina*, *E.max= E. maxima, E. ten= E. tenella,* DU=duodenum, JE=jejunum, CE=ceca, APN= Aminopeptidase N; ASCT1= Alanine, serine, cysteine and threonine transporter; $b^{0,+}AT$ and $B^0AT$= $Na^+$-independent and $Na^+$-dependent neutral amino acid transporter, respectively; CAT2= Cationic amino acid transporter-2; EAAT3= Excitatory amino acid transporter 3; GLUT1, GLUT2 and GLUT5= Glucose transporter-1, -2 and -5, respectively; LAT1=L type amino acid transorter-1; LEAP2= Liver-expressed antimicrobial peptide-2; Pept1= Peptide transporter-1; rBAT= protein related to $b^{0,+}AT$; SI= Sucrase isomaltase; ZNT1= Zinc transporter-1; ↓=downregulation; ↑=upregulation.

## LITERATURE CITED


Belli, S. I., D. J. Ferguson, M. Katrib, I. Slapetova, K. Mai, J. Slapeta, S. A. Flowers, K. B. Miska, F. M. Tomley, M. W. Shirley, M. G. Wallach, and N. C. Smith. 2009. Conservation of proteins involved in oocyst wall formation in Eimeria maxima, Eimeria tenella and Eimeria acervulina. Int J Parasitol 39:1063-1070. doi 10.1016/j.ijpara.2009.05.004

Casterlow, S., H. Li, E. R. Gilbert, R. A. Dalloul, A. P. McElroy, D. A. Emmerson, and E. A. Wong. 2011. An antimicrobial peptide is downregulated in the small intestine of Eimeria maxima-infected chickens. Poult Sci 90:1212-1219. doi 10.3382/ps.2010-01110

Christel, C. M., D. F. DeNardo, and S. M. Secor. 2007. Metabolic and digestive response to food ingestion in a binge-feeding lizard, the Gila monster (Heloderma suspectum). J Exp Biol 210:3430-3439. doi 10.1242/jeb.004820

Conway, D. P., and M. E. McKenzie. 2007. Poultry coccidiosis : diagnostic and testing procedures. 3rd ed. Blackwell Pub., Ames, Iowa.

Dalloul, R. A., T. W. Bliss, Y. H. Hong, I. Ben-Chouikha, D. W. Park, C. L. Keeler, and H. S. Lillehoj. 2007. Unique responses of the avian macrophage to different species of Eimeria. Mol Immunol 44:558-566. doi 10.1016/j.molimm.2006.02.004

Davidson, N. O., A. M. Hausman, C. A. Ifkovits, J. B. Buse, G. W. Gould, C. F. Burant, and G. I. Bell. 1992. Human intestinal glucose transporter expression and localization of GLUT5. Am J Physiol 262:C795-800.

Georgieva, N. V., M. Gabrashanska, V. Koinarski, and Z. Yaneva. 2011. Zinc Supplementation against Eimeria acervulina-Induced Oxidative Damage in Broiler Chickens. Vet Med Int 2011:647124. doi 10.4061/2011/647124

Gilbert, E. R., H. Li, D. A. Emmerson, K. E. Webb, Jr., and E. A. Wong. 2007. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. Poult Sci 86:1739-1753.

Gilbert, E. R., H. Li, D. A. Emmerson, K. E. Webb, Jr., and E. A. Wong. 2008. Dietary protein quality and feed restriction influence abundance of nutrient transporter mRNA in the small intestine of broiler chicks. J Nutr 138:262-271.

Guo, A., J. Cai, W. Gong, H. Yan, X. Luo, G. Tian, S. Zhang, H. Zhang, G. Zhu, and X. Cai. 2013. Transcriptome Analysis in Chicken Cecal Epithelia upon Infection by Eimeria tenella In Vivo. PLoS One 8:e64236. doi 10.1371/journal.pone.0064236

Leeson, S., M. L. Scott, and J. D. Summers. 2001. Nutrition of the chicken. 4th ed. University Books, Guelph, Ontario.

Lillehoj, H. S., and J. M. Trout. 1996. Avian gut-associated lymphoid tissues and intestinal immune responses to Eimeria parasites. Clin Microbiol Rev 9:349-360.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408. doi 10.1006/meth.2001.1262

Monteiro, I. M., L. Jiang, and R. P. Ferraris. 1999. Dietary modulation of intestinal fructose transport and GLUT5 mRNA expression in hypothyroid rat pups. J Pediatr Gastroenterol Nutr 29:563-570.

Nolte, C., A. Gore, I. Sekler, W. Kresse, M. Hershfinkel, A. Hoffmann, H. Kettenmann, and A. Moran. 2004. ZnT-1 expression in astroglial cells protects against zinc toxicity and slows the accumulation of intracellular zinc. Glia 48:145-155. doi 10.1002/glia.20065

Ory, R. L., and C. M. Lyman. 1955. Synthesis of tyrosine and phenylalanine by Lactobacillus arabinosus. J Bacteriol 69:508-515.

Paris, N. E., and E. A. Wong. 2013. Expression of digestive enzymes and nutrient transporters in the intestine of Eimeria maxima-infected chickens. Poult Sci 92:1331-1335. doi 10.3382/ps.2012-02966

Semenza, G. 1986. Anchoring and biosynthesis of stalked brush border membrane proteins: glycosidases and peptidases of enterocytes and renal tubuli. Annu Rev Cell Biol 2:255-313. doi 10.1146/annurev.cb.02.110186.001351

Sumners, L. H., K. B. Miska, M. C. Jenkins, R. H. Fetterer, C. M. Cox, S. Kim, and R. A. Dalloul. 2011. Expression of Toll-like receptors and antimicrobial peptides during Eimeria praecox infection in chickens. Exp Parasitol 127:714-718. doi 10.1016/j.exppara.2010.12.002

Tako, E., P. R. Ferket, and Z. Uni. 2005. Changes in chicken intestinal zinc exporter mRNA expression and small intestinal functionality following intra-amniotic zinc-methionine administration. J Nutr Biochem 16:339-346. doi 10.1016/j.jnutbio.2005.01.002

Townes, C. L., G. Michailidis, C. J. Nile, and J. Hall. 2004. Induction of cationic chicken liver-expressed antimicrobial peptide 2 in response to Salmonella enterica infection. Infect Immun 72:6987-6993. doi 10.1128/IAI.72.12.6987-6993.2004

Yu, Y. Y., C. P. Kirschke, and L. Huang. 2007. Immunohistochemical analysis of ZnT1, 4, 5, 6, and 7 in the mouse gastrointestinal tract. J Histochem Cytochem 55:223-234. doi 10.1369/jhc.6A7032.2006

## CHAPTER IV. EPILOGUE

The results from these studies indicate that upon *Eimeria* challenge, many nutrient transporters in the enterocytes were downregulated. This may be the reason for the reduced feed efficacy in *Eimeria*-challenged chickens. *E. acervulina*-challenged layers and broilers showed many common downregulated genes in the duodenum. But the responses are very different in the jejunum and ileum, as broilers did not show any gene expression changes in these sections. The layers showed more changes compared to broilers, which may be due to the divergent selection for egg laying or rapid growth of the birds.

Many statisticallt significant changes in gene expression in this thesis did not exceed the range of 50% downregulation or 2-fold upregulation, these small changes in gene expression may be considered not biologically important. But when these small changes in digestive enzymes and nutrient transporters at the enterocyte are taken cumulatively, it can result in biologically significant changes. *E. acervulina*- and *E. maxima*-challenged broilers showed numerically 42% and 30% weight gain depression, respectively, when compared to the chickens in the control group. Although these changes were not statistically significant, a 30 to 40% weight gain depression of the birds would lead to a great economical impact in a commercial flock.

In the study of different species of *Eimeria* challenge on broilers, there are many changes in the ceca regardless of the specific target tissue of the *Eimeria* species. This may be due to the structural and functional differences of the ceca compared to the small intestine. *E. acervulina, E. maxima* and *E. tenella*-challenge resulted in common downregulation of APN, GLUT5 and ZNT1 at their respective target tissues. This indicates that there may be a common host response to *Eimeria* challenge.

LEAP2 is downregulated in the duodenum of *E. acervulina*-challenged layers and broilers, and in the jejunum of *E. maxima*-challenged broilers, but not in the ceca of *E. tenella*-challenged broilers. These results imply that downregulation of LEAP2 may be common in *Eimeria* which target the small intestine, but not for the ceca targeted *E. tenella*.

In this thesis, the effect of different species of *Eimeria* was compared in broilers but not in layers. It would be interesting to study how gene expression changes in layers following different *Eimeria* challenge. Also the comparison between layers and broilers can be investigated in both *E. maxima* and *E. tenella*-challenged birds.

In these studies, only the mRNA level of the genes was analyzed. It is important to note that mRNA levels sometimes do not reflect protein levels. Further research on protein level and distribution of the digestive enzymes and nutrient transporters can provide more information on gene expression in response to *Eimeria* challenge. All of the samples in the two studies were collected on day 7 after *Eimeria* challenge. As different genes may have expression changes at different times, it would be interesting to find out the time course for gene expressions changes after *Eimeria* challenge. Many genes did not show significant expression changes in these studies but may be significantly up or downregulated at different time points.

Genes analyzed in these two studies are limited to 16 nutrient transporters, 2 digestive enzymes and one antimicrobial peptide. The change in expression profiles of other nutrient transporters and digestive enzymes present in the intestine can be further studied.

This study provides an initial characterization of some of the changes in the intestinal gene expression profiles in *Eimeria*-challenged chickens and may help elucidate a novel molecular mechanism of host response to *Eimeria* challenge.

## LITERATURE CITED


Allen, P. C., and R. H. Fetterer. 2002. Recent advances in biology and immunobiology of Eimeria species and in diagnosis and control of infection with these coccidian parasites of poultry. Clin Microbiol Rev 15:58-65.

Belley, A., K. Keller, M. Gottke, and K. Chadee. 1999. Intestinal mucins in colonization and host defense against pathogens. Am J Trop Med Hyg 60:10-15.

Belli, S. I., D. J. Ferguson, M. Katrib, I. Slapetova, K. Mai, J. Slapeta, S. A. Flowers, K. B. Miska, F. M. Tomley, M. W. Shirley, M. G. Wallach, and N. C. Smith. 2009. Conservation of proteins involved in oocyst wall formation in Eimeria maxima, Eimeria tenella and Eimeria acervulina. Int J Parasitol 39:1063-1070. doi 10.1016/j.ijpara.2009.05.004

Blachier, F., C. Boutry, C. Bos, and D. Tome. 2009. Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines. Am J Clin Nutr 90:814S-821S. doi 10.3945/ajcn.2009.27462S

Boyer, S., P. A. Sharp, E. S. Debnam, S. A. Baldwin, and S. K. Srai. 1996. Streptozotocin diabetes and the expression of GLUT1 at the brush border and basolateral membranes of intestinal enterocytes. FEBS Lett 396:218-222.

Broer, A., K. Klingel, S. Kowalczuk, J. E. Rasko, J. Cavanaugh, and S. Broer. 2004. Molecular cloning of mouse amino acid transport system B0, a neutral amino acid transporter related to Hartnup disorder. J Biol Chem 279:24467-24476. doi 10.1074/jbc.M400904200

Broer, A., C. A. Wagner, F. Lang, and S. Broer. 2000. The heterodimeric amino acid transporter 4F2hc/y+LAT2 mediates arginine efflux in exchange with glutamine. Biochem J 349 Pt 3:787-795.

Casterlow, S., H. Li, E. R. Gilbert, R. A. Dalloul, A. P. McElroy, D. A. Emmerson, and E. A. Wong. 2011. An antimicrobial peptide is downregulated in the small intestine of Eimeria maxima-infected chickens. Poult Sci 90:1212-1219. doi 10.3382/ps.2010-01110

Christel, C. M., D. F. DeNardo, and S. M. Secor. 2007. Metabolic and digestive response to food ingestion in a binge-feeding lizard, the Gila monster (Heloderma suspectum). J Exp Biol 210:3430-3439. doi 10.1242/jeb.004820

Conway, D. P., and M. E. McKenzie. 2007. Poultry coccidiosis : diagnostic and testing procedures. 3rd ed. Blackwell Pub., Ames, Iowa.

Dalloul, R. A., T. W. Bliss, Y. H. Hong, I. Ben-Chouikha, D. W. Park, C. L. Keeler, and H. S. Lillehoj. 2007. Unique responses of the avian macrophage to different species of Eimeria. Mol Immunol 44:558-566. doi 10.1016/j.molimm.2006.02.004

Daniel, H., and G. Kottra. 2004. The proton oligopeptide cotransporter family SLC15 in physiology and pharmacology. Pflugers Arch 447:610-618. doi 10.1007/s00424-003-1101-4

Danziger, R. S. 2008. Aminopeptidase N in arterial hypertension. Heart Fail Rev 13:293-298. doi 10.1007/s10741-007-9061-y

Davidson, N. O., A. M. Hausman, C. A. Ifkovits, J. B. Buse, G. W. Gould, C. F. Burant, and G. I. Bell. 1992. Human intestinal glucose transporter expression and localization of GLUT5. Am J Physiol 262:C795-800.

Devergnas, S., F. Chimienti, N. Naud, A. Pennequin, Y. Coquerel, J. Chantegrel, A. Favier, and M. Seve. 2004. Differential regulation of zinc efflux transporters ZnT-1, ZnT-5 and ZnT-7 gene expression by zinc levels: a real-time RT-PCR study. Biochem Pharmacol 68:699-709. doi 10.1016/j.bcp.2004.05.024

Fan, M. Z., J. C. Matthews, N. M. Etienne, B. Stoll, D. Lackeyram, and D. G. Burrin. 2004. Expression of apical membrane L-glutamate transporters in neonatal porcine epithelial cells along the small intestinal crypt-villus axis. Am J Physiol Gastrointest Liver Physiol 287:G385-398. doi 10.1152/ajpgi.00232.2003

Fotiadis, D., Y. Kanai, and M. Palacin. 2013. The SLC3 and SLC7 families of amino acid transporters. Mol Aspects Med 34:139-158. doi 10.1016/j.mam.2012.10.007

Garriga, C., A. Barfull, and J. M. Planas. 2004. Kinetic characterization of apical D-fructose transport in chicken jejunum. J Membr Biol 197:71-76. doi 10.1007/s00232-003-0640-0

Georgieva, N. V., M. Gabrashanska, V. Koinarski, and Z. Yaneva. 2011. Zinc Supplementation against Eimeria acervulina-Induced Oxidative Damage in Broiler Chickens. Vet Med Int 2011:647124. doi 10.4061/2011/647124

Gilbert, E. R., H. Li, D. A. Emmerson, K. E. Webb, Jr., and E. A. Wong. 2007. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. Poult Sci 86:1739-1753.

Gilbert, E. R., H. Li, D. A. Emmerson, K. E. Webb, Jr., and E. A. Wong. 2008a. Dietary protein quality and feed restriction influence abundance of nutrient transporter mRNA in the small intestine of broiler chicks. J Nutr 138:262-271.

Gilbert, E. R., E. A. Wong, and K. E. Webb, Jr. 2008b. Board-invited review: Peptide absorption and utilization: Implications for animal nutrition and health. J Anim Sci 86:2135-2155. doi 10.2527/jas.2007-0826

Green, M., and H. L. Greene. 1984. The Role of the gastrointestinal tract in nutrient delivery. Academic Press, Orlando.

Guo, A., J. Cai, W. Gong, H. Yan, X. Luo, G. Tian, S. Zhang, H. Zhang, G. Zhu, and X. Cai. 2013. Transcriptome Analysis in Chicken Cecal Epithelia upon Infection by Eimeria tenella In Vivo. PLoS One 8:e64236. doi 10.1371/journal.pone.0064236

Hediger, M. A., and D. B. Rhoads. 1994. Molecular physiology of sodium-glucose cotransporters. Physiol Rev 74:993-1026.

Hocking, P. M., B. O. Hughes, and S. Keer-Keer. 1997. Comparison of food intake, rate of consumption, pecking activity and behaviour in layer and broiler breeder males. Br Poult Sci 38:237-240. doi 10.1080/00071669708417978

Hofmann, K., M. Duker, T. Fink, P. Lichter, and W. Stoffel. 1994. Human neutral amino acid transporter ASCT1: structure of the gene (SLC1A4) and localization to chromosome 2p13-p15. Genomics 24:20-26. doi 10.1006/geno.1994.1577

Hu, Y., D. E. Smith, K. Ma, D. Jappar, W. Thomas, and K. M. Hillgren. 2008. Targeted disruption of peptide transporter Pept1 gene in mice significantly reduces dipeptide absorption in intestine. Mol Pharm 5:1122-1130.

Ivanov, A. I. 2012. Structure and regulation of intestinal epithelial tight junctions: current concepts and unanswered questions. Adv Exp Med Biol 763:132-148.

Iwanaga, T., M. Goto, and M. Watanabe. 2005. Cellular distribution of glutamate transporters in the gastrointestinal tract of mice: an immunohistochemical and in situ hybridization approach. Biomed Res 26:271-278.

Jappar, D., S. P. Wu, Y. Hu, and D. E. Smith. 2010. Significance and regional dependency of peptide transporter (PEPT) 1 in the intestinal permeability of glycylsarcosine: in situ single-pass perfusion studies in wild-type and Pept1 knockout mice. Drug Metab Dispos 38:1740-1746. doi 10.1124/dmd.110.034025

Jenkins, M., C. Parker, C. O'Brien, K. Miska, and R. Fetterer. 2013. Differing Susceptibilities of Eimeria Acervulina, Eimeria Maxima, and Eimeria Tenella Oocysts to Dessication. J Parasitol. doi 10.1645/13-192.1

Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp Parasitol 28:30-36.

Johnson, L. R. 2007. Gastrointestinal physiology. 7th ed. Mosby Elsevier, Philadelphia.

Kanai, Y., and M. A. Hediger. 1992. Primary structure and functional characterization of a high-affinity glutamate transporter. Nature 360:467-471. doi 10.1038/360467a0

Kanai, Y., H. Segawa, K. Miyamoto, H. Uchino, E. Takeda, and H. Endou. 1998. Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). J Biol Chem 273:23629-23632.

Koenen, M. E., A. G. Boonstra-Blom, and S. H. Jeurissen. 2002. Immunological differences between layer- and broiler-type chickens. Vet Immunol Immunopathol 89:47-56.

Lazaridis, K. N., L. Pham, P. Tietz, R. A. Marinelli, P. C. deGroen, S. Levine, P. A. Dawson, and N. F. LaRusso. 1997. Rat cholangiocytes absorb bile acids at their apical domain via the ileal sodium-dependent bile acid transporter. J Clin Invest 100:2714-2721. doi 10.1172/JCI119816

Le Gall, M., V. Tobin, E. Stolarczyk, V. Dalet, A. Leturque, and E. Brot-Laroche. 2007. Sugar sensing by enterocytes combines polarity, membrane bound detectors and sugar metabolism. J Cell Physiol 213:834-843. doi 10.1002/jcp.21245

Leeson, S., M. L. Scott, and J. D. Summers. 2001. Nutrition of the chicken. 4th ed. University Books, Guelph, Ontario.

Lillehoj, H. S., and J. M. Trout. 1996. Avian gut-associated lymphoid tissues and intestinal immune responses to Eimeria parasites. Clin Microbiol Rev 9:349-360.

Luan, Y., and W. Xu. 2007. The structure and main functions of aminopeptidase N. Curr Med Chem 14:639-647.

Lynn, D. J., R. Higgs, S. Gaines, J. Tierney, T. James, A. T. Lloyd, M. A. Fares, G. Mulcahy, and C. O'Farrelly. 2004. Bioinformatic discovery and initial characterisation of nine novel antimicrobial peptide genes in the chicken. Immunogenetics 56:170-177. doi 10.1007/s00251-004-0675-0

McMahon, R. J., and R. J. Cousins. 1998. Regulation of the zinc transporter ZnT-1 by dietary zinc. Proc Natl Acad Sci U S A 95:4841-4846.

Mithieux, G. 2005. The new functions of the gut in the control of glucose homeostasis. Curr Opin Clin Nutr Metab Care 8:445-449.

Monteiro, I. M., L. Jiang, and R. P. Ferraris. 1999. Dietary modulation of intestinal fructose transport and GLUT5 mRNA expression in hypothyroid rat pups. J Pediatr Gastroenterol Nutr 29:563-570.

Moran, G. W., F. C. Leslie, S. E. Levison, J. Worthington, and J. T. McLaughlin. 2008. Enteroendocrine cells: neglected players in gastrointestinal disorders? Therap Adv Gastroenterol 1:51-60. doi 10.1177/1756283X08093943

Moreto, M., and J. M. Planas. 1989. Sugar and amino acid transport properties of the chicken ceca. J Exp Zool Suppl 3:111-116.

Nolte, C., A. Gore, I. Sekler, W. Kresse, M. Hershfinkel, A. Hoffmann, H. Kettenmann, and A. Moran. 2004. ZnT-1 expression in astroglial cells protects against zinc toxicity and slows the accumulation of intracellular zinc. Glia 48:145-155. doi 10.1002/glia.20065

Noy, Y., and D. Sklan. 2001. Yolk and exogenous feed utilization in the posthatch chick. Poult Sci 80:1490-1495.

Ory, R. L., and C. M. Lyman. 1955. Synthesis of tyrosine and phenylalanine by Lactobacillus arabinosus. J Bacteriol 69:508-515.

Palacin, M., and Y. Kanai. 2004. The ancillary proteins of HATs: SLC3 family of amino acid transporters. Pflugers Arch 447:490-494. doi 10.1007/s00424-003-1062-7

Paris, N. E., and E. A. Wong. 2013. Expression of digestive enzymes and nutrient transporters in the intestine of Eimeria maxima-infected chickens. Poult Sci 92:1331-1335. doi 10.3382/ps.2012-02966

Peek, H. W., and W. J. Landman. 2011. Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. Vet Q 31:143-161. doi 10.1080/01652176.2011.605247

Reece, W. O., and W. O. Reece. 2005. Functional anatomy and physiology of domestic animals. 3rd ed. Lippincott Williams & Wilkins, Baltimore.

Romeo, E., M. H. Dave, D. Bacic, Z. Ristic, S. M. Camargo, J. Loffing, C. A. Wagner, and F. Verrey. 2006. Luminal kidney and intestine SLC6 amino acid transporters of B0AT-cluster and their tissue distribution in Mus musculus. Am J Physiol Renal Physiol 290:F376-383. doi 10.1152/ajprenal.00286.2005

Salanitro, J. P., P. A. Muirhead, and J. R. Goodman. 1976. Morphological and physiological characteristics of Gemmiger formicilis isolated from chicken ceca. Appl Environ Microbiol 32:623-632.

Semenza, G. 1986. Anchoring and biosynthesis of stalked brush border membrane proteins: glycosidases and peptidases of enterocytes and renal tubuli. Annu Rev Cell Biol 2:255-313. doi 10.1146/annurev.cb.02.110186.001351

Shariatmadari, F., and J. M. Forbes. 1993. Growth and food intake responses to diets of different protein contents and a choice between diets containing two concentrations of protein in broiler and layer strains of chicken. Br Poult Sci 34:959-970. doi 10.1080/00071669308417656

Shaw, S., E. Jayatilleke, S. Meyers, N. Colman, B. Herzlich, and V. Herbert. 1989. The ileum is the major site of absorption of vitamin B12 analogues. Am J Gastroenterol 84:22-26.

Smith, M. E., and D. G. Morton. 2010. The digestive system : basic science and clinical. 2nd ed. Churchill Livingstone, Edinburgh ; New York.

Speier, J. S., L. Yadgary, Z. Uni, and E. A. Wong. 2012. Gene expression of nutrient transporters and digestive enzymes in the yolk sac membrane and small intestine of the developing embryonic chick. Poult Sci 91:1941-1949. doi 10.3382/ps.2011-02092

Stotish, R. L., C. C. Wang, and M. Meyenhofer. 1978. Structure and composition of the oocyst wall of Eimeria tenella. J Parasitol 64:1074-1081.

Sumners, L. H., K. B. Miska, M. C. Jenkins, R. H. Fetterer, C. M. Cox, S. Kim, and R. A. Dalloul. 2011. Expression of Toll-like receptors and antimicrobial peptides during Eimeria praecox infection in chickens. Exp Parasitol 127:714-718. doi 10.1016/j.exppara.2010.12.002

Swennen, Q., P. J. Verhulst, A. Collin, A. Bordas, K. Verbeke, G. Vansant, E. Decuypere, and J. Buyse. 2007. Further investigations on the role of diet-induced thermogenesis in the regulation of feed intake in chickens: comparison of adult cockerels of lines selected for high or low residual feed intake. Poult Sci 86:1960-1971.

Tako, E., P. R. Ferket, and Z. Uni. 2005. Changes in chicken intestinal zinc exporter mRNA expression and small intestinal functionality following intra-amniotic zinc-methionine administration. J Nutr Biochem 16:339-346. doi 10.1016/j.jnutbio.2005.01.002

Terada, T., Y. Shimada, X. Pan, K. Kishimoto, T. Sakurai, R. Doi, H. Onodera, T. Katsura, M. Imamura, and K. Inui. 2005. Expression profiles of various transporters for oligopeptides, amino acids and organic ions along the human digestive tract. Biochem Pharmacol 70:1756-1763. doi 10.1016/j.bcp.2005.09.027

Townes, C. L., G. Michailidis, and J. Hall. 2009. The interaction of the antimicrobial peptide cLEAP-2 and the bacterial membrane. Biochem Biophys Res Commun 387:500-503. doi 10.1016/j.bbrc.2009.07.046

Townes, C. L., G. Michailidis, C. J. Nile, and J. Hall. 2004. Induction of cationic chicken liver-expressed antimicrobial peptide 2 in response to Salmonella enterica infection. Infect Immun 72:6987-6993. doi 10.1128/IAI.72.12.6987-6993.2004

Twietmeyer, A., and T. McCracken. 2001. Coloring guide to human anatomy. 3rd ed. Lippincott Williams & Wilkins, Philadelphia.

Uldry, M., and B. Thorens. 2004. The SLC2 family of facilitated hexose and polyol transporters. Pflugers Arch 447:480-489. doi 10.1007/s00424-003-1085-0

Uni, Z., A. Geyra, H. Ben-Hur, and D. Sklan. 2000. Small intestinal development in the young chick: crypt formation and enterocyte proliferation and migration. Br Poult Sci 41:544-551. doi 10.1080/00071660020009054

Van Beers, E. H., H. A. Buller, R. J. Grand, A. W. Einerhand, and J. Dekker. 1995. Intestinal brush border glycohydrolases: structure, function, and development. Crit Rev Biochem Mol Biol 30:197-262. doi 10.3109/10409239509085143

Verrey, F., E. I. Closs, C. A. Wagner, M. Palacin, H. Endou, and Y. Kanai. 2004. CATs and HATs: the SLC7 family of amino acid transporters. Pflugers Arch 447:532-542. doi 10.1007/s00424-003-1086-z

Wagner, C. A., F. Lang, and S. Broer. 2001. Function and structure of heterodimeric amino acid transporters. Am J Physiol Cell Physiol 281:C1077-1093.

Whittow, G. C. 2000. Sturkie's avian physiology. 5th ed. Academic Press, San Diego.

Wright, E. M., and E. Turk. 2004. The sodium/glucose cotransport family SLC5. Pflugers Arch 447:510-518. doi 10.1007/s00424-003-1063-6

Yang, J., J. Dowden, A. Tatibouet, Y. Hatanaka, and G. D. Holman. 2002. Development of high-affinity ligands and photoaffinity labels for the D-fructose transporter GLUT5. Biochem J 367:533-539. doi 10.1042/BJ20020843

Yoshikawa, T., R. Inoue, M. Matsumoto, T. Yajima, K. Ushida, and T. Iwanaga. 2011. Comparative expression of hexose transporters (SGLT1, GLUT1, GLUT2 and GLUT5) throughout the mouse gastrointestinal tract. Histochem Cell Biol 135:183-194. doi 10.1007/s00418-011-0779-1

Yu, Y. Y., C. P. Kirschke, and L. Huang. 2007. Immunohistochemical analysis of ZnT1, 4, 5, 6, and 7 in the mouse gastrointestinal tract. J Histochem Cytochem 55:223-234. doi 10.1369/jhc.6A7032.2006

Yuan, L., Y. Ni, S. Barth, Y. Wang, R. Grossmann, and R. Zhao. 2009. Layer and broiler chicks exhibit similar hypothalamic expression of orexigenic neuropeptides but distinct expression of genes related to energy homeostasis and obesity. Brain Res 1273:18-28. doi 10.1016/j.brainres.2009.03.052

Yun, C. H., H. S. Lillehoj, and E. P. Lillehoj. 2000a. Intestinal immune responses to coccidiosis. Dev Comp Immunol 24:303-324.

Yun, C. H., H. S. Lillehoj, J. Zhu, and W. Min. 2000b. Kinetic differences in intestinal and systemic interferon-gamma and antigen-specific antibodies in chickens experimentally infected with Eimeria maxima. Avian Dis 44:305-312.

Zerangue, N., and M. P. Kavanaugh. 1996. ASCT-1 is a neutral amino acid exchanger with chloride channel activity. J Biol Chem 271:27991-27994.

Zhao, F. Q., and A. F. Keating. 2007. Functional properties and genomics of glucose transporters. Curr Genomics 8:113-128.

Zwarycz, B., and E. A. Wong. 2013. Expression of the peptide transporters PepT1, PepT2, and PHT1 in the embryonic and posthatch chick. Poult Sci 92:1314-1321. doi 10.3382/ps.2012-02826