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mRNA expression of amino acid transporters, aminopeptidase, and the di- and tri-peptide transporter PepT1 in the intestine and liver of posthatch broiler chicks

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ABSTRACT Amino acid (AA) transporter proteins are responsible for the movement of amino acids in and out of cells. Aminopeptidase cleaves AAs from the N-terminus of polypeptides making them available for transport, while PepT1 is a di- and tripeptide transporter. In the intestine, these proteins are present on the brush border and basolateral membranes of enterocytes, and are essential for the uptake of AAs into enterocytes and their release into circulation. The purpose of this study was to determine the level of transcription of these genes after hatch in 3 regions of the small intestine, the ceca, and liver. Heritage broiler chicks (n = 5) were sampled at day after hatch and days 3, 5, 7, 10, 12, 14, 17, and 21 posthatch, and mRNA expression level was measured using absolute quantitation. The small intestine (duodenum, jejunum, and ileum) expressed the largest quantities of each gene tested. The expression in the ceca and liver was 1 to 3 orders of magnitude less than that of the small intestine. The expression of basolateral transporters in the small intestine was more constant over days posthatch than the expression of brush border transporters. In the ceca the expression of the brush border transporters decreased over the sampling period, while expression of basolateral genes was relatively constant. In the liver the expression of Na\textsuperscript{+} independent cationic and zwitterionic amino acid transporter (b\textsuperscript{0,+}AT), Na\textsuperscript{+} independent cationic amino acid transporter 2 (CAT2), excitatory amino acid transporter 3 (EAAT3), and the heavy chain corresponding to the b\textsuperscript{0,+} system (rBAT) significantly decreased at 12 days posthatch; however, the expression of Na\textsuperscript{+} independent cationic and Na\textsuperscript{+} dependent neutral amino acid transporter 1 (y\textsuperscript{+}LAT1), Na\textsuperscript{+} coupled neutral amino acid transporter 1; (SNAT1), and Na\textsuperscript{+} coupled neutral amino acid transporter 2 (SNAT2) significantly increased at day 5 posthatch compared to day 1 and these levels remained throughout the rest of the sampling period. The current results suggest that at 1 day posthatch chicks are capable of AA processing and transport in the intestine as well as the liver. Additionally the ability of the ceca in transporting AA from the lumen may decrease with age. The liver should be capable of amino acid transport, but its capabilities may be more specific since the expression of several transporters in this organ is either absent or very low.

Key words: amino acid transporters, chicken, intestine, liver, gene expression

INTRODUCTION The developing chicken embryo relies solely on the nutrients provided by the egg and much of those nutrients are in form of fat (Noble and Cocchi, 1990). Following hatch, the chicks must quickly adapt from a diet high in lipids to one high in protein; therefore it is important that the protein processing and amino acid (AA) transport mechanisms are functional shortly after hatch.

In the chicken, the small intestine is the main site of nutrient absorption; however it has been reported that chickens are capable of nutrient absorption throughout the length of the gut (Obst and Diamond, 1992; Ferrer et al., 1994; Awad et al., 2007). The ceca has been proposed to play a role in nitrogen recycling (Karasawa, 1999), where urea is broken down to ammonia in the colon and the resident microflora use the ammonia for amino acid production, which can then be
absorbed through the ceca (Karasawa, 1999). Moretó and Planas (1989) also hypothesized that younger chicks can absorb AAs through the ceca but this function is lost in distal and medial parts, because villi in these portions are replaced by cecal mounds and ridges.

The liver is highly metabolically active and is involved in protein metabolism (de Oliveira et al., 2008). Amino acids that are absorbed by the gut and deposited directly into the portal vein are transported to the liver, and from there can continue transport to other organs of the body (Ten Have et al., 2007). Components of AA uptake and protein synthesis mechanisms are present in the liver since it synthesizes plasma proteins such as albumin, fibrinogen, and prothrombin. Amino acids that are not used by other organs are catabolized by the liver and their byproducts excreted through kidneys. Unlike enterocytes that transport AAs via the apical or basolateral membrane, hepatocytes are thought to transport these molecules through the luminal membrane that is in contact with the circulation (Kilberg, 1982).

Proteins are broken down to di- or tripeptides, and AAs that are then transported by enterocytes via amino acid transporters (AATs). Aminopeptidase N (APN) is an exopeptidase that cleaves AAs from the N-terminus of peptides and resides on the brush border membrane (Sanderink et al., 1988). The di-and tripeptide transporter (PepT1) moves peptides from the lumen of the small intestine into the enterocyte (Chen et al., 2002). Other transporters that have different specificities for polarity and/or size are present in the brush border membrane of the enterocyte, and function in transporting free AAs into the enterocyte. Excitatory amino acid transporter 3 (EAAT3) has affinity for anionic AAs aspartate and glutamate (Hundal and Taylor, 2009). The heavy chain corresponding to the b^o,+ system (rBAT) and Na^+ independent cationic and zwitterionic amino acid transporter (b^o,+AT) form a heterodimer and transport cationic and zwitterionic AAs into the enterocyte (Bröer, 2008). Also located on the brush border membrane are the Na^+ and Cl^- dependent neutral and cationic amino acid transporter (ATB^o,+ ) and Na^+ dependent neutral amino acid transporter (B^o,AT), which transport neutral and cationic AAs, and neutral AAs, respectively (Bröer, 2008). On the basolateral membrane of the enterocyte, a number of AATs are present that can either transport AAs from inside of the enterocyte to the vascular supply or vice versa. Na^+ independent cationic amino acid transporter 1 (CAT1) and Na^+ independent cationic amino acid transporter 2 (CAT2) transporters are responsible for moving cationic AAs out of the enterocyte and into the vascular circulation (Devés and Boyd, 1998). The Na^+ independent cationic and Na^+ dependent neutral amino acid transporter 1 (y^+,LAT1) and Na^+ independent cationic and Na^+ dependent neutral amino acid transporter 2 (y^+,LAT2) are neutral and cationic amino acid transporters, while Na^+ coupled neutral amino acid transporter 1 (SNAT1) and Na^+ coupled neutral amino acid transporter 2 (SNAT2) transport neutral AAs across the basolateral membrane of the enterocyte (Bröer, 2008).

The presence of mRNAs encoding proteins involved in protein processing and AAT have been measured in the small intestine (Gilbert et al., 2007, Speier et al., 2012, Zwarycz and Wong, 2013), the ceca, as well as the liver of embryonic chicks using absolute quantitation (Miska et al., 2014). Between days 9 and 11 of incubation expression of APN, PepT1, and AATs are very low in both the small intestine and the ceca; however by day 15 of incubation these genes are highly expressed in both areas of the gut. Unlike the gut, the embryonic liver expresses APN and AATs at high levels from day 9 (Miska et al., 2014). The only gene whose expression could not be detected in the liver is PepT1. However, the presence of the components of protein processing and AAT has not been determined in the ceca or liver of posthatch chicks; Gilbert et al. (2007) determined the expression profiles of 12 AAT genes in the small intestine of late embryos and posthatch chicks of two lines of broilers selected to grow under different sources of proteins (corn versus wheat), and found some differential expression among the different regions of the gut. For example, PepT1 was expressed most abundantly in the duodenum, while APN, b^o,+AT, B^o,AT, CAT2, LAT1, and rBAT were most highly expressed in the ileum. Therefore, some region specific expression was noted. Additionally this study reported that while the expression of some genes (B^o,AT, EAAT3, and PepT1) increased with time, others (CAT1, CAT2, y^+,LAT1, and LAT1) decreased with time. In the current study, in order to expand previous work, we examined gene expression patterns of aminopeptidase N, the di- and tripeptide transporter PepT1, and 10 other AAT genes in the small intestine (duodenum, jejunum, and ileum), the ceca, and the liver of commercial (Ross heritage) broiler chicks from 1 to 21 days posthatch using absolute quantitation.

MATERIALS AND METHODS

Chicken Housing and Tissue Collection

Ross heritage broiler males (n = 45) were obtained from Longnecker’s Hatchery (Elizabethtown, PA) at 1 day posthatch. Birds were fed with 23% crude protein containing feed throughout the course of the study. Birds were fed and given water ad libitum. All procedures involving animals were approved by the Beltsville Area Animal Care and Use Committee. At days 1, 3, 5, 7, 10, 12, 14, 17, and 21 posthatch, 5 animals were euthanized by cervical dislocation. A portion of the duodenum (descending loop), jejunum, and ileum (approximately 3 to 10 cm anterior and posterior of Meckel’s diverticulum), ceca (one lobe), and liver (portion of left lobe) were taken for RNA extraction and analysis. The regions were cut into smaller
Real-Time PCR

Total RNA was extracted from 100 mg tissues using the Purelink RNA mini kit (Life Technologies, Carlsbad, CA) according to the manufacturer’s recommended protocol. Ten µg total RNA was digested using Turbo DNase (Life Technologies) for 30 min at 37°C. Complementary DNA (cDNA) was synthesized from 1 µg total RNA using the iScript Advanced cDNA synthesis kit (Bio-Rad, Hercules, CA) according to the manufacturer’s recommended protocol. To ensure that there was no possible genomic DNA contamination, negative controls for all samples were also prepared by performing reverse transcription (RT) without enzyme mix. Each of the cDNA samples was diluted 1:10 prior to quantitative real-time (qRT)-PCR, and 1 µL diluted cDNA was used per qRT-PCR reaction. All qRT-PCR reactions were performed using the SsoAdvanced SYBR green supermix (Bio-Rad, Hercules, CA). Each reaction consisted of 1 µL diluted cDNA, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 7 µL H2O, and 10 µL 2× SYBR green supermix. The following PCR conditions were used: 95°C for 30 s, and 35 cycles of 95°C for 30 s, 56°C for 1 min, and 72°C for 1 min. All qRT-PCR was carried out using a CFX96 Touch thermo cycler (Bio-Rad, Hercules, CA). Most of the primers used in this study have been previously published by Gilbert et al., 2007. SNAT1 and SNAT2 primers were designed for this study: SNAT1F 5′ GGAAACAGGCTGCATGGTAT 3′, SNAT1R 5′ CGTACCATGCCGAAAAAGRR 3′, and SNAT2F 5′ GCCATGGCTAACACTGGAAT 3′. A dilution series of 1010 to 109 molecules/µL was performed in the presence of excess yeast tRNA (Life Technologies, Carlsbad, CA) at 10 µg/mL. Each of the dilution series was subjected to cDNA synthesis using the iScript Advanced cDNA synthesis kit (Bio-Rad, Hercules, CA). A standard dilution curve was carried out on each plate with each corresponding gene using the thermocycling conditions described above. Three technical replicates were performed per sample for each PCR.

Statistical Analysis

All data were analyzed using Sigma Plot version 12.5 (Systat Software Inc., San Jose, CA). The data were log-transformed in order to maintain normality and homogeneity of variance. For each of the 5 different tissues (duodenum, jejunum, ileum, ceca, and liver), differences in level of gene expression over days posthatch were determined by one-way ANOVA. Values were expressed as means ±1 standard error and comparisons of means were made with the Holm–Sidack test. Regression analysis was done by the least-squares procedure. Correlation between level of gene expression and days posthatch was determined using Pearson product moment correlation. Unless otherwise indicated, significance was at P < 0.05.

RESULTS

Expression Analysis of Genes Encoding Brush Border AATs, PepT1, and APN in Gut Segments

The bar graph depicting expression of the 5 genes encoding AATs, the di- and tripeptide transporter PepT1,
and APN, which are associated with the brush border membrane of the gut over the 3-week sampling period is shown in Figure 1. All 7 genes were expressed at all time points (from day 1 to 21 posthatch) and regions (duodenum, jejunum, and ileum) of the small intestine. Regardless of the segment APN, was the most highly expressed gene while ATB\(^{o,+}\), a Na\(^+\) and Cl\(^-\) dependent neutral and cationic amino acid AAT, was the least expressed gene (Figure 1). In the duodenum, the segment of the small intestine which is thought to play the largest role in AA uptake, there was no correlation between the level of expression of APN, B\(^{o,+}\)AT, EAAT3, and rBAT, and days posthatch, while the level of expression of the remaining 3 genes (ATB\(^{o,+}\), b\(^{o,+}\)AT, and PepT1) were positively correlated with days posthatch (Table 1 and Figure 1A). Figure 1A shows that by day 21 posthatch all brush border associated genes with exception of APN and B\(^{o,+}\)AT were expressed in significantly higher amounts in the duodenum compared to day 1 posthatch. The expression of B\(^{o,+}\)AT was significantly lower in 21-day-old birds compared to those at day one posthatch. In the other 2 segments of small intestine sampled, the jejunum and ileum, only the level of expression of PepT1 was positively correlated with days posthatch, while there was no correlation between level of expression of the remaining genes and days posthatch (Table 1, and Figure 1B, C). Figure 1B shows that at day 21 posthatch the expression of 3 genes EAAT3, PepT1, and rBAT was significantly higher in the jejunum relative to day 1 posthatch, while Figure 1C shows that at day 21 b\(^{o,+}\)AT and EAAT3, in addition to PepT1 and rBAT, show significantly higher amounts of gene expression. In the ileum the expression of B\(^{o,+}\)AT was significantly lower at day 21 posthatch compared to day 1. Gene expression in the portion of the large intestine, the ceca, was vastly different than that of the small intestine. Generally, the level of expression across six (APN, ATB\(^{o,+}\), B\(^{o,+}\)AT, b\(^{o,+}\)AT, EAAT3, and rBAT) genes was at least 2 to 3 orders of magnitude less than observed in the regions of the small intestine. The expression of di- and tripeptide transporter PepT1 was not detected at any of the time points in the ceca. Table 1 shows that expression level of all detectable genes was negatively correlated with days posthatch, while Figure 1D shows that by days 12 to 14 posthatch expression levels were decreasing.

**Expression Analysis of Genes Encoding Basolateral Membrane AATs in Gut Segments**

The bar graph depicting expression of 6 genes encoding AATs associated with the basolateral membrane of the gut over the three-week sampling period is shown in Figure 2. The expression of all 6 genes (CAT1, CAT2, y\(^{+}\)LAT1, y\(^{+}\)LAT2, SNAT1, and SNAT2) was detected at all time periods sampled in the small as well as large intestine. Overall the expression in the duodenum, jejunum, and ileum appeared relatively unchanged over the time period sampled (Figure 2A, B, and C). There were differences in level of gene expression for some genes compared to day 1 posthatch (Figure 2A, B, C) and were most notable at days 12 and 14 posthatch, and by day 21 posthatch there were few significant differences between expression at day 1. There was no correlation between levels of expression of the 6 genes and days posthatch (Table 1). In the ceca, the expression of all 6 genes was detected; however the level of expression was 1 to 2 orders of magnitude lower than in the small intestine (Figure 1D). There was negative correlation between the level of expression of y\(^{+}\)LAT2 and days posthatch, but there was positive correlation between the expression of y\(^{+}\)LAT1 and days posthatch (Table 1). There was no correlation between the level of expression of CAT1, CAT2, SNAT1, SNAT2, and days posthatch. Most of the significant differences in gene expression occurred after day 12 posthatch (Figure 1D).

**DISCUSSION**

The present study describes the expression profiles of 13 genes that encode proteins involved in processing proteins and resulting AAs, and di- and tripeptides in the period between 1 day posthatch and 21 days posthatch. The study was conducted using the
Figure 1. Quantitative real-time–PCR analysis of genes encoding brush border amino acid transporters (ATB\textsuperscript{0,+}, B\textsuperscript{0}AT, B\textsuperscript{0,+}AT, EAAT3, and rBAT), the di- and tripeptide transporter (PepT1), and aminopeptidase N (APN) in the (A) duodenum, (B) jejunum, (C) ileum, and (D) ceca at days 1, 3, 5, 7, 10, 12, 14, 17, and 21 posthatch. Quantities represent log molecules of mRNA/nanogram total RNA ± SEM from 5 individuals. Asterisk indicates significance from a comparable segment at day 1 posthatch.

duodenum, jejunum, ileum, ceca, and the liver. Previously, Gilbert et al., 2007 conducted a study determining the expression of components of protein processing and AA uptake between incubation day 18 and 14 posthatch of chicks in the small intestine, while Miska et al., 2014 reported the expression of components of protein processing and AA uptake between days 9 and 20 egg incubation in the small and large intestine, as
well as the liver of broiler chicks. Because all 3 studies were carried out using absolute quantitation (as opposed to relative expression) the results of these studies can be easily compared and a complete analysis of protein processing, and AA uptake in the gut and liver, of embryonic and posthatch chicks, can be assembled.

Since the corn- and soybean-based diet fed to most broilers produced in the United States contains approximately 20 to 23\% crude protein, at the time of hatch the chick must be prepared to process protein and absorb the resulting peptides and AAs. It has been previously reported (Gilbert et al., 2007; Speier et al., 2012; Miska et al., 2014) that prior to hatch APN, PepT1, and genes encoding AATs are expressed in high amounts. Because Miska et al. (2014) reported expression in the same breed of broiler chick (Ross heritage) as used in the present study it is most appropriate to directly compare results immediately prior to hatch and 1 day posthatch between these 2 studies. In general the expression of brush border AATs between the day before and day after hatch either remained constant (APN, ATB<sup>+</sup>, b<sup>+</sup>+AT, and PepT1) or increased (B<sup>+</sup>AT, EAAT3, and rBAT) in the small intestine. In the ceca the expression profile was different, the expression of 6 genes (APN, ATB<sup>+</sup>, B<sup>+</sup>+AT, b<sup>+</sup>+AT, EAAT3, and rBAT) decreased. The expression of PepT1 could not be detected at any of the posthatch time points tested in the ceca and expression of B<sup>+</sup>AT decreased to nondetectable levels by day 7 posthatch. The expression of basolateral AATs (CAT1, CAT2, y<sup>+</sup>LAT1, y<sup>+</sup>LAT2) between the day before and day after hatch was similar in the small intestine, while in the ceca the expression of these genes decreased. Gilbert et al. (2007) also reported that genes associated with the brush border in the small intestine were either expressed at constant levels or increased in expression between 1 day before and 1 day posthatch, while the basolateral transporters decreased in expression (CAT1, CAT2, and y<sup>+</sup>LAT1) and y<sup>+</sup>LAT2 increased in expression. These results differ from those reported in the present study. In addition, the numbers of mRNAs detected for the basolateral transporters in heritage broilers used in this study are much larger (in some cases more than 10-fold larger, i.e., CAT2 and y<sup>+</sup>LAT1) so it is possible that difference in results between the 2 studies are due to difference in chicken breeds used.

The expression of AATs in the liver between 1 day prior to hatch and 1 day posthatch chicks is fairly constant among the genes tested. Interestingly, PepT1 was not detected in any of the embryonic chick liver samples (Miska et al., 2014; Zwarycz and Wong, 2013); however, in the present study it was detected only at days 1 and 3 posthatch. The embryonic liver does express the peptide transporter PepT2, which is generally considered to be a brain and kidney peptide transporter posthatch (Zwarycz and Wong, 2013). The Na<sup>+</sup> dependent neutral amino acid transporter B<sup>+</sup>AT has never been detected in the embryonic or posthatch liver (Miska et al., 2014).

Over the current period of sampling (1 to 21 days posthatch) a number of interesting trends in gene expression were noted that could be related to intestinal function. Amino acid uptake in birds is carried out in the small intestine, and the current data show that highest expression of APN, PepT1, and AATs are expressed in the 3 segments of the small intestine. The expression in the duodenum of several of the enzymes associated with the brush border membrane of enterocytes was positively correlated with days posthatch (ATB<sup>+</sup>, b<sup>+</sup>+AT, and PepT1), and expression of all brush border genes (with exception of APN and B<sup>+</sup>AT) was significantly higher in 21-day-old chicks when compared to 1-day-old chicks, suggesting that the chick grows and develops the capacity to uptake AAs from the lumen of the gut does as well. The number of brush border genes whose expression positively correlated with days posthatch was less in the jejunum and ileum; however in the ileum expression of 6 genes was significantly higher in 21-day-old chicks when compared to 1-day-old chicks, possibly indicating an increase in capacity for AA uptake. In contrast to the expression of genes associated with the brush border the expression of genes associated with the basolateral surface of enterocytes was less variable. There was no correlation between expression of any of these genes in any of the 3 segments of the small intestine and days posthatch, and there was also little significant difference between gene expressions from 1-day-old chicks compared to 21-day-old chicks. These data suggest that the transport of

### Table 1. Correlation between level of gene expression and age for 11 AATs, the di- and tripeptide transporter PepT1, and APN in 3 segments of the small intestine, ceca, and liver.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>APN</th>
<th>ATB&lt;sup&gt;+&lt;/sup&gt;</th>
<th>B&lt;sup&gt;+&lt;/sup&gt;AT</th>
<th>b&lt;sup&gt;+&lt;/sup&gt;+AT</th>
<th>EAAT3</th>
<th>PepT1</th>
<th>rBAT</th>
<th>CAT1</th>
<th>CAT2</th>
<th>y&lt;sup&gt;+&lt;/sup&gt;LAT1</th>
<th>y&lt;sup&gt;+&lt;/sup&gt;LAT2</th>
<th>SNAT1</th>
<th>SNAT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>NS</td>
<td>0.71</td>
<td>NS</td>
<td>0.79</td>
<td>NS</td>
<td>0.78</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Jejunum</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.80</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Ileum</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.76</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ceca</td>
<td>−0.77</td>
<td>−0.77</td>
<td>−0.70</td>
<td>−0.81</td>
<td>−0.81</td>
<td>NE</td>
<td>−0.78</td>
<td>NS</td>
<td>0.85</td>
<td>−0.83</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Liver</td>
<td>NS</td>
<td>NS</td>
<td>NE</td>
<td>−0.85</td>
<td>NS</td>
<td>−0.68</td>
<td>NS</td>
<td>NS</td>
<td>0.91</td>
<td>−0.72</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NE = Not expressed.

NS = No significant correlation found.

Values are considered significant if correlation coefficient is greater than 0.70 or \( P < 0.05 \).
Figure 2. Quantitative real-time–PCR analysis of genes encoding basolateral amino acid transporters (CAT1, CAT2, y⁺LAT1, y⁺LAT2, SNAT1, and SNAT2) in the (A) duodenum, (B) jejunum, (C) ileum, and (D) ceca at days 1, 3, 5, 7, 10, 12, 14, 17, and 21 posthatch. Quantities represent log molecules of mRNA/nanogram total RNA ± SEM from 5 individuals. Asterisk indicates significance from a comparable segment at day 1 posthatch.
AAs from the enterocyte to the circulation is relatively constant over days posthatch. Rapidly growing birds have rapidly growing guts (Lilja, 1983, Konarzewski et al., 1990) and over a period of 3 weeks following hatch the length of the small intestine increases by a factor of 2.5 and its mass increases approximately 5-fold (Obst and Diamond, 1992). Therefore it is likely that the transport of AAs through the brush border increases to keep up with rapid growth, but the transport mechanism of AAs through the basolateral membrane is already sufficiently expressed at hatch to accommodate rapid growth in other parts of the body. Studies of these molecules at the protein level will be necessary to determine whether their behavior at the protein level corresponds to their mRNA expression.

Chickens possess a bilobed ceca which is part of the large intestine. It is known to function in electrolyte transport and is inhabited by complex populations of microbiota (Mead, 1989). The capacity of the ceca in transporting AAs has been largely uninvestigated. It has also been hypothesized that the resident microflora would use any metabolically useful AAs present in the ceca (Obst and Diamond, 1989). Moretó and Planas (1989) hypothesized that younger chicks can absorb AAs through the ceca but this ability is lost in distal and medial parts, because eventually villi in these regions are replaced by cecal mounds and ridges; however even older birds should have the ability to transport AAs through the neck or proximal regions of the ceca. In the present study, a single intact lobe of the ceca from 1 to 21-day-old chicks was used to carry out the mRNA expression analysis; therefore all 3 segments of the ceca (proximal, medial, and distal) were used in the analysis. In general, both brush border as well as basolateral enzymes in the ceca were expressed at 1 to 3 orders of magnitude less than in the small intestine,
suggesting that even though most of the components may be present (PepT1 was not expressed by the ceca) they are expressed at much lower levels, thus the capacity of the ceca as a whole in AAT is likely less than that of the small intestine. Additionally, the expression of the brush border associated genes was negatively correlated with days posthatch, and their expression decreased significantly over the course of the sampling period. These results are consistent with those reported by Moretó and Planas (1989) who hypothesized that the ability of the ceca to transport AA decreases with time in the distal and medial portions. The expression of the basolateral enzymes was more stable compared to brush border enzymes over time, and the expression of none of the 6 genes tested was significantly less at 21 days posthatch than in 1-day old chicks. This suggests that AAT from the enterocytes present in the ceca to other cells or capillaries remains steady over 3 weeks posthatch.

There has been very little research in investigating AAT in the liver of chickens, even though the liver is highly metabolically active, and is the site for the synthesis of many proteins found in the bloodstream, like albumin, lipoproteins, and clotting factors (Romanoff, 1960). It is also the site for protein metabolism like deamination, transamination, and gluconeogenesis (Yadgary et al., 2010). Unlike enterocytes that transport AAs via the apical or basolateral membrane, hepatocytes are not polarized and are thought to transport AA molecules through the luminal membrane that is in contact with the circulation (Kilberg, 1982). Even though polarity is not expected in hepatocytes in the present study it was found that most genes associated with gut brush border have a tendency to decrease with age (the expression of APN remained steady). On the other hand many of the genes associated with the basolateral membrane of the gut increased or remained fairly constant in expression over time. The significance of these results is not known at present but most of the genes associated with AA processing and transport (with the exception of B’AT and PepT1) are expressed in the liver but at levels lower than in the small intestine.

At this point the expression profiles of many of the genes involved in AA processing and transport, in the gut and liver of embryos and posthatch chicks, have been described; now research must be carried out at the protein level in order to obtain a better grasp of the functional aspects of these proteins.

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