Dietary protein composition influences abundance of peptide and amino acid transporter messenger ribonucleic acid in the small intestine of 2 lines of broiler chicks[1]

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ABSTRACT This study evaluated the effect of dietary protein composition on mRNA abundance of a peptide transporter (peptide transporter 1, PepT1), amino acid (AA) transporters [Na^+-independent cationic and zwitterionic AA transporter ($b^{o,+}AT$), excitatory AA transporter 3 (EAAT3), Na^+-independent cationic and Na^+-dependent neutral AA transporter 2 (y^+LAT2), L-type AA transporter 1 (LAT1), and cationic AA transporter 1 (CAT1)], and a digestive enzyme (aminopeptidase N) in 2 lines (A and B) of broilers that differentially express PepT1 mRNA (line B > line A). From d 8 to 15 posthatch, birds were fed 1 of 3 diets. Protein sources included whey protein concentrate, a whey partial hydrolysate (WPH), or a mixture of free AA (AA) identical to the composition of whey. Quantities of mRNA were assayed by real-time PCR in the small intestine of males at d 8, 9, 11, 13, and 15. For all genes except LAT1, abundance of mRNA was greatest in line B birds that consumed the WPH diet ($P < 0.006$). When mRNA abundance was normalized to β-actin quantities, this effect disappeared, demonstrating a generalized effect on gene expression in line B birds that consumed the hydrolysate. There was a greater villus height:crypt depth ratio ($P < 0.05$) in line B birds fed the WPH diet as compared with line A. In conclusion, line B birds, which express greater PepT1, displayed enhanced intestinal mucosal absorptive surface area and differential regulation of PepT1, AA transporters, and aminopeptidase N in response to dietary protein composition.

Key words: amino acid transporter, broiler, hydrolysate, peptide transporter 1, whey

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INTRODUCTION

The first 2 wk posthatch represent a period of rapid intestinal development in the commercial broiler chick. During this time, activities of digestive enzymes and nutrient transporters increase and dramatic morphological changes occur, including increases in the number and proliferation rate of enterocytes, widening and lengthening of the villi, and deepening of crypts (Nitsan et al., 1991; Uni et al., 1998, 2000; Sklan and Noy, 2000; Moran, 2007). Digestive enzymes are thought to be the rate-limiting step for utilization of nitrogen from dietary protein during the early posthatch period. Nitrogen digestibility increases during the first 2 wk posthatch, but from immediately posthatch onward, there is a high capacity for amino acid (**AA**) absorption from easily digested proteins (Noy and Sklan, 1996; Batal and Parsons, 2002b). The presence of luminal substrate provides the major stimulus for development of the intestinal mucosal layer, and as such, the type of feed and molecular form of nutrients in the intestine may dramatically influence growth and development.

Amino acids may be absorbed in the gut in their free form or as small peptides. The intestinal peptide transporter 1 (**PepT1**) has been characterized in multiple species and to date is the only peptide transporter known to be expressed in enterocytes (Fei et al., 1994; Chen et al., 2002; Daniel, 2004). It has been found that PepT1 is proton-dependent, has broad substrate specificity, and is able to transport several di- and tripeptides. Amino acids in their free form are transported into enterocytes by AA transporters that have various ion dependencies and show a narrower substrate specificity and higher affinity than PepT1 (Albritton et al., 1989; Chairoungdua et al., 1999; Rajan et al., 2000; Kanai and Hediger, 2004).

Peptide and AA transporters in the small intestine of chickens are regulated by a variety of factors, including

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intestinal development, genetic selection, and dietary protein quantity and quality (Chen et al., 2005; Gilbert et al., 2007, 2008). Our laboratory has used 2 lines of broilers, A and B (Aviagen, Huntsville, AL), for several studies of intestinal nutrient transporter gene expression. We consistently observe greater expression of PepT1 mRNA in line B birds under a variety of conditions and hypothesize that they may have a greater capacity to absorb AA that are available in the intestinal lumen as small peptides. Thus, feeding a diet containing a partial protein hydrolysate may lead to enhanced and more efficient AA assimilation and subsequent growth in line B broilers. Additionally, feeding diets containing AA with a potentially greater bioavailability at an early age (within the first 2 wk posthatch) may help alleviate the bottleneck that exists in AA assimilation in the young broiler due to incomplete maturation of digestive enzyme machinery.

To begin testing this hypothesis, the objective of this study was to determine the influence of dietary protein composition on intestinal morphology and gene expression of peptide and AA transporters and a digestive enzyme, aminopeptidase N (**APN**), which plays a role in maintaining the balance between peptides and AA (Sanderink et al., 1988). Chicks from the 2 genetic lines were fed 1 of 3 diets that differed only in composition of the protein source. In one diet, protein was supplied by whey protein concentrate (**WPC**; Lacprodan 80, Arla Foods, Viby, Denmark). In the second diet, whey protein partial hydrolysate (**WPH**; Lacprodan 3065, Arla Foods) was used, in which most of the AA were present as oligopeptides (~95%). In the third diet, a mixture of crystalline AA (AA) was used that matched the composition of the whey protein and, hence, should be immediately available for absorption by free AA transporters. Thus, we generated a model to compare dietary protein supplied by an intact protein to a partial hydrolysate containing mostly oligopeptides and a third diet containing a mixture of free AA.

MATERIALS AND METHODS

Feeding Trial and Tissue Collection

All animal procedures were approved by the Institutional Animal Care and Use Committee at Virginia Tech. Lines A and B are 2 commercial broiler lines that have been selected under different nutritional conditions. Detailed information on these lines has been published (Gilbert et al., 2007, 2008). Briefly, both lines originate from the same genetic stock; however, for at least 10 generations, line A has been selected on a corn-based diet, whereas line B has been selected on a wheat-based diet and higher AA concentrations than for line A. Over the years, this has led to different growth responses in the 2 lines. Our study of these lines is based on the premise that their difference in growth response to dietary protein may be related to differences in digestive enzyme and nutrient transporter gene expression that may have emerged as a result of selection under different nutritional environments.

We obtained sexed line A and line B day-of-hatch chicks from Aviagen. Chicks were transported from the hatchery in Alabama to the poultry farm at Virginia Tech in Blacksburg under thermoneutral conditions. Chicks were placed in heated floor pens with pine shavings and were given free access to drinking water and ad libitum access to a commercial corn-soy-based diet that has been described previously (Gilbert et al., 2007). Birds were fed a standard diet for the first week posthatch to allow ample time for the transition to floor pens and feed consumption. At d 8, 17 male and 12 female birds from both lines were killed by cervical dislocation and sampled for intestinal tissue as described previously (Chen et al., 2005) and the remaining chicks were randomly assigned to 1 of 3 experimental diets (Table 1). Diets were formulated to be similar with the exception of dietary protein composition (intact protein vs. partial hydrolysate vs. mixture of free AA). The whey hydrolysate was chosen because its AA composition is similar to the whey protein and most of its AA (~95%) are in the form of oligopeptides (Table 2). Diets were supplemented with L-arginine at the recommended ideal ratio to lysine (Emmert and Baker, 1997) because preliminary trials using diets not supplemented with L-arginine resulted in poor performance (data not shown). Diets were formulated to meet or exceed recommended vitamin and mineral concentrations for chemically defined diets fed to chicks (NRC, 1994). Purina TestDiet (Richmond, IN) assisted with diet formulation and prepared the experimental diets.

Pen weights were similar before consumption of experimental diets. Feed intake of all diets was equalized by restricting intake to the amount of the diet consumed the least during the previous 8 h (line B female, AA diet). Body weights were recorded daily and feed consumption was measured at 3 intervals (0700, 1500, and 2300 h) daily. At d 9, 11, 13, and 15, 17 males and 12 females from each line and each diet were killed and sampled for intestinal tissue. For each sampling day, intestine was divided into duodenum, jejunum, and ileum. Intestinal segments were squeezed and rinsed in ice-cold PBS (NaH_2PO_4, 1.47 m*M*; Na_2HPO_4, 8.09 m*M*; NaCl, 145 m*M*). Rinsed segments were weighed, minced with a razor blade, and mixed. Aliquots were frozen at −80°C. Five males were selected from each group for RNA isolation. Sex of birds was confirmed by PCR as described previously (Gilbert et al., 2007). Remaining birds were used for other analyses.

Histological Analyses

Five male birds from each group were used for histology at d 15. Approximately 1-cm sections were dissected out of the tract at the mid-region of each segment and were rinsed as described above and fixed in neutral-buffered 10% formalin. Vials were incubated at 4°C overnight on a rocker. Sections were then subjected

Table 1. Composition (as-fed basis) of the whey-, hydrolysate-, and amino acid (AA)-based diets

	Diet[1]		
Ingredient, %	Whey	Hydrolysate	AA
Corn starch	50.09	50.09	50.09
Whey protein concentrate[2]	26.02	—	—
Whey hydrolysate[3]	—	25.57	—
AA mixture[4]	—	—	28.53
Soybean oil	6.00	6.00	6.00
Sucrose	5.00	5.00	5.00
Milk fat	—	2.04	2.10
Powdered cellulose	4.57	2.94	—
Mineral premix[5]	5.37	5.37	5.37
Vitamin premix[6]	0.20	0.20	0.20
Choline chloride	0.17	0.17	0.17
BMD-50[7]	0.05	0.05	0.05
DL-α-Tocopheryl acetate	0.002	0.002	0.002
L-Arginine	2.53	2.56	2.53

[1]The CP values for the whey and hydrolysate diets were formulated to be 24%. The analyzed CP values for all diets are as follows: whey diet, 24%; hydrolysate, 25%; AA diet, 27%.

[2]Lacprodan DI-8090 whey protein concentrate (Arla Foods, Viby, Denmark).

[3]Lacprodan DI-3065 whey protein hydrolysate (Arla Foods).

[4]Amino acid mixture (% of the diet): glutamic acid, 4.61; L-arginine, 3.38; L-lysine hydrochloride, 3.04; L-asparagine, 2.95; L-leucine, 2.80; L-threonine, 1.96; L-isoleucine, 1.76; L-valine, 1.64; L-proline, 1.61; L-serine, 1.46; L-alanine, 1.34; L-phenylalanine, 0.84; L-tyrosine, 0.84; L-histidine, 0.64; L-cystine, 0.62; DL-methionine, 0.57; glycine, 0.50; and L-tryptophan, 0.47.

[5]Provided the following per kilogram of diet: $Ca_3(PO_4)_2$, 28.0 g; K_2HPO_4, 9.0 g; NaCl, 8.89 g; $MgSO_4{\cdot}7H_20$, 3.5 g; $ZnCO_3$, 0.10 g; $CaCO_3$, 3.0 g; $MnSO_4{\cdot}H_20$, 0.65 g; $FeSO_4{\cdot}7H_20$, 0.42 g; KI, 40 mg; $CuSO_4{\cdot}5H_2O$, 20 mg; $Na_2MoO_4{\cdot}2H_2O$, 9 mg; H_3BO_3, 9 mg; $CoSO_4{\cdot}7H_2O$, 1 mg; and Na_2SeO_3, 0.22 mg.

[6]Provided the following per kilogram of diet: thiamin-HCl, 20 mg; niacin, 50 mg; riboflavin, 10 mg; D-Ca pantothenate, 30 mg; vitamin B_{12}, 0.04 mg; pyridoxine-HCl, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; menadione dimethylpyrimidinol bisulfate, 2 mg; cholecalciferol, 15 μg; retinyl acetate, 1,789 μg; and ascorbic acid, 250 mg.

[7]BMD-50 provided by Alpharma Inc. (Fort Lee, NJ). The addition of BMD-50 provides 1 g of bacitracin activity/kg of diet.

to three 30-min washes in PBS. After the final wash, sections were set in 70% ethanol and sent to Histo Scientific Research Laboratories (Mount Jackson, VA) for embedding, 5-μm sectioning, and hematoxylin and eosin staining. For each sample, images were collected using a Nikon Eclipse 50i microscope and Infinity 1 camera (Nikon, Tokyo, Japan). Morphometric measurements were performed using SigmaScan Pro 5.0 software (SPSS Scientific, Chicago, IL). Images were calibrated using 2-pt rescaling based on the number of pixels equal to 1 mm, obtained using a micrometer. For each section, 12 well-oriented villi and 12 crypts were chosen. Villus height was defined as the distance from the tip of the villus to the crypt-villus junction. Villus width was defined as the distance from one side of the villus to the other midway up the length. Crypt depth was defined as the distance from the crypt-villus junction to the base of the crypt. The villus height:crypt depth ratio (**VCR**) was calculated based on distance measurements.

Real-Time PCR Analyses

Total RNA was isolated from frozen tissue aliquots using the RNeasy Mini Kit (Qiagen, Germantown, MD) according to the manufacturer's protocol (Gilbert et al., 2007). Total RNA was quantified at 260/280 nm using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and integrity was verified by gel electrophoresis. Total RNA was stored at −80°C. A RNA standard curve was made for each gene of interest based on modification of a previously described protocol (Fronhoffs et al., 2002). Primers used for cloning were described previously (Gilbert et al., 2007, 2008). Total RNA from jejunum and gene-specific primers were used to perform real-time PCR followed by subcloning into the pGEM-T Easy vector (Promega, Madison, WI). The cloning procedure was performed as already described (Gilbert et al., 2007). Purified plasmid cDNA samples were sequenced at the Virginia Bioinformatics Institute at Virginia Tech. Gene-specific

Table 2. Molecular weight (MW) distribution of peptides in the whey hydrolysate[1]

Item	Whey hydrolysate,[2] % of hydrolysate
MW, Da	
<175	<5
<375	18.5
375 to 750	40.4
750 to 1,250	24.2
1,250 to 2,500	14.6
>2,500	2.4
Maximum MW	6,000 Da

[1]Based on gel permeation chromatography elution profile monitored by UV at 214 nm. Analysis performed by Arla Foods (Viby, Denmark).

[2]Lacprodan DI-3065 (Arla Foods).

standard curves were generated as described previously (Gilbert et al., 2007). Briefly, plasmids containing amplified chicken cDNA were linearized opposite a T7 or SP6 promoter depending on the orientation of the insert sequence. In vitro transcription reactions were performed on linearized plasmids using the MEGAscript T7 or SP6 in vitro transcription kit (Ambion, Austin, TX; Gilbert et al., 2007) and cRNA was precipitated with lithium chloride and quantified using the RiboGreen assay (Molecular Probes, Eugene, OR) and a FLUOstar Optima microplate reader (BMG Labtech, Offenburg, Germany). A dilution series of 10^{11} to 10^4 molecules/μL was performed in the presence of yeast tRNA at 10 mg/L.

For real-time PCR, nested primers were designed (Gilbert et al., 2007, 2008) within cloned chicken cDNA sequences using Primer Express software (Applied Biosystems, Foster City, CA). Total RNA samples and RNA standard curves were reverse-transcribed using the High Capacity cDNA Archive Kit (Applied Biosystems; Gilbert et al., 2007). Each reverse transcription reaction contained 2,000 ng of RNA at a concentration of 100 ng/μL or an equal volume of the dilution series of cRNA. The cDNA was diluted 1:30 before addition to the PCR reaction that contained primers (5 μM) and SYBR Green Master Mix (Applied Biosystems). Polymerase chain reaction was performed under the following conditions: 50°C for 10 min and 40 cycles of 95°C for 1 min and 60°C for 1 min using an Applied Biosystems Real-Time PCR 7300 system. A dissociation step consisting of 95°C for 15 s, 60°C for 30 s, and 95°C for 15 s was performed at the end of each PCR to verify amplification of a single product.

Statistical Analyses

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, Cary, NC). The model for BW and intestinal segment weight data included the main effects of age, genetic line, diet, and 2-way interactions. Morphometric measurement data were subjected to a log base-10 transformation before statistical analysis. Morphometry data are presented in the results as nontransformed data. The model included the main effects of genetic line and diet and the 2-way interaction. All gene expression data were analyzed as both absolute quantities and normalized values. Normalized values were calculated as a ratio of target gene mRNA to β-actin mRNA quantities and these data were transformed before statistical analysis. The model for gene expression data included the main effects of age, genetic line, diet, intestinal segment, and 2- and 3-way interactions. Significant differences were further evaluated by Tukey's test for multiple comparisons. The d 8 time point was not included in this model because consumption of the experimental diets was not initiated until after intestinal sampling on d 8. Differences were considered significant at $P < 0.05$.

RESULTS

Growth Performance and Feed Intake

Body weights did not differ across dietary treatments ($P = 0.4$), although a main effect of genetic line on BW was observed where line B birds were heavier than line A (Table 3; $P = 0.02$). The line difference was accentuated with age (Figure 1A; $P = 0.02$). Pen feed intakes were controlled to be equal to the dietary group with the lowest consumption (line B female AA) and increased with age to intakes of approximately 36 g/bird for d 14. Weights of intestinal segments were affected by both genetic line and diet (Table 3). Duodenum, both as an individual weight and as a percentage of BW, was greater ($P < 0.01$) in line B birds compared with line A and line-specific expression changed with time (Figure 1B). Ileum weight was greater ($P = 0.02$) in line B, although when expressed as a percentage of BW, there was no difference. Jejunum and ileum, both as individual weights and as percentages of BW, were affected by diet. Jejunum was largest ($P < 0.03$) in birds that consumed the AA diet, intermediate in those that consumed the WPH diet, and smallest in birds that consumed the WPC diet. Ileum was largest ($P < 0.03$) in the birds that consumed the AA or WPH diet, in comparison with birds that consumed the WPC diet. Relative jejunal weight changed differently with age depending on the dietary group (Figure 1C).

Influence of Dietary Protein Composition on Intestinal Morphology

Morphometric measurements were performed on cross-sections of the duodenum, jejunum, and ileum of birds at d 15 of the trial. There were no main effects of genetic line on villus height, villus width, crypt depth, or VCR (Table 4). Dietary protein composition influenced villus width and VCR. Villus width and VCR were greater ($P < 0.02$) in birds consuming the WPH diet as compared with birds that consumed the AA diet. Villus height, crypt depth, and VCR were greatest ($P < 0.03$) in the duodenum, lowest in the ileum, and intermediate in the jejunum. Villus width was greater ($P < 0.03$) in the duodenum as compared with the ileum. There was an interaction of genetic line × diet for villus height, crypt depth, and VCR (Figure 2; $P < 0.05$). In birds consuming the WPC diet, there was little difference between genetic lines for these parameters. In birds consuming the AA diet, villus height was greater in line A birds compared with line B, whereas for crypt depth, there was little difference between genetic lines, resulting in a slightly higher VCR for line A birds consuming AA compared with line B. In birds that were given the WPH diet, there was little difference in villus height between the 2 genetic lines; however, line A birds had deeper crypts, resulting in a greater VCR in line B birds consuming the WPH diet.

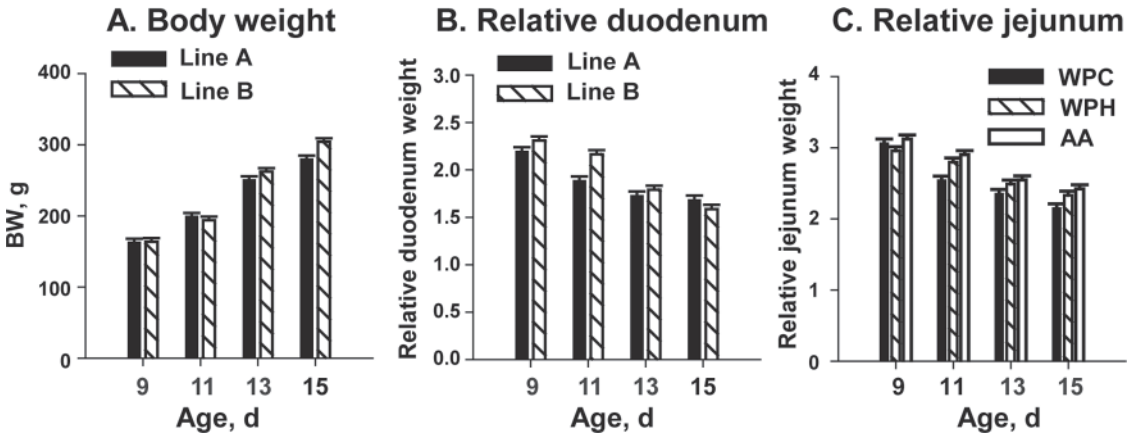

Figure 1. Body and intestinal weights of male birds from 2 genetic lines at d 9, 11, 13, and 15 posthatch in birds fed whey protein concentrate (WPC), a whey partial hydrolysate (WPH), or a mixture of amino acids identical to the composition of whey (AA). Bars on the graph represent means of male birds ± SEM. Relative weights are expressed as the individual segment weight as a percentage of total BW. There was an interaction of age × genetic line for BW and relative duodenum ($P < 0.03$) and an interaction of age × diet for relative jejunum ($P < 0.05$).

Influence of Genetic Line and Dietary Protein Composition on Transporter and Enzyme mRNA

Real-time PCR and the absolute quantification method were used to determine mRNA abundance of PepT1, AA transporters, APN, and a housekeeping gene, β-actin. Results are summarized in Tables 5 and 6 with separate rows for main effects of genetic line, diet, intestinal segment, age, and 2-way interactions. Three-way interactions were not significant. The absolute quantities, expressed as molecules of RNA per nanogram of total RNA, are shown in Table 5. The mRNA quantities normalized against β-actin for main effects of genetic line and diet and the 2-way interaction are shown in Table 6. There are several reasons why tables for both absolute and normalized quantities are included in the results. One is to highlight the dramatic differences in transcript abundance for the different genes using absolute quantity data. A second reason is to make the key point that the combined influence of genetic line and diet resulted in a similar effect on absolute expression of all genes. Third, showing normalized data illustrates that results are generalized

Table 3. Effect of dietary protein composition on BW and intestinal segment weights in 2 lines of broilers from d 9 to 15 posthatch[1]

Item	BW	Duodenum	Relative duodenum	Jejunum	Relative jejunum	Ileum	Relative ileum
Line							
A	223.2	4.07	1.87	5.77	2.65	3.93	1.81
B	231.0	4.35	1.96	5.88	2.63	4.11	1.84
SEM	2.5	0.05	0.03	0.07	0.02	0.06	0.02
P-value	0.02	0.0002	0.005	0.20	0.70	0.02	0.20
Diet[2] (n = 10)							
WPC	225.7	4.10	1.88	5.51^{c}	2.53^{c}	3.79^{b}	1.74^{b}
WPH	225.1	4.21	1.93	5.81^{b}	2.64^{b}	4.11^{a}	1.88^{a}
AA	230.5	4.31	1.94	6.15^{a}	2.75^{a}	4.15^{a}	1.86^{a}
SEM	3.0	0.06	0.03	0.08	0.03	0.07	0.03
P-value	0.40	0.08	0.30	0.0001	0.0001	0.0002	0.0001
Age (n = 10)							
d 9	163.3	3.65	2.25	4.95	3.05	3.53	2.17
d 11	196.6	3.94	2.03	5.37	2.75	3.71	1.90
d 13	256.5	4.50	1.76	6.31	2.46	4.20	1.64
d 15	292.0	4.73	1.64	6.67	2.30	4.62	1.59
SEM	3.5	0.07	0.03	0.09	0.03	0.08	0.03
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction[3]	*P*-value						
A × L	0.02	0.50	0.0005	0.50	0.09	0.70	0.10
A × D	0.30	0.30	0.50	0.04	0.04	0.30	0.40
L × D	0.50	0.30	0.60	0.60	0.40	0.20	0.10

[a–c]Means in a column without a common superscript differ ($P < 0.03$, Tukey's test).

[1]Body weight, duodenum, jejunum, and ileum expressed as weight (g). Relative duodenum, relative jejunum, and relative ileum expressed as individual segment weight as a percentage of total BW.

[2]WPC = whey protein concentrate; WPH = whey partial hydrolysate; AA = mixture of free amino acids.

[3]For the interactions, A, L, and D represent the main effects of age, genetic line, and diet, respectively.

Table 4. Effect of dietary protein composition on intestinal morphometry in 2 lines of broiler chicks at d 15 posthatch[1]

Item	Villus height	Villus width	Crypt depth	VCR
Line (n = 5)				
A	0.965	0.135	0.160	6.07
B	0.925	0.140	0.150	6.26
SEM	0.020	0.005	0.005	0.16
P-value	0.15	0.30	0.08	0.42
Diet[2] (n = 10)				
WPC	0.925	0.135^{ab}	0.145	6.31^{ab}
WPH	0.990	0.150^{a}	0.155	6.48^{a}
AA	0.920	0.130^{b}	0.160	5.71^{b}
SEM	0.025	0.005	0.005	0.20
P-value	0.06	0.02	0.18	0.02
Segment (n = 10)				
Duodenum	1.28^{x}	0.150^{x}	0.185^{x}	7.04^{x}
Jejunum	0.90^{y}	0.140^{xy}	0.150^{y}	6.09^{y}
Ileum	0.66^{z}	0.130^{y}	0.130^{z}	5.36^{z}
SEM	0.025	0.005	0.005	0.20
P-value	0.0001	0.02	0.0001	0.0001
Interaction[3]	*P*-value			
L × D	0.04	0.92	0.04	0.0002
D × S	0.50	0.76	0.87	0.26

[a,b]Means in a column without a common superscript differ ($P < 0.02$, Tukey's test).

[x–z]Means in a column without a common superscript differ ($P < 0.03$, Tukey's test).

[1]Distance measurements expressed as distance (mm). Villus height to crypt depth (VCR) expressed as ratio.

[2]WPC = whey protein concentrate; WPH = whey partial hydrolysate; AA = mixture of free amino acids.

[3]For the interactions, L, D, and S represent the main effects of genetic line, diet, and intestinal segment, respectively.

and nonspecific, leading to a dampening of all effects after normalizing to β-actin.

In terms of absolute quantities, there are striking differences among enterocyte-associated genes. Aminopeptidase N constantly appears as an abundant transcript with mRNA levels that are comparable to housekeeping genes, suggesting that it plays an important role in the final hydrolysis of small peptides in the gut. The Na^+-independent cationic and zwitterionic AA transporter (**$b^{o,+}$AT**) also consistently appears as an abundant mRNA, with quantities greater than 100,000 molecules. The other brush-border membrane transporters evaluated in this study, PepT1 and excitatory AA transporter 3 (**EAAT3**), both show up in considerably smaller quantities, on the order of 10,000 molecules. In sharp contrast, the basolateral transporters, cationic AA transporter 1 (**CAT1**), Na^+-independent cationic and Na^+-dependent neutral AA transporter 2 (**y^+LAT2**), and L-type AA transporter 1 (**LAT1**), are expressed in lower quantities than brush-border transporters, particularly CAT1 and LAT1 at hundreds of molecules.

For all genes except y^+LAT2, there was a main effect of dietary protein composition on mRNA abundance (Table 5). Abundance of $b^{o,+}$AT, EAAT3, APN, and β-actin mRNA in birds consuming the WPH diet was greater than in birds that consumed the WPC ($P <$

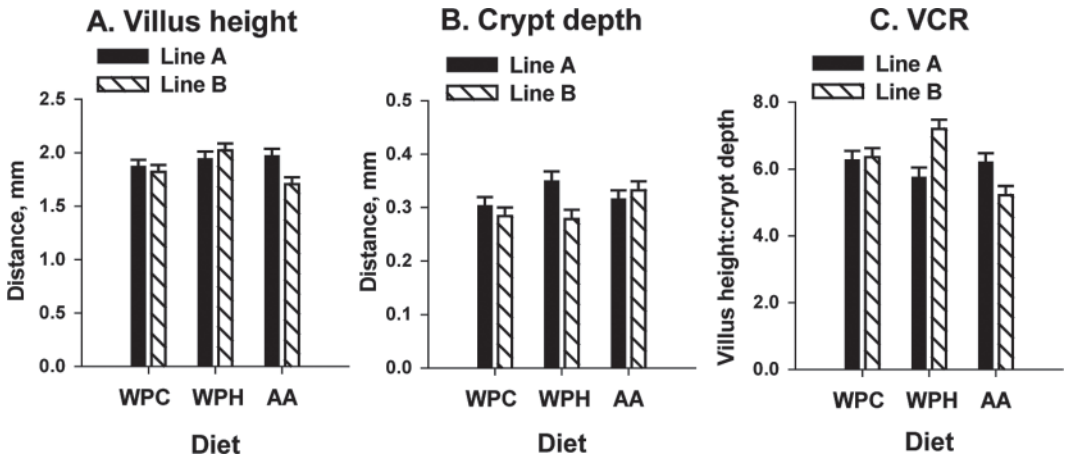

Figure 2. Effect of genetic line and dietary protein composition on intestinal morphometry. Line × diet interaction ($P < 0.05$) for A) villus height, B) crypt depth, and C) villus height:crypt depth ratio (VCR) in broilers fed whey protein concentrate (WPC), a whey partial hydrolysate (WPH), or a mixture of amino acids identical to the composition of whey (AA) from d 9 to 15 posthatch. Bars represent the mean of male birds (n = 5) ± SEM.

Table 5. Effect of dietary protein composition on mRNA abundance of peptide and amino acid (AA) transporters, a digestive enzyme, and β-actin[1]

	Gene[2]							
Item	PepT1	$b^{o,+}AT$	EAAT3	y^+LAT2	CAT1	LAT1	APN	β-actin
Line (n = 5)								
A	7,640	127,610	5,020	5,775	465	525	261,320	693,180
B	11,940	151,180	6,400	8,380	645	560	284,545	815,500
SEM	345	5,170	220	350	20	20	8,235	25,800
P-value	0.0001	0.001	0.0001	0.0001	0.0001	0.20	0.04	0.0009
Diet[3] (n = 10)								
WPC	$9,500^{ab}$	$132,580^{b}$	$5,150^{b}$	6,405	480^{b}	495^{b}	$251,525^{b}$	$675,340^{b}$
WPH	$10,845^{a}$	$161,800^{a}$	$6,930^{a}$	7,875	600^{a}	585^{a}	$303,905^{a}$	$849,450^{a}$
AA	$9,030^{b}$	$123,800^{b}$	$5,045^{b}$	6,950	580^{a}	540^{ab}	$263,360^{b}$	$738,220^{b}$
SEM	425	6,335	270	430	25	20	10,090	31,600
P-value	0.008	0.0001	0.0001	0.05	0.003	0.02	0.0007	0.0005
Segment (n = 10)								
Duodenum	$11,065^{x}$	$120,320^{y}$	$2,620^{z}$	6,470	470^{y}	490^{y}	$168,130^{z}$	$611,425^{y}$
Jejunum	$10,035^{x}$	$112,250^{y}$	$4,430^{y}$	7,350	545^{y}	555^{xy}	$247,420^{y}$	$848,990^{x}$
Ileum	$8,275^{y}$	$185,615^{x}$	$10,075^{x}$	7,410	650^{x}	575^{x}	$403,245^{x}$	$802,600^{x}$
SEM	425	6,335	270	430	25	20	10,090	31,600
P-value	0.0001	0.0001	0.0001	0.20	0.0001	0.03	0.0001	0.0001
Age (n = 10)								
d 9	8,285	98,471	4,175	5,585	580	530	227,150	601,380
d 11	11,035	157,355	5,830	7,900	620	555	278,650	888,970
d 13	10,120	155,050	6,845	7,840	475	500	308,070	780,580
d 15	9,730	146,705	5,985	6,980	540	580	277,855	746,430
SEM	490	7,315	310	500	30	25	11,650	36,490
P-value	0.001	0.0001	0.0001	0.003	0.005	0.10	0.0001	0.0001
Interaction[4]	*P*-value							
A × L	0.005	0.20	0.04	0.005	0.0001	0.0001	0.60	0.0002
A × D	0.08	0.01	0.01	0.10	0.003	0.10	0.04	0.0001
L × D	0.005	0.005	0.003	0.004	0.005	0.40	0.005	0.0003
D × S	0.90	0.02	0.0005	0.70	0.80	0.70	0.50	0.80

[a,b]Means in a column without a common superscript differ ($P < 0.04$, Tukey's test).

[x–z]Means in a column without a common superscript differ ($P < 0.03$, Tukey's test).

[1]All means expressed as molecules of mRNA per nanogram of total RNA.

[2]PepT1 = peptide transporter 1; $b^{o,+}AT$ = Na^+-independent cationic and zwitterionic AA transporter; EAAT3 = excitatory AA transporter 3; y^+LAT2 = Na^+-independent cationic and Na^+-dependent neutral AA transporter 2; CAT1 = cationic AA transporter 1; LAT1 = L-type AA transporter 1; APN = aminopeptidase N.

[3]WPC = whey protein concentrate; WPH = whey partial hydrolysate; AA = mixture of free AA.

[4]For the interactions, A, D, L, and S represent the main effects of age, diet, genetic line, and intestinal segment, respectively.

Table 6. Effect of dietary protein composition on normalized mRNA abundance of peptide and amino acid (AA) transporters and aminopeptidase N (APN)[1]

	Gene[2]						
Item	PepT1	$b^{o,+}AT$	EAAT3	y^+LAT2	CAT1	LAT1	APN
Line (n = 5)							
A	0.013	0.19	0.0075	0.0095	0.00075	0.00085	0.41
B	0.017	0.20	0.0081	0.011	0.00087	0.00078	0.38
SEM	0.0006	0.040	0.00024	0.0004	0.000033	0.000027	0.011
P-value	0.0001	0.68	0.002	0.019	0.0001	0.06	0.07
Diet[3] (n = 10)							
WPC	0.016	0.21^{a}	0.0079^{ab}	0.010	0.00077	0.00082	0.40
WPH	0.015	0.21^{a}	0.0084^{a}	0.010	0.00076	0.00081	0.39
AA	0.014	0.18^{b}	0.0073^{b}	0.010	0.00089	0.00082	0.3899
SEM	0.00075	0.0064	0.00029	0.00052	0.00004	0.000033	0.014
P-value	0.08	0.0003	0.005	0.99	0.05	0.99	0.48
Interaction[4]	*P*-value						
L × D	0.05	0.40	0.42	0.29	0.13	0.0001	0.05

[a,b]Means in a column without a common superscript differ ($P < 0.04$, Tukey's test).

[1]Ratio of absolute quantities (molecules of mRNA/ng of total RNA) to β-actin absolute quantities of mRNA.

[2]PepT1 = peptide transporter 1; $b^{o,+}AT$ = Na^+-independent cationic and zwitterionic AA transporter; EAAT3 = excitatory AA transporter 3; y^+LAT2 = Na^+-independent cationic and Na^+-dependent neutral AA transporter 2; CAT1 = cationic AA transporter 1; LAT1 = L-type AA transporter 1; APN = aminopeptidase.

[3]WPC = whey protein concentrate; WPH = whey partial hydrolysate; AA = mixture of free AA.

[4]For the interaction, D and L represent the main effects of diet and genetic line, respectively.

0.01) or AA diet ($P < 0.02$). Expression of PepT1 in birds that were fed the WPH diet was greater ($P = 0.008$) as compared with birds that consumed the AA diet, whereas for CAT1 and LAT1, expression in the birds fed the WPH diet was greater ($P < 0.02$) than in the WPC diet-fed group. Dietary protein composition influenced gene expression between the 2 genetic lines. Expression of PepT1 (Figure 3A), $b^{o,+}AT$ (Figure 3B), EAAT3 (Figure 3C), y^+LAT2 (Figure 3D), CAT1 (Figure 3E), APN (Figure 3F), and β-actin (Figure 3G) varied little among dietary treatments in line A birds ($P < 0.006$). In line B birds, PepT1, $b^{o,+}AT$, EAAT3, CAT1, y^+LAT2, APN, and β-actin showed a similar expression pattern where mRNA was greatest in the birds consuming the WPH diet compared with the other 2 diets ($P < 0.006$). As shown in Table 6, this common line × diet interaction disappears after normalization to β-actin, indicating a generalized upregulation in transcription.

For all genes except LAT1, mRNA quantities were greater ($P < 0.05$) in line B birds as compared with line A (Table 5). Age influenced the response in genetic lines. Abundance of PepT1 mRNA (Figure 4A) varied little with age in line A birds, whereas in line B birds, expression increased with age to d 13 followed by a slight decline to d 15 ($P = 0.005$). For EAAT3 (Figure 4B), y^+LAT2 (Figure 4C), CAT1 (Figure 4D), LAT1 (Figure 4E), and β-actin (Figure 4F), mRNA quantities increased with age from d 9 to 15 in line A birds ($P < 0.05$). In line B birds, expression peaked at d 13 for EAAT3 and at d 11 for LAT1, y^+LAT2, and β-actin. For CAT1, expression was greatest at d 9 and 11 ($P < 0.05$).

For all genes except for y^+LAT2, there was a main effect of intestinal segment on gene expression (Table 5). Abundance of PepT1 mRNA was greater ($P < 0.03$) in the duodenum and jejunum as compared with the ileum, whereas for $b^{o,+}AT$, EAAT3, CAT1, and APN, mRNA quantities were greatest ($P < 0.03$) in the ileum. Quantities of LAT1 and β-actin mRNA were greater ($P < 0.03$) in the jejunum and ileum as compared with the duodenum. The 2 brush-border membrane AA transporters, $b^{o,+}AT$ (Figure 5A) and EAAT3 (Figure 5B), showed a similar effect of diet on their spatial pattern of gene expression in the small intestine ($P < 0.03$). Expression of both genes increased from proximal to distal small intestine and this proximal to distal gradient was accentuated in birds consuming the WPH diet.

All genes evaluated except for LAT1 showed a main effect of age on mRNA abundance ($P < 0.006$). In general, expression was greatest at d 9 or 11 and plateaued. There was a different age-specific pattern of gene expression of $b^{o,+}AT$ (Figure 6A), EAAT3 (Figure 6B), CAT1 (Figure 6C), APN (Figure 6D), and β-actin (Figure 6E) in birds consuming the different diets. Quantities of $b^{o,+}AT$, EAAT3, CAT1, APN, and β-actin mRNA in birds consuming the WPC diet were greatest at d 11, decreased to d 13, and plateaued ($P < 0.05$). Expression of EAAT3, $b^{o,+}AT$, and APN increased to d 13 and plateaued in birds consuming the WPH diet ($P < 0.05$). Abundance of CAT1 mRNA decreased from d 9 to 13 and maintained constant levels to d 15 in birds consuming the WPH diet ($P = 0.003$). β-Actin mRNA quantities were greatest at d 11 and 15 in birds consuming the WPH diet. Expression of $b^{o,+}AT$, EAAT3, APN, and β-actin in birds consuming the AA diet peaked at d 13 ($P < 0.05$), whereas CAT1 mRNA changed little between d 11 and 15 in birds consuming the AA diet ($P = 0.003$).

DISCUSSION

Our objective was to determine the effect of dietary protein composition on peptide and AA transporter gene expression in 2 genetically selected lines of broilers that differ in intestinal expression of the peptide transporter, PepT1. We focused on transporter and APN expression to determine if the anticipated difference in AA availability among the 3 diets would lead to different transcript levels of free AA or peptide transporters and aminopeptidases. We observed in prior studies that expression of PepT1 is influenced by both diet and genetic line (Chen et al., 2005; Gilbert et al., 2007, 2008). Others have demonstrated that peptide transport is a faster and more energetically efficient route of AA absorption compared with free AA uptake (Silk et al., 1980; Steinhardt and Adibi, 1986; Daniel, 2004). Collectively, results from these studies suggest that peptide uptake may be very important from a nutritional standpoint, although feeding studies involving inclusion of peptides as a source of AA in animal diets are limited. Additionally, we observed in 2 independent studies that line B birds consistently express greater quantities of PepT1 mRNA compared with line A birds. Although reagents are not yet available for confirming these findings at the protein level, we hypothesize that this difference may translate into a difference in peptide absorption capacities between the 2 broiler lines.

Although nutrient digestibility values were not reported in this study, it is likely that AA digestibility was different across diets. In chicks fed corn-soybean meal or corn-canola meal diets, digestibility of essential AA increased with age from hatch to 21 d (Batal and Parsons, 2002b). In chicks fed a dextrose-casein or starch-crystalline AA diet, AA digestibility was not affected by age and values for essential AA were greater in these diets compared with corn-soybean meal or corn-canola meal at all time points. During the first 2 wk posthatch, the digestive and absorptive capacities of the chick are still developing and digestibility values for casein and the AA diet were high because of a high capacity for AA absorption and ability to digest easily digested proteins. Chicks fed a crystalline AA-based diet for the first week or 3 wk posthatch consumed less compared with chicks offered a standard corn-soybean meal diet (Batal and Parsons, 2002a). In the gut of chicks fed the crystalline AA for the first 7 or 21 d, there was reduced villus height and crypt depth, particularly in

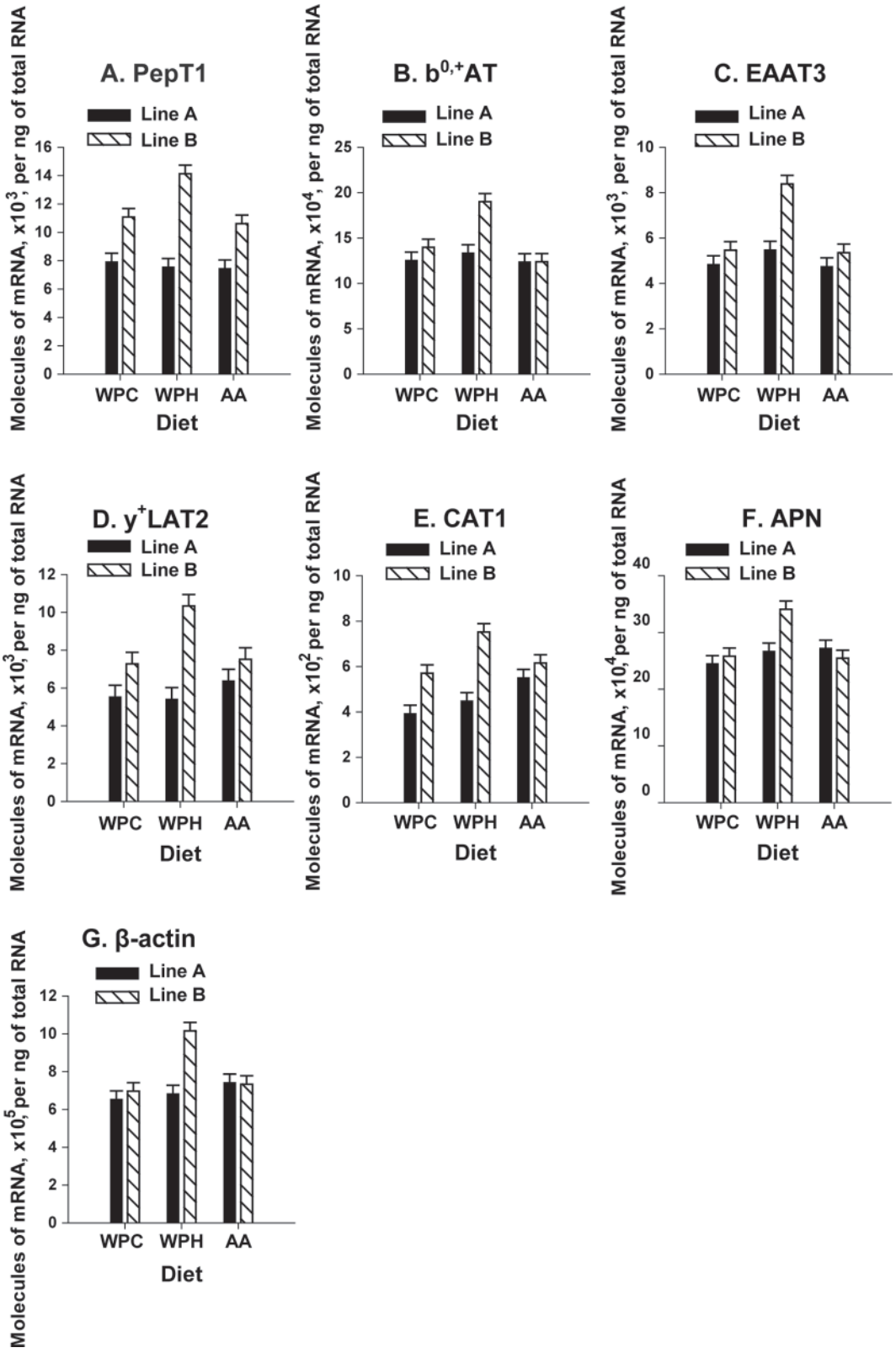

Figure 3. Effect of diet on peptide and amino acid (AA) transporter and aminopeptidase N (APN) mRNA abundance in 2 lines of broilers. The number of A) peptide transporter 1 (PepT1), B) Na^+-independent cationic and zwitterionic AA transporter ($b^{o,+}AT$), C) excitatory AA transporter 3 (EAAT3), D) Na^+-independent cationic and Na^+-dependent neutral AA transporter 2 (y^+LAT2), E) cationic AA transporter 1 (CAT1), F) APN, and G) β-actin mRNA molecules per nanogram of total RNA. Bars represent the mean of male birds (n = 5) from line A or line B ± SEM that consumed whey protein concentrate (WPC), whey partial hydrolysate (WPH), and AA mixture identical to the composition of whey (AA) from d 9 to 15 posthatch. There was an interaction of genetic line × diet for all 7 genes ($P < 0.006$).

the distal region. The lower intake and high AA digestibility of the AA diet provided less luminal stimulation in the gut, with little substrate entering the lower small intestine and colon, explaining the stunted morphology. In our study, feed intake was equalized across treatments by restricting intake to the group that consumed the least, which was the group of females from line B consuming the AA diet. We did not observe a significant dietary main effect on villus height or crypt depth but did observe an effect on villus width.

To our knowledge, results from feeding hydrolysates or peptide-based diets to poultry have not been reported. Because of the relevance to clinical nutrition, there have been multiple reports of feeding hydrolysates and AA-based diets to rats. When casein or whey-based diets were compared with their respective hydrolysates, there was no difference in nitrogen digestibility, but nitrogen retention was greater in rats fed the hydrolysate diets (Boza et al., 1995). The difference in absorption rate of AA from a peptide-based diet versus the intact protein may influence subsequent utilization of AA for protein synthesis. When a peptide-based diet was compared with a free AA-based diet, there was greater energy conversion efficiency, protein efficiency ratio, nitrogen protein utilization, and biological value reported for rats fed the peptide-based diet, whereas there was a higher apparent digestibility reported for rats fed the free AA-based diet (Boza et al., 2000). There was greater plasma total and essential AA in rats consuming the peptide diet, whereas there was greater plasma urea in rats fed the AA diet, suggesting that rats consuming the AA diet were using the AA for energy rather than protein deposition. Numerous factors can affect utilization of dietary AA, including insolubility of free AA, absorption or interference of the molecular form of AA with nutrient transporters, availability of AA in the free or peptide-bound form to gut microflora, effect of the form of AA on osmolarity, and effect of rate of AA absorption on liver AA oxidation (Boza et al., 2000).

Based on these findings, we expected to find effects of diet on gene expression of AA and peptide transporters in this study. Although we observed multiple effects of age, line, and diet on peptide and AA transporter mRNA in this study, no differences in BW were observed. This may in part be due to the fact that diets were fed for only 1 wk (d 8 to 15) and this may not

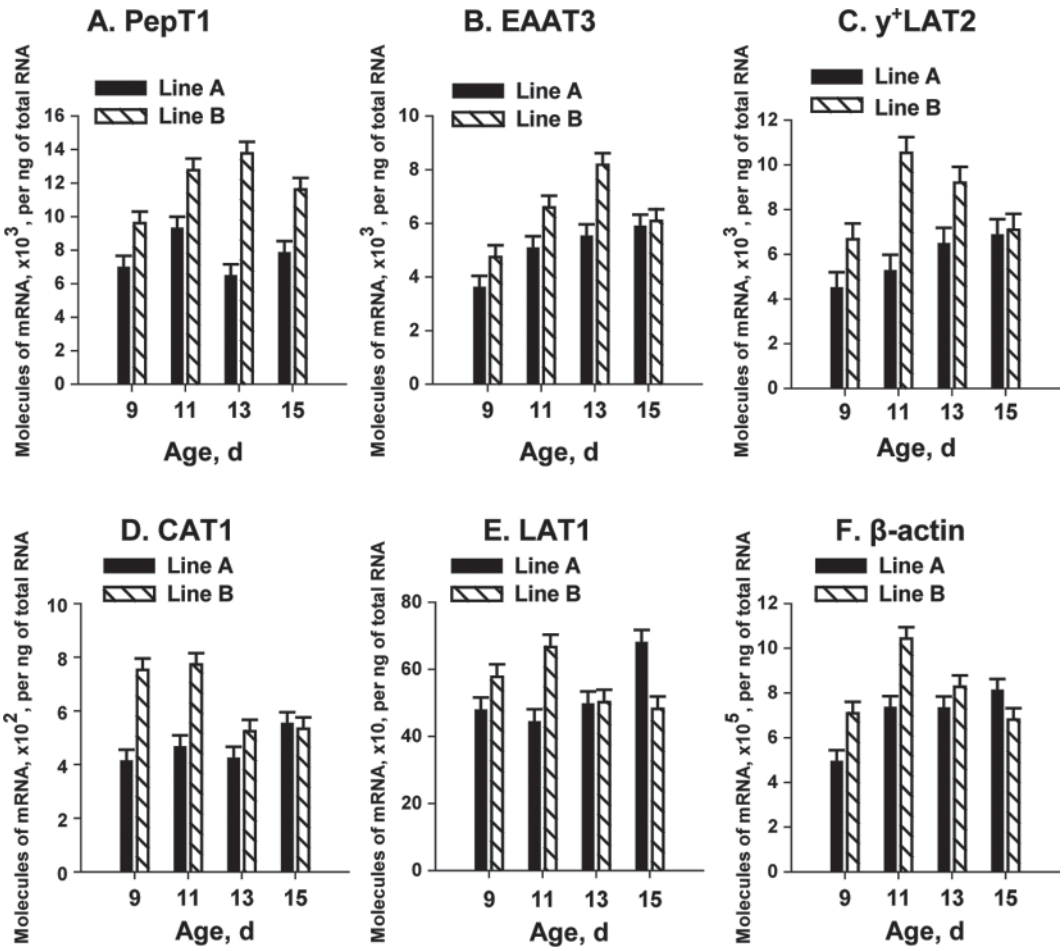

Figure 4. Effect of age on peptide and amino acid (AA) transporter and aminopeptidase N mRNA abundance in 2 lines of broilers from d 9 to 15 posthatch. The number of A) peptide transporter 1 (PepT1), B) excitatory AA transporter 3 (EAAT3), C) Na^+-independent cationic and Na^+-dependent neutral AA transporter 2 (y^+LAT2), D) cationic AA transporter 1 (CAT1), E) L-type AA transporter 1 (LAT1), and F) β-actin mRNA molecules per nanogram of total RNA. Bars represent the mean of male birds (n = 15; 5 from each dietary group) from line A or line B ± SEM. There was an interaction of genetic line × age for all 6 genes ($P < 0.05$).

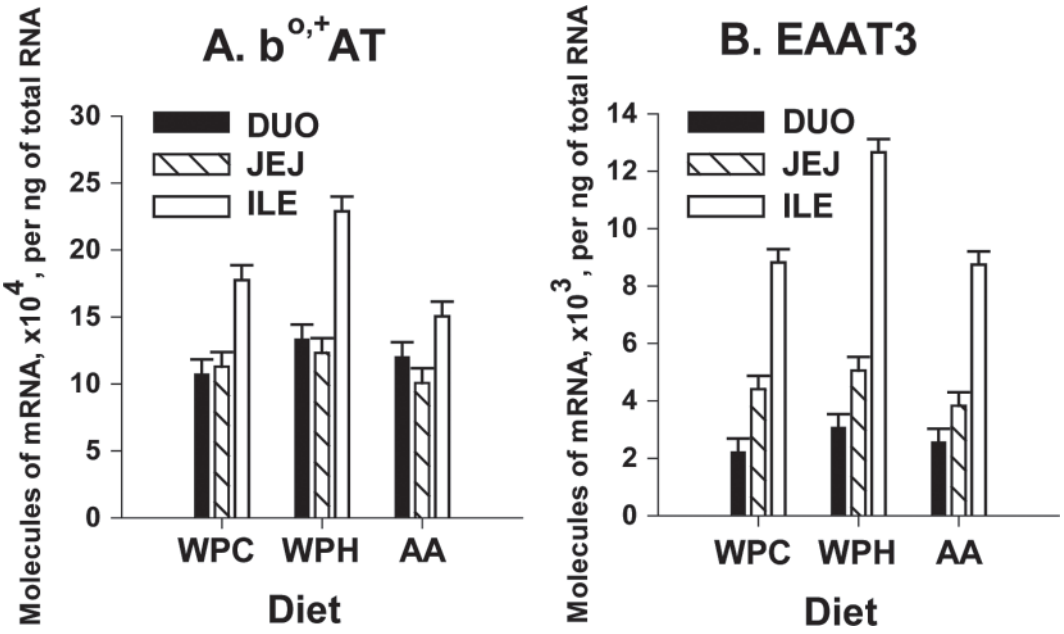

Figure 5. Effect of dietary protein composition on spatial pattern of amino acid (AA) transporter mRNA abundance in broilers. The number of A) Na^+-independent cationic and zwitterionic AA transporter ($b^{o,+}AT$) and B) excitatory AA transporter 3 (EAAT3) mRNA molecules per nanogram of total RNA. Bars represent the mean of male birds (n = 10; 5 line A and B) ± SEM in the duodenum (DUO), jejunum (JEJ), and ileum (ILE) of birds that consumed whey protein concentrate (WPC), whey partial hydrolysate (WPH), and AA mixture identical to the composition of whey (AA) from d 9 to 15 posthatch. There was an interaction of intestinal segment × diet for both genes ($P < 0.03$).

have been enough time to see differences in gene expression translate into differences in growth performance. A commercial diet was fed for the first week posthatch to provide an adequate stimulus for gastrointestinal development because others have demonstrated that crystalline AA-based diets fed early in life to broilers may hinder growth and development of the gut (Batal and Parsons, 2002a).

Effect of Dietary Protein Composition on Intestinal Histology

The first 2 wk posthatch represent a time of dramatic changes in gastrointestinal development, with the most dramatic changes occurring during the first 24 h (Uni et al., 2000; Geyra et al., 2001). Changes include cell proliferation, enterocyte hypertrophy, and changes in morphology, crypt invagination, and increased villus surface area, with the rate of these changes being segment-specific. All of these changes would be expected to influence digestive and absorptive functions in the gut, and we would anticipate that changes in luminal stimulus would affect intestinal morphology as well. We did not observe an effect of genetic line on villus height, villus width, crypt depth, or VCR on d 15 of this study. We did, however, observe several main effects of diet and several genetic line × diet interactions. Villi were wider in the birds that consumed the WPH and WPC diets as compared with birds fed the AA diet. In birds fed the AA diet, a mixture of crystalline AA was used to provide AA to the diet, with a much different anticipated availability in the intestine. A change in villus width may be a reflection of differences in the gut microflora. Immediate absorption of AA by the intestine provides less substrate for the bacteria. Lamina propria is the largest determinant of villus width and is populated with fibroblasts, endothelial cells, lymphocytes, macrophages, and IgA-secreting plasma cells. In germ-free animals, there is delayed development of the lamina propria characterized by less germinal centers and IgA-secreting cells (Dibner et al., 2008). Thus, villus width can provide some insight into the intestinal immune status of the animal.

There was no difference in villus height between line A and B birds consuming the WPH diet, but crypt depths were shallower in line B birds consuming the WPH diet. This resulted in a larger VCR in line B birds that were fed the WPH diet. This may indicate that more mature enterocytes inhabited the villi of these birds, with less cell recruitment and hence less energy expenditure needed to maintain the absorptive function. More mature enterocytes surrounding the villi implies that there is a greater enterocyte functionality and, hence, greater absorptive surface. This combined with less energy needed to maintain cells on the villi potentially allows for more nutrients to be allocated to different regions of the body to support growth.

Effect of Genetic Line, Intestinal Segment, and Dietary Protein Composition on Expression of Transporter and APN mRNA

Consistent with what we observed in previous studies (Gilbert et al., 2007, 2008), PepT1 mRNA was greater

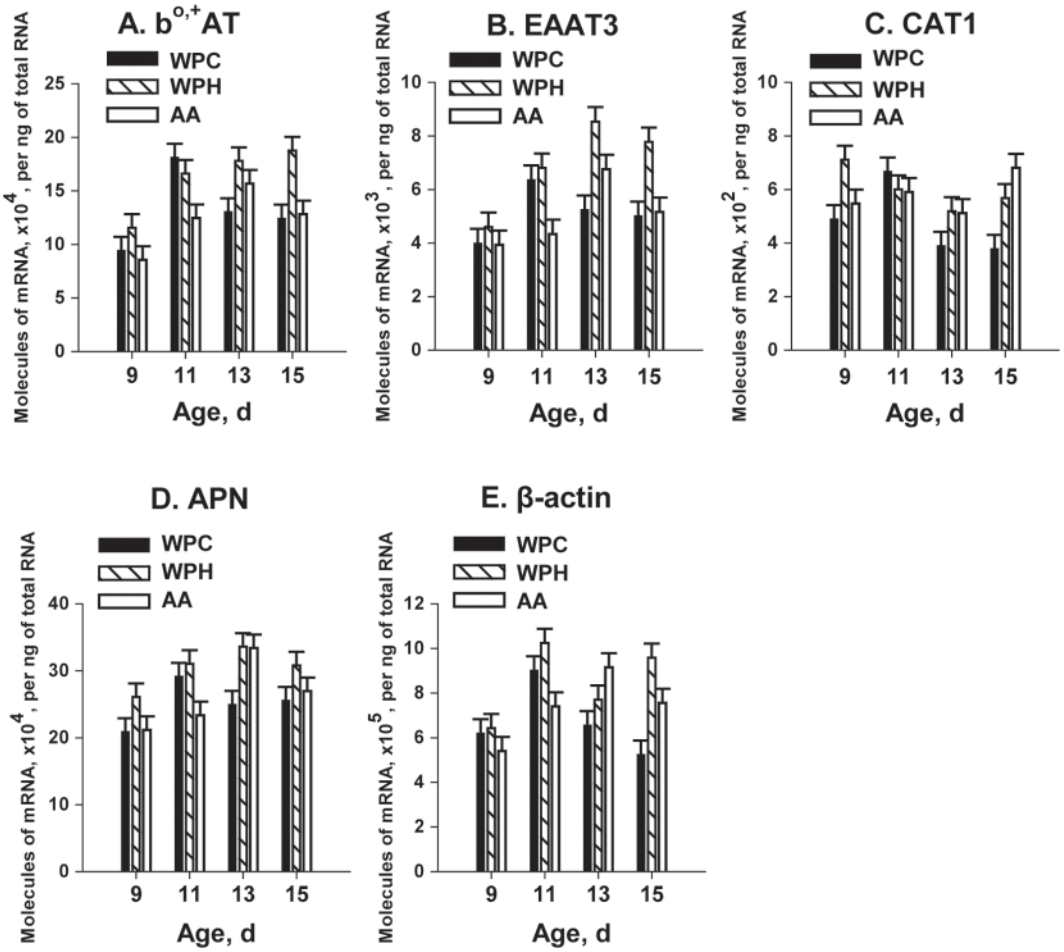

Figure 6. Effect of dietary protein composition on the age-related change in mRNA abundance of amino acid (AA) transporters and a digestive enzyme in broilers. The number of A) Na^+-independent cationic and zwitterionic AA transporter ($b^{o,+}AT$), B) excitatory AA transporter 3 (EAAT3), C) cationic AA transporter 1 (CAT1), D) aminopeptidase N (APN), and E) β-actin mRNA molecules per nanogram of total RNA. Bars represent the mean of male birds (n = 10; 5 line A and B) ± SEM that consumed whey protein concentrate (WPC), whey partial hydrolysate (WPH), and AA mixture identical to the composition of whey (AA) from d 9 to 15 posthatch. There was an interaction of age × diet for all 5 genes ($P < 0.05$).

in line B birds. Expression was also greater in line B for $b^{o,+}AT$, EAAT3, y^+LAT2, CAT1, APN, and β-actin. The magnitude of difference was greatest for PepT1, where line B birds had 56% greater intestinal expression than line A birds, whereas for the other genes, the difference was between 9 and 39%. The genetic line difference in PepT1 expression is the only consistent difference that we have observed in nutrient transporters between lines A and B and suggests that the process of genetic selection has altered expression of the peptide transporter. Expression of PepT1 and the AA transporters examined in this study appear to be more responsive to the diets containing greater quantities of absorbable AA, and both age and genetic line influenced this response.

The segment-specific patterns of expression for the different transporters and APN are consistent with what we have previously reported with PepT1 most abundant in the proximal small intestine and the AA transporters and APN most abundant in the distal small intestine (Gilbert et al., 2007, 2008). These segmental differences did not change after normalization to β-actin (data not shown). For both of the brush-border membrane AA transporters, $b^{o,+}AT$ and EAAT3, mRNA abundance was greatest in the ileum and interestingly, the difference between the ileum and other 2 segments was most accentuated in birds that consumed the WPH diet (Figure 5). This may relate to the presence of more available AA in the ileum of birds consuming the WPH diet because the presence of more peptides entering the duodenum would also provide more substrate for brush-border peptidases such as APN, in turn yielding more substrate for brush-border transporters. In the WPC and AA diet, less and more free AA relative to the WPH diet, respectively, would in principle be available to the duodenum. In birds consuming the WPC diet, this may lead to a slower release of free AA, reducing the need for upregulation in the ileum, whereas in birds consuming the AA diet, much of the AA absorption may be complete by the time the digesta reaches the ileum, reducing the need for upregulation of AA transporters in the distal small intestine. This segment

× diet interaction was not observed for the basolateral AA transporters, which are unlikely to respond in the same manner to luminal AA.

Before normalizing mRNA quantities to β-actin, we evaluated changes in absolute abundance for all genes, including β-actin. In doing this, we observed a common trend whereby genetic line and diet influenced the transporters and the housekeeping gene in a similar manner. For all genes except LAT1, mRNA quantities were greatest in line B birds that consumed the WPH diet. This response of line B birds, which express greater PepT1 mRNA levels, may have led to an overall increase in cellular metabolism and hence a generalized upregulation of all enterocyte-associated genes. To further illustrate this point, normalization of transporter and APN mRNA to β-actin mRNA diminished the differences that were common among these genes. The histological findings support the idea that line B birds were able to assimilate more AA, which in turn lead to enhanced enterocyte functionality. The greater VCR in line B birds that consumed the WPH diet suggests that the enterocytes populating the villi were more mature and hence likely to have greater expression levels of digestive enzymes, nutrient transporters, as well as cytoskeleton-associated genes such as β-actin.

Because we knew that line B chicks express greater quantities of PepT1 compared with line A chicks, we hypothesized that expression of PepT1 in line B birds would be more responsive to a peptide-based diet. In other words, we anticipated that a greater availability of AA in the enterocyte would have an effect on cellular metabolism and perhaps lead to a substrate-induced upregulation of PepT1. Elements were identified in the 5′ upstream region of mammalian PepT1 responsive to peptides and free AA (Shiraga et al., 1999; Fei et al., 2000), whereas none were identified upstream of the chicken PepT1 promoter (Frazier et al., 2008). Addition of dipeptides, Gly-Sar or Gly-Gln, to the medium of Caco-2 cells caused an increase in the expression of PepT1 mRNA and protein (Thamotharan et al., 1998; Walker et al., 1998). Uptake of dipeptides also caused stimulation of AA uptake by the $b^{o,+}$ system (Wenzel et al., 2001), suggesting that free AA uptake may also be regulated by PepT1 activity. It is difficult to relate findings from a mammalian model system because the structure of the chicken PepT1 promoter is different from mammals and therefore may be regulated differently.

In conclusion, we demonstrated that intestinal morphology and expression of genes associated with the digestion and absorption of AA are influenced by dietary protein composition. Line B birds that consumed the peptide-based protein source showed a generalized upregulation of all genes and displayed greater VCR, indicative of greater absorptive surface area and efficient utilization of nutrients for gut turnover. These results have the very interesting implication that genetic selection can indeed alter expression levels of nutrient transporters in response to dietary protein composition and that diets can in turn be formulated to best match these changes in gut function to maximize nutrient utilization efficiency. Diets were fed for only 1 wk, which was most likely insufficient time for differences in growth to become apparent. These results clearly point to an effect of diet on transcriptional regulation of intestinal genes, however; protein abundance and activity data are also needed to corroborate these findings and provide a more complete picture of digestive enzyme and nutrient transporter regulation and turnover in enterocytes.

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