Dietary Protein Level and Stage of Development Affect Expression of an Intestinal Peptide Transporter (cPepT1) in Chickens

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ABSTRACT The objective of this study was to evaluate the effect of dietary protein intake and stage of development on chicken intestinal peptide transporter (cPepT1) mRNA abundance. Chicks were sampled at embryonic d 16 (e 16) until the day of hatch (d 0). After hatch, mixed sex Cobb chicks were randomly assigned to diets containing 12, 18, or 24% crude protein (CP). Intakes of the 18 and 24% CP-fed birds were restricted to that consumed by the 12% CP-fed birds. Chicks were sampled on d 0, 1, 3, 5, 7, 10, 14, 21, 28, and 35. The experiment was repeated with the addition of a 4th group with free access to the 24% CP diet. PepT1 mRNA abundance in the duodenum, jejunum, and ileum was quantified by Northern blot. PepT1 mRNA was barely detectable between E 16 and 20 but increased 14- to 50-fold by d 0 (P < 0.001). In chickens fed 12% CP, cPepT1 mRNA abundance decreased decreased throughout the experiment, whereas those fed the restricted 18 or 24% CP diets showed an increase in cPepT1 mRNA abundance (protein × time interaction, P < 0.01). In chicks with free access to the 24% CP diet, cPepT1 mRNA decreased until d 14 but returned to an intermediate level at d 35 (protein × time interaction, P < 0.01). The relative abundance of cPepT1 mRNA varied among intestinal segments with greater abundance occurring in the duodenal or jejunal sections (P < 0.05). Our results indicate that expression of cPepT1 mRNA is regulated by both dietary protein intake and developmental stage. J. Nutr. 135: 193–198, 2005.

KEY WORDS: • gene expression • chickens • PepT1 • dietary protein

Peptide transporters (PepT)4 are membrane proteins responsible for selective translocation of small peptides across the cell membrane. The cloning and characterization of the peptide transporters, PepT1 and PepT2, provided valuable information on the mechanism of peptide transport in mammalian species (1). PepT1 and PepT2 are distinct in their tissue distributions, substrate kinetics, and specific roles in different tissues. PepT1, which is expressed predominantly in the small intestine, and PepT2, which is expressed in kidney, central nervous system, and several other peripheral tissues, were shown to have nutritional, clinical, and pharmaceutical importance (2–4). In the small intestine, PepT1 is differentially expressed in different sections of the small intestine as well as in different areas along the crypt-villus axis (5).

The expression of PepT1 varies in response to changes in diet and developmental age. Selective amino acids and peptides (6) and growth factors (7) can regulate PepT1 gene expression. Under various conditions of malnourishment, expression of PepT1 is greatly enhanced (8). Shen et al. (9) reported that intestinal expression of PepT1 in rats is induced postpartum. Although PepT1 is located exclusively in the apical brush border of enterocytes in both prenatal and mature rats, it could be detected in the subapical cytoplasm and basolateral membrane of enterocytes immediately after birth (10).

A chicken intestinal peptide transporter (cPepT1) was cloned and characterized in our laboratory (11). Northern blot analysis showed that cPepT1 mRNA was expressed mainly in the small intestine, and at lower levels in the kidney and cecum, but not in the liver, crop, proventriculus, or pectoralis and fibularis longus muscles. The developmental and nutritional changes in PepT1 mRNA abundance have not been investigated in chickens. The objective of the present study was to determine the relative abundance of cPepT1 mRNA from the embryonic d 16 (e 16) to d 35 in broilers fed diets containing different dietary protein levels.

MATERIALS AND METHODS

Chemicals. All chemicals and reagents were purchased from Fisher Scientific unless otherwise stated. TriReagent was purchased from Molecular Research Center. Transferring nylon membranes were purchased from Osmonics. [α-32P]-dATP was purchased from ICN Pharmaceutical. DNA polymerase I/DNase I was purchased from Invitrogen.

Animals and tissue sampling. Cobb-Cobb eggs and day of hatch Cobb-Cobb chicks were obtained from a commercial hatchery. For
eggs, the entire small intestine was collected from the embryos. After hatch but before feeding, chicks were sampled and designated d 0. Day of hatch chicks were randomly assigned to heated floor pens with wood shavings. All pens had 24-h lighting and the chicks had free access to water. For a given sampling day, 10–14 chicks from each group were killed by cervical dislocation and sexed by visual inspection of the gonads. Intestinal tissues were collected and separated into duodenum, jejunum, and ileum, washed with ice-cold PBS, frozen in liquid nitrogen, and stored at −80°C. The proximal enlarged loop of the small intestine was taken as the duodenum. The rest of the small intestine was divided by Meckel’s diverticulum into upper and lower portions, corresponding to the jejunum and ileum, respectively.

Growth data and tissue samples from male birds only were analyzed for d 1 through 35. All animal procedures were approved by the Institutional Animal Care and Use Committee at Virginia Tech.

**Expt. 1.** Ten eggs were sampled at e 18. After hatch but before feeding, 10 chicks were sampled (d 0). Other chicks (n = 339) were randomly divided among 3 pens. Chicks in a pen were randomly assigned to diets containing 12, 18, or 24% crude protein (CP). Daily feed intake of the chicks fed diets containing 18 or 24% crude protein was restricted to the intake of chicks fed the 12% CP diet. This was accomplished by feeding the groups of chicks receiving 18 and 24% CP diets an amount of feed equivalent to the feed consumed by the group of chicks fed the 12% CP diet the previous day. The day of the experimental diets is shown in Table 1. On d 1, 3, 5, 7, 10, 14, 21, 28, and 35, 10–12 chicks from each group were killed by cervical dislocation and intestinal tissues were collected immediately. Total feed consumption was monitored daily and body weight was determined for chickens sampled at each time point.

**Expt. 2.** Chicks, 6 males and 6 females, were sampled daily from e 16 until d 30. The entire small intestine was removed and minced. Chicks in 3 pens were randomly assigned to the main effects of diet and time and all two-way interactions. The main effect of time was further tested for linear and quadratic fit using orthogonal contrast statements in the General Linear Models procedure (SAS/STAT version 7.12 for Windows, SAS Institute). For the variables feed intake and body weight (Expts. 1 and 3), the model included the main effects of diet and time and the diet × time interaction. For hybridization intensity comparisons (Expts. 1 and 3), the model included the main effects of diet, tissue, and time and all two-way interactions. For Expt. 3, the model included the main effects of time and sex and the time × sex interaction. The main effect of time was further tested for linear and quadratic fit using orthogonal contrast statements in the General Linear Model procedure. Significant differences among diets, tissues, or sex was tested using Tukey’s Honestly Significant Difference.

**RESULTS**

**Feed intake and growth rate.** In Expt. 1, feed intake was equalized among the 3 treatments. Body weight increased quadratically (P < 0.0001) with time. The body weight of the group fed the 12% CP diet was lower (P < 0.05) than that of the groups fed 18 and 24% CP diets throughout the experiment. Final body weights for the groups fed the 12, 18, and 24% CP diets were 632, 862, and 949 g, respectively. An interaction (P < 0.0001) between crude protein level and time was the result of a slower rate of growth in chicks fed the 12% CP diet. In Expt. 3, the intake of the chicks with free access to the 24% CP diet was 40% greater at d 14 [50 g/(bird · d)] than that of the chicks fed the 12, 18 and 24% CP diets throughout the experiment. The body weight of the group fed the 12% CP diet was lower (P < 0.005) than that of the groups fed 18 and 24% CP diets [36.4 g/(bird · d)] and 1.5-fold greater by d 28 [50.5, 50.0, and 50.0 g/(bird · d) for chicks fed 12, 18, and 24% CP diets, respectively, and 129.4 g/(bird · d) for chicks with free access to the 24% CP diet]. Body weight increased quadratically (P < 0.0001) throughout the feeding period, with the chicks with free access to the 24% CP diet growing the fastest, chicks fed the 12% CP diet growing the slowest, and intermediate growth in the chicks fed the 18 and 24% CP diets (P < 0.05). Final body weights for the groups with equalized intakes of 12, 18, and 24% CP diets and those with free access to the 24% CP diet were 504, 746, 877, and 1854 g, respectively. These differing rates of growth account for the interaction observed between crude protein level and time (P < 0.0001).1

**Effects of developmental age and diet on cPepT1 mRNA abundance in the small intestine.** In Expt. 1, PepT1 mRNA abundance was barely detectable at e 18 and increased ~50-
fold at d 0 (P < 0.05) in the duodenum, jejunum, and ileum (Fig. 1). After hatch, there was a linear increase (P < 0.0001) in cPepT1 mRNA abundance with time and there was a protein × time interaction (P < 0.0001; Fig. 2). The interaction resulted from cPepT1 mRNA abundance generally decreasing throughout the study in all 3 sections of the small intestine in chicks fed the 12% CP diet (Fig. 2A, upper panel), whereas cPepT1 mRNA abundance increased with time in all 3 sections of the small intestine of chicks fed the 18% CP diet and the 24% CP diet (Fig. 2A, middle and lower panels, respectively). The major change in cPepT1 abundance occurred by d 21 for chicks fed the 12% CP diet (Fig. 2A, upper panel), d 14 for those fed the 18% CP diet (Fig. 2A, middle panel), and d 10 for those fed the 24% CP diet (Fig. 2A, lower panel). Data from the 3 sections were combined to show the main effect of diet (Fig. 2B, upper panel). cPepT1 mRNA level was lowest (P < 0.05) in chickens fed the low-protein diet (12%), highest in chickens fed the 24% CP diet, and intermediate for chicks fed the 18% CP diet (P < 0.05). Data from the chicks fed the 3 diets were combined to show the main effect of tissue (Fig. 2B, lower panel). cPepT1 mRNA abundance was higher in the jejunum (P < 0.05) than in the other 2 tissues.

To examine more closely the ontogeny of cPepT1 gene expression before hatch, expression of cPepT1 was examined daily in Expt. 2 in male and female broilers from e 16 to d 0 (Fig. 3). Expression of cPepT1 increased 14-fold from e 16 to d 0, with the rapid rise occurring just before hatch. The higher fold increase in Expt. 1 was due to the fact that cPepT1 was barely detectable at e 18, which resulted in a greater calculated fold-increase relative to d 0. No differences were observed (P > 0.10) in PepT1 abundance between male and female birds.

In Expt. 3, there was a general decrease in cPepT1 mRNA abundance in the jejunum and ileum of chickens fed the 12% CP diet throughout the duration of the experiment (Fig. 4A, upper panel); this was observed also in Expt. 1. In the duodenum, however, cPepT1 mRNA abundance generally increased to d 14 before declining to d 35. Groups fed restricted diets containing 18 or 24% CP again had an increase in cPepT1 mRNA abundance with time in all 3 sections of the small intestine (Fig. 4A, middle panels). Interestingly, in chickens with free access to the 24% CP diet, a decrease in cPepT1 mRNA abundance was observed in the 3 sections of the small intestine during the first 14 d. By the end of the feeding period (d 35), cPepT1 mRNA abundance in chickens with free access to the 24% CP diet approached or exceeded the level of cPepT1 mRNA at d 0 (Fig. 4A, lower panel). Data from the 3 sections of the small intestine of chicks in each treatment group were combined to show the main effect of diet (Fig. 4B, upper panel). cPepT1 mRNA abundance for chickens fed the 12% CP diet and for those with free access to the 24% CP diet was lower (P < 0.05) than that of chickens fed the 18 or 24% CP diets. cPepT1 mRNA abundance was the highest in chick-

![FIGURE 1](https://academic.oup.com/jn/article-abstract/135/2/193/4663626)  
Northern blot of cPepT1 mRNA abundance in duodenal (Duo), jejunal (Jej), and ileal (Ile) tissues at e 18, at day of hatch (d 0), and at 1, 3, 5, 7, 10, 14, 21, 28, and 35 d after hatch of male broilers fed diets containing 12, 18, or 24% crude protein (Expt. 1). The blot represents 1 of the 5 replicate gels. Each gel consisted of 1 replicate including each of the 3 small intestinal sections from 1 of the 5 chickens of each of the treatment groups from each sampling time. Each lane represents a RNA sample from 1 section of the small intestine of 1 chicken sampled at the indicated time point.

![FIGURE 2](https://academic.oup.com/jn/article-abstract/135/2/193/4663626)  
cPepT1 mRNA abundance (from densitometric analysis of Northern blots) in duodenal (Duo), jejunal (Jej), and ileal (Ile) tissues at e 18, at day of hatch (d 0), and at 1, 3, 5, 7, 10, 14, 21, 28, and 35 d following hatch of male broilers fed diets containing 12 (A, upper panel), 18 (A, middle panel), or 24% crude protein (A, lower panel) and the main effects of diet and tissues (B, Expt. 1). For panel A, values are means ± SEM from 1 section of the small intestine, n = 5. For the main effects of diet (B, upper panel), values are means ± SEM from the 3 sections of the small intestine from 5 chickens (n = 15) fed 1 of the 3 diets that were sampled at the indicated time point. For the main effects of tissue (B, lower panel), values are means ± SEM from 1 section of the small intestine from 15 chickens (n = 15) fed the 3 diets that were sampled at the indicated time point. Diets or tissues without a common letter differ (P < 0.05). There was a linear effect with time (P < 0.0001). There was a crude protein × time interaction (P < 0.0001).
ens with restricted access to the 24% CP diet \((P < 0.05)\).

There was a decrease in cPepT1 mRNA abundance by d 14 in chickens with free access to the 24% CP diet, which then returned to an intermediate level by d 35. Data from the chicks in all 4 treatment groups were combined to show the main effect of tissue (Fig. 4B, lower panel). cPepT1 mRNA abundance was the highest in the duodenum, lowest in the ileum, and intermediate in the jejunum \((P < 0.05)\).

**DISCUSSION**

The growth and development of chicks depend on the uptake of nutrients. The appearance and development of intestinal digestive enzymes and transport proteins are important factors that affect the uptake of nutrients and therefore the starting point of growth. In this report, we examined cPepT1 mRNA abundance from late embryogenesis (e 16) until d 0. During this period, the abundance of cPepT1 mRNA increased 50-fold from e 18 to d 0 in Expt. 1 and 14-fold from e 16 to d 0 in Expt. 2, with the rapid rise occurring just before hatch. A similar pattern of development-specific expression of PepT1 was observed in rats (9). The rat PepT1 \((rPepT1)\) mRNA was present at d 20 of fetal life but increased rapidly at birth with maximal expression occurring at d 3 to 5. The authors concluded that the rPepT1 gene was induced postpartum by suckling and later weaning. In chickens, the induction occurred before food consumption; thus there should be little nutritional change during late incubation to the time of hatch except for possible contributions from yolk and albumen (12). The expressions of many genes involved in digestion such as trypsin, amylase, and lipase are stimulated several days after hatch by feed intake (13). Obst and Diamond (14) reported a dramatic increase in glucose transport 2 wk posthatch in chicks. Uni et al. (15) observed that the relative abundance of mRNA for maltase, aminopeptidase, sodium-glucose transporter \((SGLT)-1\), and ATPase was low in e 15 and 17 broiler embryos and increase 9- to 25-fold on e 19 followed by a dramatic decrease on the day of hatch. Activity of these enzymes and transporters, however, increased from e 15 to the day of hatch. In broiler embryos, the mRNA of sucrase-isomaltase was observed at e 15, increased from e 17 to a peak on e 19, decreased to the day of hatch, and had a further peak of expression 2 d posthatch (16). In contrast, the mRNA of SGLT-1 was not detected until e 19 when a major peak of expression was observed followed by a decrease to low levels at the day of hatch with subsequent small increases occurring posthatch. Therefore, cPepT1, like other genes, appears to be regulated developmentally and may be induced before or during hatch for immediate uptake of peptide substrates when feed intake begins.
To improve our understanding of the mechanisms involved in the nutritional regulation of transporter gene expression, we investigated the effects of dietary protein on the abundance of cPepT1 mRNA to determine whether an increase in dietary protein level was associated with a similar increase in cPepT1 mRNA. In a normal production setting, dietary protein levels would be varied in a stepwise manner beginning at ~23% at hatch and decreasing to 20% and 18% at 21 and 42 d of age, respectively (17). Depending on the production system, an even more refined step-wise change may involve 4 or 5 steps. For the current study, we chose not to vary dietary protein percentage with age; rather, we held it constant at 12, 18, or 24% for the duration of the experiment. A number of dietary models could have been chosen for this study, each having its weaknesses and strengths. In the model used, intake was equalized and protein content was varied. Therefore, although protein intake varied, so did the intake of other macronutrients. This point must be considered as the results are evaluated. In Expt. 1, an increase in cPepT1 mRNA abundance in chickens fed 18 and 24% CP diets and a decrease in chickens fed a low 12% CP diet was observed. To investigate the possibility that the increase in cPepT1 mRNA abundance in chickens fed 18 or 24% CP diets may be due to a combination of diet restriction and protein intake rather than dietary protein intake alone, a 4th group with free access to the 24% CP diet in Expt. 3 was added. In chickens with free access to the 24% CP diet, the cPepT1 mRNA abundance declined during the first 14 d and rose from d 14 to 35, but remained lower than that of chickens fed diets containing 18 or 24% CP.

These results are similar to those from studies of PepT1 expression in rats. rPepT1 mRNA abundance dropped suddenly after d 5 to 11% of the level of an adult and rose by d 75 to 25% of the level of an adult (9). Ihara et al. (8) examined the expression of rPepT1 in response to starvation. In those studies, the level of rPepT1 mRNA increased to 179, 164, and 161% of control in rats that were food deprived (no food intake for 4d), partially deprived (50% of control intake), or administered total parenteral nutrition, respectively. rPepT1 protein expression showed similar changes with mRNA. In contrast, there was no change in SGLT1 mRNA level in these treatment groups. Therefore, the higher cPepT1 mRNA abundance in the present study may have been due to the restricted feeding for these 2 groups.

Adaptation to a protein diet was correlated with changes in the activity of enzymes involved in protein digestion (18). A high-protein diet enhanced the levels of intestinal peptidases (19), and a protein-sufficient diet induced lactate synthesis in protein-starved pigs (20). These are consistent with reports on dietary regulation of PepT1 in rats showing that the rPepT1 mRNA abundance was ~2-fold higher in rats fed a 50% casein diet than in rats fed a protein-free diet (6). The kinetic analysis from the studies also showed that rPepT1 activity was induced by a 50% casein diet with an increase in Vmax but not Km. The comparable patterns of the induced PepT1 mRNA abundance with increased dietary protein level suggested that dietary protein might have a regulatory effect on cPepT1 expression at the transcriptional level. Furthermore, both PepT1 mRNA abundance and transport rate of Gly-Gln by PepT1 increased when cells were treated with the dipeptide Gly-Sar for 24 h (21), whereas Gly-Gln treatment of the cells also caused an increase in both PepT1 mRNA abundance and Gly-Sar uptake (22). Promoter analysis of rPepT1 showed that the 5′-upstream region of rPepT1 contains elements that respond to peptide substrates such as Asp-Lys, Phe-Val, and free amino acids including Phe, Lys, and Arg (6). Therefore, end products of protein digestion, small peptides and amino acids, may participate in pathways that control expression of intestinal transporters like PepT1 and eventually cause increased transporter capacity in the intestine. Mechanisms by which dietary protein regulates cPepT1 gene expression have yet to be determined.

There are reports regarding amino acid transporters showing that gene expression of system A, system L, and system XAG was upregulated under conditions of amino acid restriction (23,24). It was suggested that gene expression is upregulated through amino acid sensory pathways at times of amino acid starvation (25). Although the regulatory networks have not been fully revealed, a potential amino acid-responsive element was found in the promoter regions of several genes, including mouse PepT1 (25). At present, we do not know whether the upregulatory effect was due to a response to peptide restriction or amino acid restriction derived from dietary protein limitation. Both amino acid-activating and peptide-activating pathways have to be investigated to fully elucidate the regulatory effects of dietary protein limitation. Peptide transporters may be one of the essential proteins induced by a stress response such as starvation for cell survival.

Further studies are required to establish the mechanism of transcriptional control of the cPepT1 gene, particularly through identification of the factors involved in the regulation of the transporters. Amino acids, peptide substrates, insulin, and growth factors were reported as factors regulating peptide transport through PepT1. Information on how cPepT1 is regulated at the molecular level will provide further insight into the basic mechanisms controlling body nitrogen and amino acid homeostasis in chickens.

LITERATURE CITED


