African Journal of Biotechnology Vol. 8 (9), pp. 1973-1978, 4 May, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Responses of mRNA expression of PepT1 in small intestine to graded duodenal soybean small peptides infusion in lactating goats

Liu Hui[1,2], Wang Ling[1,2,3], Cao Zhi-jun[1,2], Li Sheng-li[1,2]* and Wang Li-bing[1,2]

[1]College of Animal Science and Technology, China Agricultural University, Beijing 100193, P. R. China.
[2]National Key Laboratory of Animal Nutrition, Beijing, 100193, China.
[3]College of Agriculture, Ningxia University, Ningxia, 750021, China.

Accepted 20 April, 2009

To study the effect of circulation small peptides concentration on mRNA expression in small intestine, graded amount of soybean small peptides (SSP) were infused into lactating goats through duodenal fistulas. Peptide-bound amino acid (PBAA) concentration in arterial plasma and the mRNA expression of PepT1 was detected in the current study. The results showed that concentrations of all peptide-bound amino acids (PBAA) increased and the activity of PepT1 in duodenum tissue was enhanced by SSP infusion. The PepT1expression in duodenum tissue was significantly increased with the increment of amounts of SSP infusion (P<0.05). That in ileum tissue was increased with the increment of the amounts of SSP infusion; the increment of Pep T1 expression was significant only in the 180 g/d treatment (P<0.05). mRNA abundance of PepT1 in jejunum tissue was 1.1 to 1.8 to 2.4 times in SSP infusion group as compared with the control group. In the 120 and 180 g/d treatment, the difference of PepT1 expression in jejunum was significant (P<0.05). The data suggested Pep T1 expression in duodenum, ileum and jejunum tissue was enhanced by an increase in circulation small peptide concentration, and the duodenum might be a main position of small peptides absorption in intestine.

Key words: PepT1, mRNA expression, soybean small peptides, small intestine, lactating goats.

INTRODUCTION

Recent studies showed that much of digestive products of proteins were small peptides, and these peptides were absorbed completely by intestines (Ganapathy et al., 1994). However, absorption of small peptides in intestines depend on peptides transporters that are membrane proteins responsible for selective translocation of small peptides across the cell membrane. Peptides transporters are present in tissues of sheep, cows, pigs and chickens (Mathews et al., 1996a, b; Pan et al., 1997, 2001; Chen et al., 1999), and a 2.8 kb mRNA transcript for the peptide transporter (PepT1) in duodenum, jejunum and ileum tissues from sheep and lactating Holsten cows (Chen et al., 1999). Pep T1 expression was regulated by many factors, such as dietary protein level, protein quality, availability of transportable substrate, amino acid composition, etc.

Small bowel activity of Pep T1 is increased in rats fed high protein or dipeptide-enriched diets (Erichson et al., 1995; Shirage et al., 1999). Broiler chicks fed a diet containing soybean meal showed expression of Pep T1 rose continuously with age from d 3 to d 14 (Gilbert et al., 2008). However, few studies about the effect of small peptides level in diet on expression of Pep T1 have been reported.

In the present study, we infused soybean small peptides by duodenal fistula, and changed the concentration of circulation peptides, and then monitored how mRNA expression of Pep T1 in duodenum, ileum and jejunum tissue was affected.

MATERIALS AND METHODS

Experimental animals and diets

Sixteen Chinese Saanen dairy goats, with an average bodyweight of 38 ± 2 kg, were used for trail. Animals were surgically fitted with duodenal fistulas and unilateral skin-covered carotid artery which

*Corresponding author. E-mail: lisheng0677@vip.sina.com. Tel:/Fax: +86-10-6273-1254.

Table 1. Ingredients and nutrient levels of the experiment diet.

Ingredients (%)		Nutrient levels	
Alfalfa hay	40	DM (%)	90.06
Chinese wildrye hay	20	ME (MJ/Kg)[2]	8.86
Corn	28	CP (%)	10.94
Wheat bran	9	NDF (%)	45.11
Soybean meal	2	Ca (%)	0.75
Dicalcium phosphate	0.2	P (%)	0.34
Salt	0.4		
Minerals and vitamins[1]	0.4		
Total	100.00		

[1]Contained 15,000,000 IU of vitamin A/kg, 15,000,000 IU of vitamin D/kg, 3000 mg of vitamin E/kg, 4000 mg/kg of Mn, 6000 mg/kg of Zn, 2000 mg/kg of Fe, 3000 mg/kg of Cu, 150 mg/kg of I, 100 mg/kg of Se and 40 mg/ kg of Co.
[2]ME is calculated value. Other nutrient levels are measured values.

Table 2. The total amino acids content of SSP (n=3).

Amino acid	Concentration (mg/g)
Valine (Val)	27.29±3.02
Isoleucine (Ile)	30.60±3.65
Leucine (Leu)	38.50±3.92
Threonine (Thr)	20.28±2.17
Methionine (Met)	8.37±0.62
Phenylalanine (Phe)	23.76±2.31
Lysine (Lys)	33.55±3.62
Histidine (His)	15.71±1.55
Arginine (Arg)	44.70±4.29
Serine (Ser)	24.31±2.63
Glutamic acid (Glu)	101.76±11.58
Glycin (Gly)	27.67±2.39
Alanine (Ala)	33.43±3.41
Tyrosine (Tyr)	17.27±1.87
Aspartic acid (Asp)	59.41±5.32
Proline (Pro)	21.26±2.04
Total amino acids (TAA)	519.54±51.29

Values present the means ± SE.

were used to infuse SSP (molecular weight <1.47 KD; ZS Duqing Biotechnology, China) solution and collected blood samples. The composition of AA in the SSP is shown in Table 2. Note that the PBAA content of SSP was calculated as the difference between the AA content of hydrolyzed samples and the FAA content of the same sample before hydrolysis.

Goats were placed in metabolism crates individually and allowed 14 d of adaptation stage. Feed was delivered in 12 equal portions at 2 h intervals, and water was available *ad libitum*. The goats were hand-milked twice daily (07:00 and 19:00 h). The use of animals and all surgical and experimental procedures in this study were approved by the Animal Care and Use Committee, Animal Science and Technology College China Agricultural University.

The diet formulated contained 60% forage and 40% concentrate feeds including vitamin and mineral mixes (Table 1). Feed intake was adjusted to allow 5% refusals.

Experimental procedures

There were four different treatments in this experiment: 1) control, duodenal infusion of 0 g/d SSP (0.9% sodium chloride solution 700 ml), 2, 3 and 4) duodenal infusion of 60, 120 and 180 g/d SSP which was dissolved in 700 ml/d of 0.9% sodium chloride solution, respectively. Infusion was conducted using peristaltic pump. Infusion period lasted for 14d. On the last infusion day, blood sample were collected from carotid artery. All goats were kept standing during the blood sampling period.

Plasma was immediately separated from the blood samples as following by centrifugation at 3000 ×*g* for 15 min at 4°C and was stored at -20°C for further analysis. The plasma was prepared for free amino acid (FAA) and hydrolyzed AA analysis. Plasma was mixed with 1 *M* per chloric acid (1:1 vol/vol) thoroughly, centrifuged at 1500 × *g* at 4℃ for 15 min. The supernatant was re-centrifuged under the same condition to remove any residual protein. The supernatant was then neutralized (pH 7 to 8) by adding 2 M K_2CO_3, and allowed to stand for 2 h at 4℃ before the precipitated per chlorate salt were removed by centrifugation as described above (Backwell et al., 1997). The samples were analyzed for FAA and hydrolyzed AA using amino acid analysis apparatus (SYKAM, Germany). The PBAA content of plasma was calculated as the difference between the AA content of hydrolyzed samples and the FAA content of the same sample before hydrolysis. The small intestines (duodenum, ileum and jejunum) tissue samples (about 1 g) were taken and immediately frozen in liquid N and then stored at -80°C for further analysis.

RNA Preparation

Total RNA was extracted from the duodenum, ileum and jejunum tissue using the Trizol kit according to the manufacture's protocol, and total RNA was qualified spectrophotomerrically at 260/280 nm, then stored at -80℃.

cDNA Preparation

The cDNA synthesis reactions were performed in a 20 µl total volume containing 1 µg RNA, 5×M-MLV buffer 4 µl, 10 mmol dNTP, 20 U Rnasein, M-MLV 100 U and 20 pmol Oligo dT. The reactions mixture was incubated at 20℃ for 5 min, followed 42℃ for 60min and 70℃ for 5 min. The cDNA samples were stored -20℃ after cooled.

Real-time PCR

The following primers were designed according to the published gene sequences in GenBank (Table 3). The number of molecules of mRNA present for each gene of interest per nanogram of total RNA starting template was determined by Real-time PCR (Gilbert, 2007). The cDNA was diluted to 10^4 - 10 molecules before addition to PCR that contained primers and SYBR green master mix (Applied Biosystems, America). This was followed by product melt to confirm single PCR products. Amplification of cDNA samples was performed under the following conditions: 15 min denaturation at 95℃, 40 cycles of 15 s at 94℃, 30 s at 55℃ and 45 s at 72℃ and 1 cycle at 72℃ for 10 min. The products of Real-time PCR were examined on a 1.5% agarose gel. The relative quantity of targeted mRNA expression was analyzed using GAPDH as a house keeping gene.

Table 3. The primer sequence used for real-time PCR.

Name	Oligo	Primer sequence	Predicted size (bp)	Genbank accession
PepT1	Forward primer	5'-CCATTGGGCAGGTAGTCA-3'	156	NM_001009758
	Reverse primer	5'-ACACGCAAGGCTTTATCC-3'		
GAPDH	Forward primer	5'-GCAAGTTCCACGGCACAG-3'	249	AJ431207
	Reverse primer	5'-GGTTCACGCCCATCACAA-3'		

PepT1; Peptide transporter, GAPDH; Glyceraldehyde-3-phosphate dehydrogenase.

Table 4. Effect of duodenal infusion of SSP on PBAA concentration of arterial plasma

Amino acid	PBAA (mg/L)			
	0 g/d	60 g/d	120 g/d	180 g/d
Val	47.04±0.82^{a}	56.64±1.13^{b}	60.20±1.10^{c}	61.61±1.10^{c}
Ile	27.58±0.68^{a}	46.19±0.97^{b}	49.75±1.42^{b}	48.94±2.97^{b}
Leu	63.90±4.15^{a}	76.61±3.07^{b}	100.38±0.58^{c}	110.99±5.83^{c}
Phe	37.31±2.22^{a}	46.12±2.98^{a}	57.15±3.84^{b}	63.26±4.30^{b}
Thr	10.54±0.36^{a}	18.86±0.27^{b}	21.42±0.67^{bc}	22.50±1.85^{c}
Met	8.56±0.52^{a}	12.55±1.68^{ab}	16.81±2.47^{b}	17.14±1.93^{b}
Lys	37.22±0.80^{a}	54.05±0.81^{b}	76.04±5.52^{c}	80.01±5.24^{c}
His	35.33±0.98^{a}	43.50±0.85^{b}	50.23±5.52^{bc}	52.43±2.15^{c}
Arg	28.85±0.95^{a}	36.39±3.01^{ab}	41.23±2.49^{b}	53.99±4.39^{c}
Ser	26.27±2.59^{a}	47.84±0.58^{b}	48.18±1.53^{b}	56.67±3.56^{c}
Glu	135.86±8.56^{a}	158.754±5.44^{b}	194.89±2.84^{c}	173.23±6.67^{b}
Gly	34.43±1.90^{a}	35.74±1.23^{a}	42.80±0.86^{b}	45.66±1.31^{b}
Ala	24.56±0.54^{a}	29.86±0.86^{b}	36.26±1.50^{c}	33.54±2.11^{bc}
Tyr	20.43±0.80^{a}	24.32±2.47^{a}	30.46±0.56^{b}	35.24±2.69^{b}
Asp	69.53±3.17^{a}	78.12±2.57^{a}	90.07±6.19^{b}	94.07±1.02^{b}
Pro	28.75±0.91^{a}	38.52±1.21^{b}	43.60±1.08^{c}	45.50±1.51^{c}
TPEAA	296.33±6.56^{A}	390.89±7.75^{B}	473.20±14.44^{C}	510.86±13.66^{C}
TPNEAA	329.93±12.25^{a}	407.68±6.80^{b}	477.23±8.69^{c}	471.17±7.93^{c}

Values present the means ± SE.
Alphabet in the same row differ; lower-case letters: $P<0.05$; upper-case letter letters: $P<0.01$.
PBAA: Peptide-bound amino acid, TNEAA: Total peptide-bound essential amino acid.
TPNEAA: Total peptide-bound nonessential amino acid.

Statistical analyses

Data were analyzed by the one–way ANOVA procedure using SPSS 10.0. Multiple comparisons were done by Duncan's test. Significance was declared at $P < 0.05$.

RESULTS

PBAA concentration in arterial plasma

Among peptide-bound amino acid (PBAA) pool, in comparison to the control treatment, concentration of all PBAA were increased by SSP infusion, and those of other PBAA, apart from PB-Ile, PB-Glu, and PB-Ala, were increase with the increment of amounts of SSP infusion. However, the increment of PB-Met, PB-Phe, PB-Arg, PB-Gly, PB-Tyr and PB-Asp concentration in 60 g/d treatment was not significant (P>0.05). Total peptide-bound essential amino acid concentration in arterial plasma had significant increase by SSP infusion (P<0.01), significant difference for total peptide-bound nonessential amino acid was P<0.05 (Table 4).

cDNA fraction of PepT1 gene

The product of PCR Amplifying was detected via 1.5% agarose gel, the result showed Pep T1 fraction was ob-

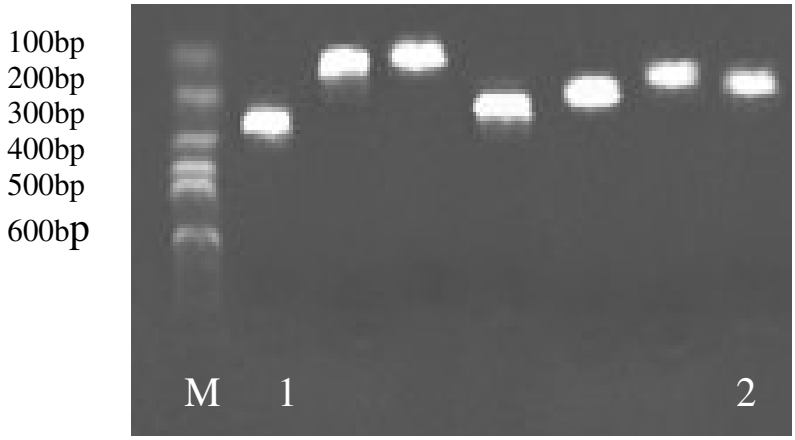

Figure 1. Special amplified PCR PepT1 band. M. DNA marker, 1. PepT1, 2. GAPDH. PepT1: Peptide transporter, GAPDH: Glyceraldehyde-3-phosphatedehydrogenase.

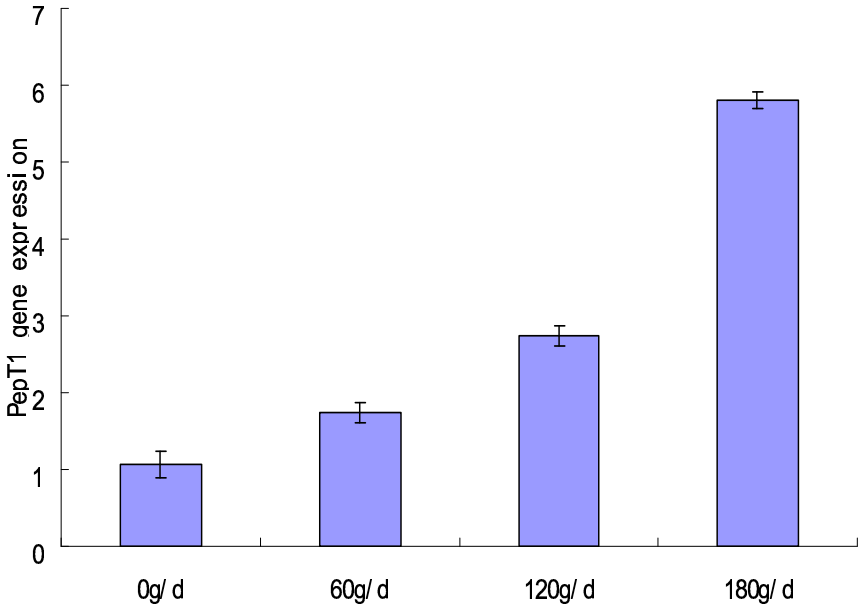

Figure 2. The effect of duodenal soybean small peptides infusion on PepT1expression in duodenal tissue. Real-time PCR analysis of PepT1expression in duodenal tissue taken from goats n=4 infused with 0, 60, 120 and 180 g/d soybean small peptides. Results are presented as means ± SE.

obtained, and the size of Pep T1 was between 100 and 200 bp, accorded with the size expected (Figure 1).

PepT1 expression in duodenum tissue

As Figure 2 showed, the activity of PepT1 in duodenum tissue was enhanced by SSP infusion. PepT1 expression in 60, 120 and 180 g/d treatment was 1.6, 2.6 and 5.4-fold as compared with the control treatment, respectively. The mRNA activity of Pep T1 was significantly increased with the increment of the amounts of SSP infusion ($P<0.05$).

PepT1 expression in ileum tissue

In comparison to the control treatment, the mRNA expression of Pep T1 in ileum tissue was increased with the increment of amounts of SSP infusion, and that in 60, 120 and 180g/d group was 1.1 to 1.4 to 3.3-fold. However, the increment of Pep T1 expression was significant only in

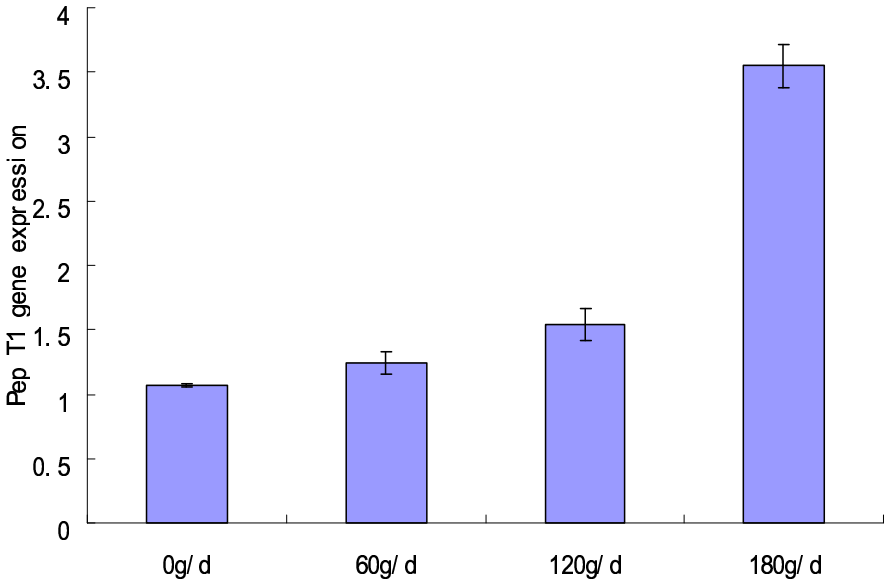

Figure 3. The effect of duodenal soybean small peptides infusion on PepT1expression in ileum tissue. Real-time PCR analysis of PepT1expression in ileum tissue taken from goats n=4 infused with 0, 60, 120 and 180 g/d soybean small peptides. Results are presented as means ± SE.

the 180 g/d treatment (P<0.05) (Figure 3).

PepT1 expression in jejunum tissue

The PepT1 expression was 1.51 in the control group, and it was increased to 1.72, 2.67 and 3.68 by SSP infusion, respectively. mRNA abundance of PepT1 in SSP infusion group was 1.1 to1.8 to 2.4 times as compared with the control group. When infused with 120 and 180 SSP, the difference of PepT1 expression in jejunum was significant (P<0.05), and in the 60 g/d treatment, the activity of PepT1 increased, but was not significant (P>0.05) (Figure 4).

DISCUSSION

Pep T1 is a member of the proton-coupled oligopeptide transporter superfamily (Daniel, 2004), and can transport most of di- and tripeptides (Ganapathy et al., 1994). In general, Pep T1 is very responsive to changes in dietary protein, and its expression and activity increases with dietary protein and peptide level. Shirage et al. (1999) maintained rats on a 20% casein diet for 1 week, a group of rats was switched to a protein-free diet, and others were switched to diets consisting of 50, 20 and 5% casein, 20% of a dipeptide, or 10% a single AA. The results showed that greater levels of dietary protein were associated with greater expression of Pep T1 mRNA. There was significantly greater Pep T1 mRNA for dipeptide-fed as compared with rats fed the protein-free diet. In our experiment, the results were consistent with Shirage' report; we found Pep T1 expression in duodenum, ileum and jejunum tissue were enhanced by SSP infused, and Pep T1 abundance was increased with the increment of the amounts of SSP infusion, which suggested the increment of substrate concentration could enhance activity of PepT1. Most of PBAA concentration in arterial plasma was significantly increased (P<0.05), and the change accorded with increment of Pep T1 expression, which suggested Pep T1 expression might be related to absorption of small peptides. However, Pep T1 mRNA in small intestine was influenced by not only CP in diets but also manner of intake. In chicken, an increase in Pep T1 expression was observed in chickens fed 18 and 24% crude protein (CP) diets with restricted food intake, and a decrease in Pep T1 expression was observed in chickens fed with 12% of CP diet (Chen et al., 2005). In chickens fed the 24% CP diet *ad libitum,* there was lower in abundance of Pep T1 as compared to chickens consuming restricted amounts of the diets. In our study, the intake of animals was not restricted, and we speculated the influence of Pep T1 expression was not significantly affected by manner of intake.

Although Pep T1 expression in duodenum, ileum and jejunum tissue was increased by infusion, it was highest in duodenum tissue. This suggests that duodenum might be a main site of small peptides absorption in small intestine.

In conclusion, when small peptides were infused, an increase in PBAA absorption was observed, and Pep T1 expression in duodenum, ileum and jejunum tissue was enhanced. We are of the opinion that the increment of

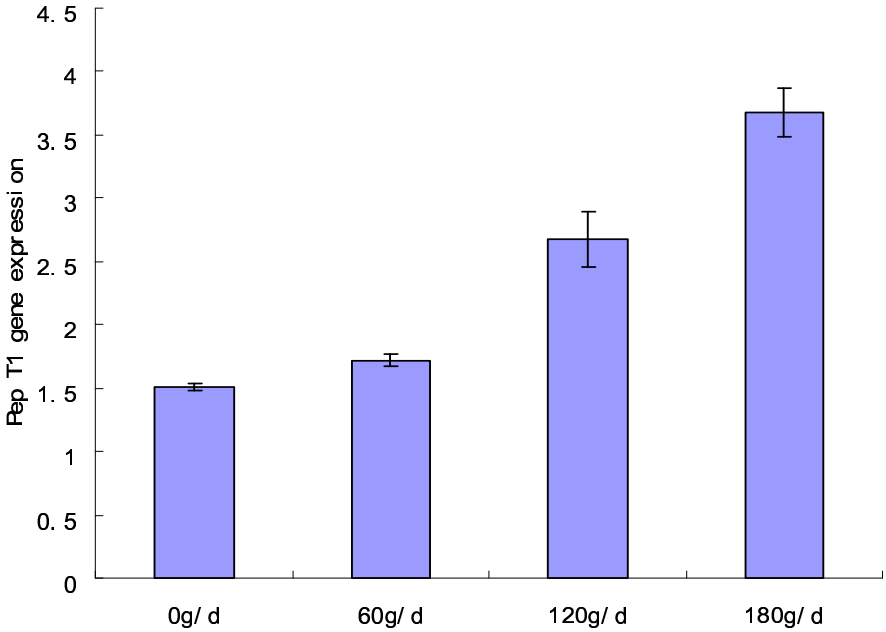

Figure 4. The effect of duodenal soybean small peptides infusion on PepT1expression in jejunum tissue. Real-time PCR analysis of PepT1expression in jejunum tissue taken from goats n=4 infused with 0, 60, 120 and 180g/d soybean small peptides. Results are presented means ± SE.

small peptides circulation concentration could improve Pep T1 expression absorption and the duodenum might be the main position of small peptides absorption in intestine.

ACKNOWLEDGEMENT

This research was funded by the National Natural Science Foundation of China (Grant no. 30671521).

REFERNCES

Backwell FRC, Hipolito-Reis M, Wilson D, Bruce LA, Buchan V, MacRae JC (1997). Quantification of circulating peptides and assessment of peptide uptake across the gastrointestinal tract of sheep. J. Anim. Sci. 75: 3315-3322.

Chen H, Pan YX, Wong EA, Webb Jr KE (2005). Dietary protein level and stage of development affect expression of an intestinal peptide transporter (cPep T1) in chickens. J. Nutr. 135: 193-198.

Chen H, Wong EA, Wedd Jr KE (1999). Tissue distribution of a peptide transporter mRNA in sheep, dairy cows, pigs, and chickens. J. Anim. Sci. 77: 1277-1283.

Daniel H (2004). Molecular and integrative physiology of intestinal peptide transport. Annu. Rev. Physiol. 66: 361-384.

Erichson RH, Gum JRG, Lindstrom MM, McKean D, Kim YS (1995). Regional expression and dietary regualation of rat small intestinal peptide and amino acid transporter mRNA. Biophys. Res. Commun. 216: 249-257.

Ganapathy V, Brandsch M, Leibach FH (1994). Intestinal transport of amino acids and peptides. Page, 1773-1794 in Physiology of Gastrointestinal Tract. 3rd ed. Johnson LR, Alpers DH, Jacobson ED, Walsh JH (ed.). Raven press, New York.

Gilbert ER, Li H, Emmerson DA, Webb Jr KE, Wong EA (2007). Developmental regulation of nutrient transporter and enzyme mRNA in the small intestine of broiler chicks. Poult. Sci. 86: 1739-1753.

Gilbert ER, Li H, Emmerson DA, Webb Jr KE, Wong EA (2008). Dietary protein quality and feed restriction influence abundance of nutrient transporter mRNA in the small intestinal of broiler chicks. J. Nutr. 138: 1-10.

Mathews JC, Wong EA, Bender PK, Bloomquist JR, Webber Jr KE (1996). Demonstration and characterization of dipeptide transport system activity in sheep omasal epithelium by expression of mRNA in *Xenopus laevis oocytes*. J. Anim. Sci. 74: 1720-1727.

Pan YX, Wong EA, Bloomquist JR (1997). Poly $(A)^+$ RNA from sheep omasal epithelium induces expression of a peptide transport protein in *Xenopus laevis oocytes.* J. Anim. Sci. 75: 3323-3330.

Pan YX, Wong EA, Bloomquist JR, Webber Jr KE (2001). Expression of a cloned ovine gastrointestinal peptide transporter (oPep T1) in *Xenopus laevis oocytes* in duces uptake of oligopeptides *in vitro*. J. Nutr. 131: 1264-1270.

Shirag T, Miyamoto KL, Tanaka H, Yamamoto H, Taketani Y, Morita K, Tamai I, Tsuji A, Takeda E (1999). Cellular and molecular mechanisms of dietary regulation on rat intestinal H+/peptide transport Pep T1. Gastroenterol. 116: 354-362.