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Short Communication

# Molecular cloning, tissue distribution and the expression of cystine/glutamate exchanger (xCT, SLC7A11) in different tissues during development in broiler chickens

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ABSTRACT

The cystine/glutamate exchanger (xCT, SLC7A11) is a component of the system $X_c$ amino-acid antiporter that is able to export glutamate and import cysteine into cells. The xCT amino acid exchanger has received a lot of attention, due to the fact that cysteine is an essential substrate for the synthesis of glutathione (GSH), an endogenous antioxidant in cells. The objective of this research was to clone the full-length cDNA of chicken xCT, and to investigate the gene expression of xCT in different tissues, including intestinal segments of broiler chickens during development. The full-length cDNA of chicken xCT (2,703 bp) was obtained from the jejunum by reverse transcription-PCR and sequenced. Homology tests showed that chicken xCT had 80.4%, 80.2%, and 71.2% homology at the nucleotide level with humans, cattle, and rats, respectively. Likewise, amino acid sequence analysis showed that chicken xCT protein is 86.4%, 79.3%, and 75.6% homologous with humans, cattle, and rats, respectively. Additionally, phylogenetic analysis indicated that chicken xCT genes share a closer genetic relationship with humans and cattle, than with rats. The chicken xCT protein has 12 transmembrane helixes, 6 extracellular loops, and 5 intracellular loops. The mRNA of xCT was detected in all tissues, including intestinal segments, in which the mRNA expression of xCT was significantly higher ($P < 0.05$) within the colon, compared to the jejunum and ileum. During development, a linear pattern of changes regarding the levels of the xCT mRNA was found, indicating that there was an abundance of xCT within the duodenum ($P < 0.05$). Furthermore, there were changes of the xCT mRNA abundance in the colon during development, which displayed linear and cubic patterns ($P < 0.05$). These results indicated that xCT is widely expressed both in intestinal segments, as well as other organs that are not associated with nutrient absorption. Further investigation is needed to characterize the functional relevance of xCT activity in oxidative stress and inflammation in the small intestine of broiler chickens.



## 1. Introduction

Oxidative stress is generally referred to as an imbalance between oxidants and antioxidants at the cellular level, which can cause the oxidative modification of cellular macromolecules, apoptosis, necrosis, and structural tissue damage (Lykkesfeldt and Svendsen, 2007; Lauridsen, 2018). Oxidative stress may be due to several factors, including the contamination of feed with mycotoxins (Lee et al., 2017), impaired fat quality (Leskovec et al., 2018; Lindblom et al., 2018), high dietary polyunsaturated fatty acids

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(PUFA) (Cherian and Hayat, 2009; Gao et al., 2010), heat stress (Gu et al., 2012; Sahin et al., 2017), insufficient intake of antioxidants (Voljč et al., 2011), fast growth rate (Zambonelli et al., 2016), increased activity of the immune system (e.g., vaccination and infection), pulmonary hypertension (Iqbal et al., 2001), and coccidiosis (Georgieva et al., 2006). Several studies have shown that oxidative stress may be associated with the pathogenesis of some intestinal diseases in broiler chickens, such as diarrhea and enteritis, as well as the decreased growth performance of broiler chickens (Estévez, 2015; Leskovec et al., 2018). As a result, antioxidant-based therapeutic effects have been examined in broiler chickens (Leskovec et al., 2018; Min et al., 2018).

Amino acids are key nutrients that are essential for maintaining gut mucosal growth and functions, serving as both metabolic fuels and precursors for the syntheses of various monomer and polymer nitrogenous compounds, which are essential to the gut and body (Burrin and Reeds, 1997; Ziegler et al., 2003). Cysteine is an essential substrate for the synthesis of glutathione (GSH). It is a tripeptide of cysteine, glutamate, and glycine, and is a major endogenous antioxidant in cells (Bannai, 1986; Patel et al., 2004; Koppula et al., 2018). Cystine uptake is closely associated with the expression of the cystine/glutamate exchanger (xCT, SLC7A11), which is a component of the system $X_c^-$ sodium-independent amino acid antiporter (Bannai and Kitamura, 1980; Burdo et al., 2006). As shown in Fig. 1, the essential roles of the apical membrane-associated xCT and excitatory amino acid carrier 1 (EAAC1) are associated with other membrane amino acid (AA) transporters and intracellular biochemical steps, which provide intracellular L-glutamate, L-cysteine and glycine. These amino acids are utilized in maintaining intestinal epithelial homeostasis, de novo biosynthesis of GSH, and redox balance in broiler chickens. According to Tang et al. (2016), cystine deprivation depleted intracellular GSH in cells and induced cell death. In broiler chickens, it is evident that the antioxidative capacity is closely correlated with the intracellular GSH levels (Banerjee et al., 2008; Zhang et al., 2011, 2018). It has been shown that the expression level of xCT could be upregulated by oxidative stress induced by enhanced GSH biosynthesis in animals (Conrad and Sato, 2012). Thus, the expression of xCT at the cell surface may be an important regulator of intracellular redox balance (Lo et al., 2008), and chicken xCT may be a potential therapeutic target in treating complications associated with oxidative stress in the poultry industry. However, information about chicken xCT is limited. Therefore, the objective of this research was to gain more insight about chicken xCT by cloning the full-length coding region of xCT and to investigate the expression of xCT in different tissues, including intestinal segments of broiler chickens during development.

## 2. Materials and methods

The animal protocol for this study was approved by the University of Guelph Animal Care Committee.

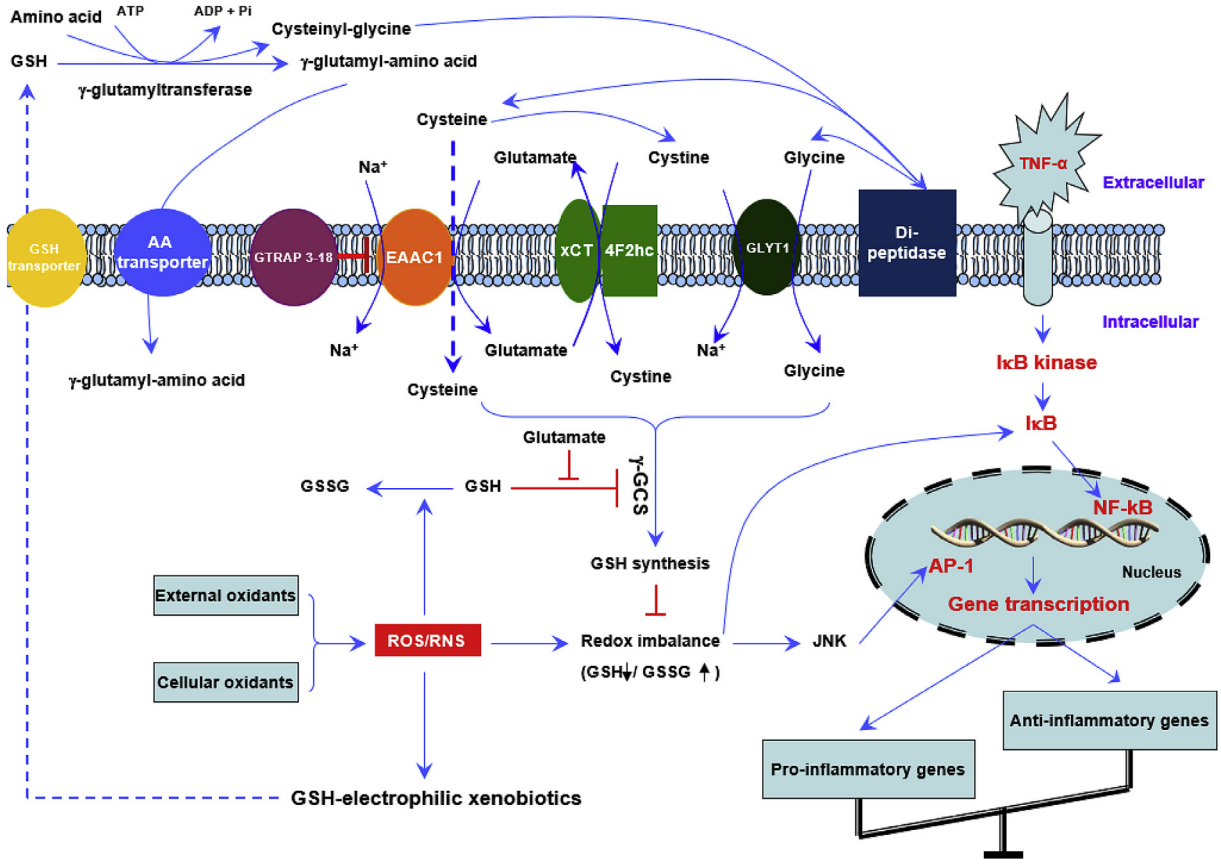


**Fig. 1.** Schematic illustration of essential roles of the apical membrane-associated cysteine/glutamate exchanger (xCT) and excitatory amino acid carrier 1 (EAAC1) in relationships to other membrane amino acid (AA) transporters and intracellular biochemical steps in providing intracellular L-glutamate, L-cysteine, and glycine for maintaining intestinal epithelial homeostasis, de novo biosynthesis of glutathione and redox balance in broiler chickens. GSH = glutathione; EAAC1 = excitatory AA carrier 1; GTRAP3-18 = EAAC1-associated protein; xCT and 4F2hc, subunits of system $Xc^-$; GLyT1 = glycine transporter 1; TNF-α = tumor necrosis factor alpha; IkB kinase; γ-GCS = gammaglutamylcysteine synthetase; GSSG = glutathione disulfide; ROS = reactive oxygen species; RNS = reactive nitrogen species; JNK = Jun N-terminal kinases; AP-1 = activator-protein-1.

### 2.1. Animals and diets

For the study of xCT cloning, expression and distribution in different tissues, six 35-day-old broiler chickens were obtained from the Arkell Poultry Research Station of the University of Guelph. The chickens were euthanized by $CO_2$, and tissues from the upper palate, tongue, heart, lung, proventriculus (true stomach), ventriculus (gizzard), liver, kidney, duodenum, jejunum, ileum, cecum, and colon were cut into small pieces, quickly immersed in RNAlater (Thermo Fisher Scientific, Waltham, MA) and stored at −20 °C until RNA was extracted. Intestinal tissue included the mucosa, submucosa, muscularis, and serosa, which were sampled for further analyses.

In order to study xCT gene expression during development, twenty 1-day-old male Ross 308 broiler chickens were obtained from a local hatchery in Manitoba (Carleton Hatchery, Grunthal, Manitoba). Corn-soybean basal diets were formulated in order to exceed the requirements of the National Research Council (1994), as shown in Table 1. The 20 chickens were randomly allotted to 2-floor pens (2.13 m × 1.52 m) containing straw bedding, and allowed free water and feed during the experiment period. The chickens were housed in a facility with controlled temperature and light conditions. The temperature was maintained at 31 °C from d 1 to 3, 30 °C from d 3 to 7, 28 °C from d 7 to 14, 25 °C from d 21 to 28, and 24 °C from d 28 to 35. The lighting program during the study was as follows: 24 h light (L): 0 h darkness (D) from d 0 to 3, 22L:2D from d 4 to 7, and 18L:6D from d 8 to 35. Four chickens were randomly selected from the 2 pens, and euthanized by cervical dislocation at the ages of 7, 14, 21, 28, and 35 d. Tissues (5 cm long) were collected from the duodenum, jejunum, ileum, and colon. The tissues were quickly immersed in RNAlater, and stored at −20 °C until RNA was extracted. The animal protocol for this experiment was approved by the University of Manitoba Animal Care Committee.

### 2.2. RNA extraction

Total RNA was isolated from 50 mg of tissue samples using an RNAqueous total RNA isolation kit (Ambion Inc., Foster City, CA), and DNA contamination was eliminated by DNase I (Invitrogen, Carlsbad, CA) treatment according to the manufacturer's instructions. The RNA integrity was checked by a 1% agarose gel electrophoresis stained with SYBR Green (Invitrogen). OD260:OD280 and OD260:OD230 were measured using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE) to determine RNA concentrations. The RNA samples were stored at −80 °C and kept for further analysis.

### 2.3. Full-length chicken xCT coding region cloning

The first strand of cDNA was synthesized using oligo (dT) 20 primers, and Superscript II reverse transcriptase (Invitrogen), according to the manufacturer's instructions. Primers for amplifying the full-length of the chicken xCT coding region were designed with Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome) based on the predicted chicken xCT mRNA sequence (GenBank: XM_426289.5); the primer sequences are listed in Table 2. The expected size of the reverse transcription (RT)-PCR product was 2,703 bp. PCR thermocycler conditions were as follows: 95 °C denature 2 min, 40 cycles at 95 °C for 30 s, 54 °C for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 10 min. Each PCR reaction mixture contained 38.1 μL of 0.1% diethylpyrocarbonate (DEPC)-treated water, 1 μL each of forward and reverse primer (10 μmol/L), 5 μL of 10 × PCR Buffer; 1 μL of dNTP mix (10 mmol/L), 0.4 μL of TaqDNA polymerase (5 U/μL), 1.5 μL of $MgCl_2$ (50 mmol/L), and 2 μL cDNA; the total volume was 50 μL. All PCR products were electrophoresed on a 2% agarose gel in Tris-borate-EDTA buffer, and visualized by staining with SYBR Green (Invitrogen). To confirm the identity of the PCR products, the PCR products were purified and sequenced by dideoxy-mediated chain termination sequencing at the University of Manitoba Molecular Supercenter. The nucleotide sequence of the xCT gene reported in this paper resides in the GenBank/EMBL data bank (accession no. MH760782.1).

### 2.4. Bioinformatics analyses

The homology of xCT mRNA and amino acid sequences among chicken (MH760782.1), humans (XM_011531802.2/XP_011530104.1), cattle (XM_015475398.1/XP_015330884.1), and rats (XM_006232323.3/XP_006232385.1) was analyzed by Clustal Omega, which is a multiple sequence alignment online-tool (http://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers et al., 2011). The

**Table 1**
Ingredients of diets used in this study (g/kg).

| Item | 0 to 14 d of age | 15 to 28 d of age | 28 to 35 d of age |
|---|---|---|---|
| Corn ground | 522.29 | 529.38 | 566 |
| Soybean meal | 305 | 261 | 225 |
| Corn gluten meal (60% CP) | 35 | 35 | 35 |
| Shorts wheat | 25 | 30 | 30 |
| Canola meal | 25 | 30 | 30 |
| Soya oil | 22.6 | 43.8 | 45.8 |
| Corn distillers dried grains with soluble | 20 | 30 | 30 |
| Limestone | 15 | 13 | 13 |
| Vitamin premix[1] | 10 | 10 | 10 |
| Monocalcium phosphate | 9 | 7 | 5 |
| Mineral premix[2] | 5 | 5 | 5 |
| DL-methionine 99% | 2.65 | 2.37 | 2.05 |
| Lysine-HCl | 2.25 | 2.46 | 2.31 |
| Threonine | 0.71 | 0.49 | 0.34 |
| Xylanase 8000 G[3] | 0.2 | 0.2 | 0.2 |
| Quantum blue 5000 G[4] | 0.3 | 0.3 | 0.3 |
| Total | 1000 | 1000 | 1000 |

[1] Provided per kilogram of diet: retinyl acetate, 8,250 IU; cholecalciferol 1,000 IU; DL-alpha-tocopherol, 11 IU; cyanocobalamin, 0.012 mg; phylloquinone, 1.1 mg; niacin, 53 mg; choline, 1,020 mg; folacin, 0.75 mg; biotin, 0.25 mg; riboflavin, 5.5 mg.
[2] Provided per kilogram of diet: manganese, 55 mg; zinc, 50 mg; iron, 80 mg; copper, 5 mg; selenium, 0.1 mg; iodine, 0.36 mg; sodium, 1.6 g.
[3] Xylanase 8000 G: 8,000 U/g; Danisco Animal Nutrition, Marlborough, United Kingdom.
[4] Quantum blue 5000 G: 5,000 FTU/g; AB Vista, Plantation, FL, United States.

**Table 2**
Primers used in this study.

| Genes | Sequence (5′-3′) | Size, bp | Tm, °C | Location | Product size, bp | Purpose | GenBank ID |
|---|---|---|---|---|---|---|---|
| xCT | FP: TGAGCTGGGAACGTGCATTA | 20 | 59.68 | Exon 2 | 115 | Real-time RT-PCR | MH760782.1 |
| | RP: AGGGCGAATAACCAGCAGTT | 20 | 59.67 | Exon 3 | | | |
| β-actin | FP: AATGGCTCCGGTATGTGCAA | 20 | 60.03 | Exon 1 | 112 | Real-time RT-PCR | NM_205518.1 |
| | RP: GGCCCATACCAACCATCACA | 20 | 60.03 | Exon 2 | | | |
| xCT | FP: GACTAACTTGGTACGACAAG | 20 | 47.00 | | 2,703 | Clone | XM_426289.5 |
| | RP: TTTATTGCCGTGTCTATAC | 19 | 45.90 | | | | |

FP = forward primer; RP = reverse primer; Tm = melting temperature; xCT = cystine/glutamate exchanger; real-time RT-PCR = real-time reverse transcription PCR.

phylogenetic relationship of the xCT amino acid sequences among chickens, mice, humans, and pigs was further analyzed, and the phylogenetic tree was constructed by the unweighted pair group method with arithmetic mean (UPGMA) method in the MEGA 5.05 software (Tamura et al., 2011). Visualization of the secondary structure of chicken xCT protein was conducted using Protter (http://wlab.ethz.ch/protter/start).

### 2.5. Real-time reverse transcription-PCR analysis

One microgram RNA was used to synthesize the first strand of cDNA using the iScript cDNA Synthesis Kit (Bio-Rad, Mississauga), according to the manufacturer's instructions. Primers for chicken xCT and β-actin genes were designed with Primer-Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table 2) and were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). Real-time RT-PCR was performed using SYBR Green Supermix (Bio-Rad) on a CFX Connec Real-Time PCR Detection System (Bio-Rad) (Omonijo et al., 2018). One microgram cDNA was added to make a total volume of 20 μL containing 10 μL SYBR Green mix, and 1 μL each of forward and reverse primers. The following thermocycler conditions were used: denaturation 15 s at 95 °C, annealing 15 s at 56 °C, extension 30 s at 72 °C, and repeat cycle 45 times. Beta-actin was used as the internal control to normalize the amount of starting RNA used in the real-time RT-PCR. A melting curve program was conducted to confirm the specificity of each product, and the size of the products was verified by agarose gel electrophoresis. A duplicate assay was done for all the samples. The target gene expression was normalized with that of a selected reference gene, and relative gene expression was determined by using $R = 2^{[Ct(reference)-Ct(test)]}$ (Kleta et al., 2004). The efficiencies of PCR were acquired by amplification of the dilution series of the DNase-treated RNA according to formula $10^{(-1/slope)}$ (Pfaffl, 2001). The efficiencies of all primers used in this study were between 96% and 105%.

### 2.6. Statistical analysis

Statistical Analysis System (SAS 9.4, SAS Institute, Cary, NC) was used to perform statistical analyses. The relative gene expression was statistically analyzed by ANOVA, followed by equally spaced orthogonal polynomial contrast, and Tukey's test for individual comparison. GraphPad Prism 7 (GraphPad Software Inc, San Diego, CA) was used to make figures. All data were expressed as means ± SEM, and the level of statistically significant difference was set at $P < 0.05$.

## 3. Results

As shown in Fig. 2, the expected 2,703 bp chicken cDNA fragment of xCT gene was obtained from the jejunum through RT-PCR. The PCR product was further purified, sequenced, and submitted to GenBank/EMBL data bank (accession no. MH760782.1). Compared

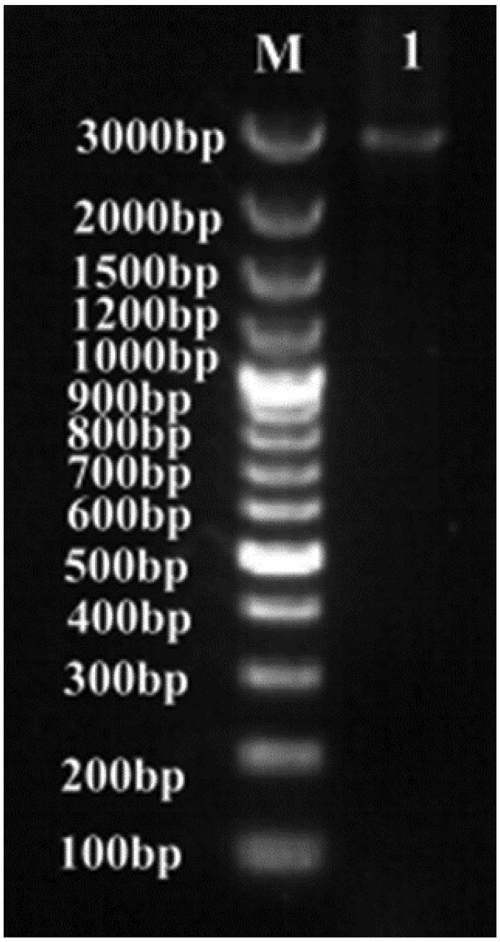


**Fig. 2.** Electrophoretic pattern of chicken cystine/glutamate exchanger (xCT) PCR product in 1% agarose gel. M: GeneRuler 100 bp Plus DNA Ladder; lane 1: chicken xCT PCR product. The size of PCR product of the full-length coding region of chicken xCT was 2,703 bp.

with other species, the coding region cDNA sequence of chicken xCT had 80.4%, 80.2%, and 71.2% homology with humans, cattle, and rats, respectively. Chicken xCT amino acid primary structure had 86.4%, 79.3% and 75.6% homology with humans, cattle, and rats, respectively. Phylogenetic analysis indicated that chicken xCT share a closer genetic relationship with humans and cattle, compared to rats (Fig. 4). Protter results revealed that chicken xCT protein contains 12 transmembrane helixes, 6 extracellular loops, and 5 intracellular loops (Fig. 5).

As shown in Fig. 3A, the chicken xCT gene was expressed in the tissues including the upper palate, tongue, heart, lung, proventriculus (true stomach), ventriculus (gizzard), liver, kidney, duodenum, jejunum, ileum, cecum, and colon of broiler chickens. As shown in Fig. 3B, there was no difference observed in the xCT mRNA

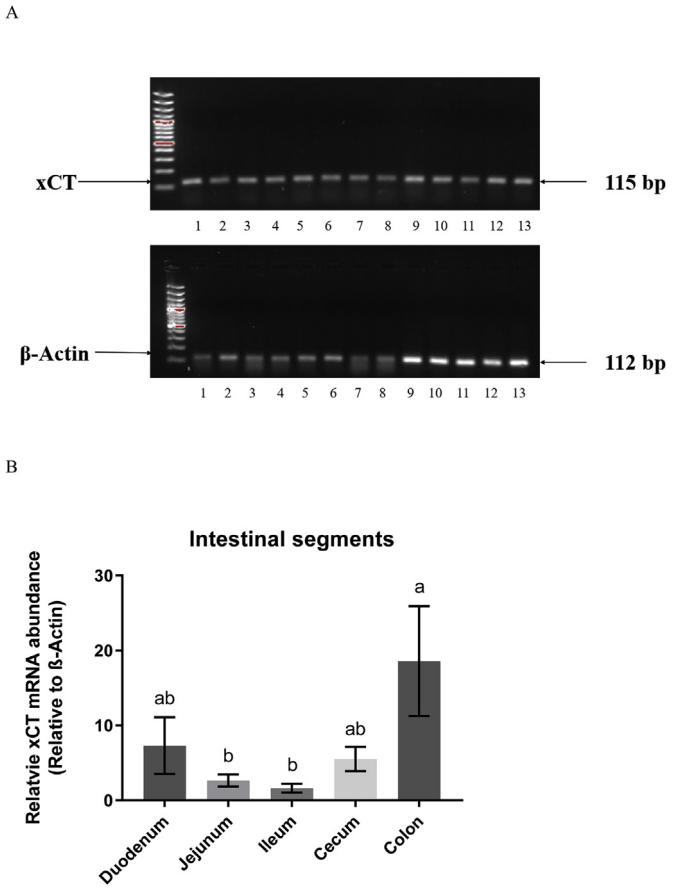


**Fig. 3.** Expression of cystine/glutamate exchanger (xCT) in different tissues of broiler chickens. **(A)** Total RNA was extracted from 13 different tissues of 35-d-old broiler chickens. Cystine/glutamate exchanger mRNA abundance was detected by reverse transcription (RT)-PCR and β-actin was used as internal control. The end PCR products were verified by agarose gel electrophoresis. The number of bands represents as, 1: cecum, 2: bcolon, 3: ileum, 4: jejunum, 5: duodenum, 6: kidney, 7: gizzard, 8: true stomach, 9: liver, 10: heart, 11: lung, 12: tongue and 13: upper palate. **(B)** Gene expression of xCT in the duodenum, jejunum, ileum, cecum and colon. Total RNA was extracted from different intestinal segments and xCT mRNA abundance was detected by RT-PCR. Each value represents the mean ± SEM and different letters represent a significant difference ($P < 0.05$).

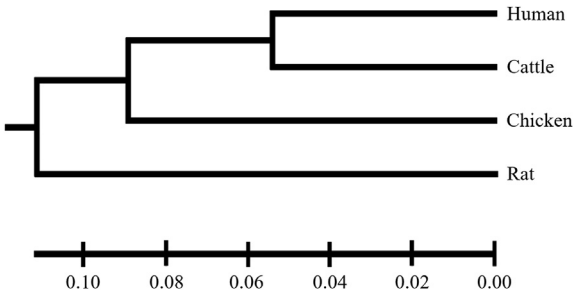


**Fig. 4.** The phylogenetic trees of cystine/glutamate exchanger (xCT) amino acid sequences among chicken, cat, cattle and humans. The phylogenetic tree was constructed by the unweighted pair group method with arithmetic mean (UPGMA) method in the MEGA 5.05 software (Tamura et al., 2011). GenBank accession no.: chicken, XM_426289.5; human, XM_011531802.2; cattle, XM_015475398.1; and rat, XM_006232323.3.

abundance among the duodenum, jejunum, ileum, and cecum. However, the mRNA abundance of xCT in the colon was significantly higher ($P < 0.05$) than that of the jejunum and ileum.

As shown in Table 3, xCT mRNA abundance in the duodenum changes occurred in a linear pattern ($P < 0.05$) during development. The mRNA abundance at the age of 7 d was significantly higher than that at the age of 35 d. Both linear and cubic patterns of changes of xCT mRNA abundance were found in the colon during development ($P < 0.05$). The xCT mRNA abundance in the colon at the age of 14 d was significantly higher ($P < 0.05$) when compared with those at the ages of 28 and 35 d. There was no difference in the xCT mRNA abundance in the jejunum and ileum during development.

## 4. Discussion

Previous studies have indicated that the expression of xCT is most likely associated with the redox balance in animals (Conrad and Sato, 2012), since xCT transports cystine into the intracellular space, and then the cystine is further utilized for the synthesis of GSH, which is a natural endogenous antioxidant (Bannai, 1986; Patel et al., 2004; Koppula et al., 2018). A low level of cystine in the in vitro circumstance decreased the amount of GSH, and had similar effects on cytotoxicity (Tan et al., 2001). Thus, xCT plays a vital role in regulating the ability of cells to cope with oxidative stress, and is a potential novel therapeutic target for diseases associated with oxidative stress, due to xCT enhancing intracellular GSH levels (Burdo et al., 2006; Maher et al., 2008). The functions of xCT in humans and mice have been widely investigated (Conrad and Sato, 2012; Nasca et al., 2017; Kobayashi et al., 2018). However, the information about chicken xCT is still unclear. In this study, the full-length of the coding region of chicken xCT was cloned, and the expression of xCT in different tissues including intestinal segments of broiler chickens during development was investigated. The amino acid sequence of the chicken xCT had 75% to 86% homology with other species, which indicated that the xCT was highly conserved among chickens, humans, cattle, and rats. Moreover, the chicken xCT may have similar functions with the xCT of other species. This has also been confirmed by bioinformatics analyses, which has shown that chicken xCT protein has a similar secondary structure that is consistent with the 12 transmembrane helixes, 6 extracellular loops, and 5 intracellular loops in other species (Sato et al., 2000; Gasol et al., 2004; He et al., 2012; Tian et al., 2015). According to the phylogenetic tree, chicken xCT shares a closer genetic relationship with humans and cattle compared to rats. Therefore, further studies are needed to characterize the functions of chicken xCT. The results of the current study also showed that the xCT mRNA was widely detectable in the chicken tissues such as the upper palate, tongue, heart, lung, proventriculus (true stomach), ventriculus (gizzard), liver, kidney, duodenum, jejunum, ileum, cecum, and colon. This result indicates that xCT is widely expressed both in the intestinal segments and other organs that are not related to nutrient absorption, including the proventriculus. The function of xCT in non-digestive organs is unclear, and may be involved in the modulation of intracellular redox balance by regulating GSH synthesis. For example, GSH synthesis is active and rich in the liver of mammals and fish, which indicates that the expression of xCT in chicken liver may play an important role in the regulation of GSH synthesis (Lu, 2009; Ojopagogo et al., 2013). The results from the current study are somewhat consistent with the results from Fagerberg et al. (2014), showing that human xCT was detectable in 25 different human tissues (not including the kidney and liver), using RNA-seq analysis. The study conducted by Bassi et al. (2001) also demonstrated that human xCT mRNA was not expressed in the kidney and liver. However, xCT mRNA was detectable in the brain, kidney, and duodenum of mice (Burdo et al., 2006). The discrepancies among the various studies may be a result of the different species used. It would be beneficial to further investigate the protein abundance and localization of chicken xCT

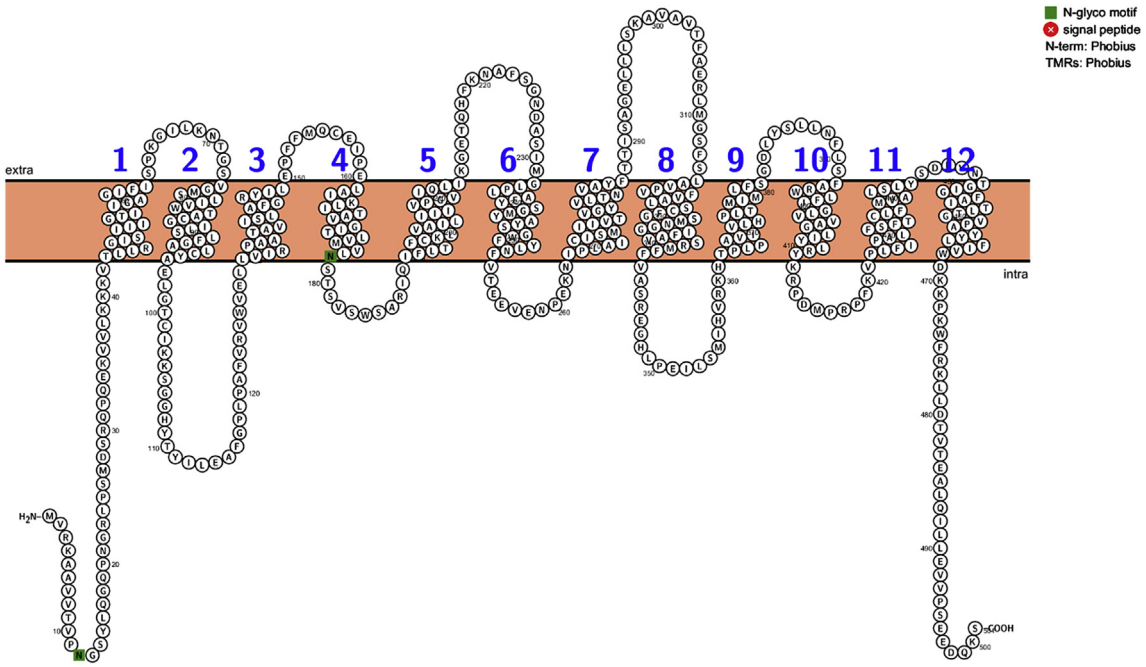


**Fig. 5.** A secondary structure model of chicken cystine/glutamate exchanger (xCT) protein produced by Protter (http://wlab.ethz.ch/protter/start) based on the amino acid sequence. The protein of chicken xCT is composed of 501 amino acids which form 12 transmembrane helixes, 6 extracellular loops and 5 intracellular loops.

**Table 3**
Relative cystine/glutamate exchanger (xCT) mRNA abundance in the intestinal segments during the development between 7 and 35 d of age of broiler chickens.[1]

| Item | 7 d of age | 14 d of age | 21 d of age | 28 d of age | 35 d of age | Pooled SE |
|---|---|---|---|---|---|---|
| Duodenum[2] | 0.73$^{a}$ | 0.47$^{ab}$ | 0.38$^{ab}$ | 0.47$^{ab}$ | 0.26$^{b}$ | 0.02 |
| Jejunum | 0.61$^{a}$ | 0.63$^{a}$ | 0.39$^{a}$ | 0.35$^{a}$ | 0.39$^{a}$ | 0.04 |
| Ileum | 0.93$^{a}$ | 0.68$^{a}$ | 0.55$^{a}$ | 0.71$^{a}$ | 0.64$^{a}$ | 0.02 |
| Colon[2,3] | 2.16$^{ab}$ | 3.25$^{a}$ | 1.47$^{ab}$ | 1.20$^{b}$ | 1.39$^{b}$ | 0.52 |

$^{a,b}$ Means with different superscript letters in the same row differ ($P < 0.05$) among the age groups.
[1] The xCT mRNA expression was determined by real-time reverse transcription (RT)-PCR. Values represent means ± pooled SE (standard error).
[2] Orthogonal polynomial contrasts represented a linear pattern ($P < 0.05$) of the age effects.
[3] Orthogonal polynomial contrasts represented a cubic pattern ($P < 0.05$) of the age effects.

in the intestine, although it is believed that xCT is located at the basolateral membrane of the intestine in humans (Bassi et al., 2001).

It is evident that the small intestine is rich with amino acid transporters because the small intestine is the main area for nutrient uptake, and amino acid transporters are highly expressed in the ileum (Gilbert et al., 2007). However, our results indicated that the colon had relatively higher levels of xCT mRNA than the jejunum and ileum. These are consistent with the results from a previous study which showed the expression of colonic xCT (1.216 ± 0.585) was higher than that of duodenal xCT (0.217 ± 0.072) in humans (Fagerberg et al., 2014). One possible explanation for this is that the colon may need to transport more cysteine from the blood in order to carry out synthesis of GSH. Compared with relatively high intracellular concentrations of glutamate and glycine, intracellular cysteine concentrations are very low, and intracellular cysteine availability for GSH synthesis is believed to be the rate-limiting factor (McBean, 2002). Cysteine for intracellular GSH synthesis may come from 2 different mechanisms: 1) cystine can be transported into cells through xCT and then converted into cysteine (Sido et al., 2008); 2) there could be a direct uptake of cysteine through the EAAC1 (Zerangue and Kavanaugh, 1996). A study from Sido et al. (1998) suggested that decreased availability of csyt(e)ine in the distal ileum and colon for synthesis of mucosal GSH may contribute to the pathogenesis of Crohn's disease, an inflammatory bowel disease in the distal ileum and colon in humans. Moreover, the lower gut of broiler chickens (e.g. colon) is the place where most pathogens reside and propagate, which could lead to more infection-associated oxidative stress or inflammation. Likewise, it is hypothesized that the expression of xCT in the colon is crucial for attenuating gut oxidative stress and inflammation in broiler chickens. However, this theory needs to be further investigated.

In the current study, the expression of xCT in intestinal segments in broiler chickens during development was also investigated. Although the xCT mRNA abundance in the jejunum and ileum during development did not show a significant difference, the expression of xCT mRNA in the duodenum changed, following a linear pattern during development. The xCT mRNA abundance in duodenum at the age of 7 d was significantly higher than that at the age of 35 d. There were linear and cubic patterns of changes in the expression of xCT in the colon during development. The colonic xCT mRNA abundance was significantly higher at the age of 14 d when compared with the ages of 28 and 35 d. In this study, abrupt changes of xCT mRNA abundance in the duodenum and colon between the first and second week were found, and according to Nakao et al. (2015), abrupt changes of nutrient transporters in the early ages of broiler chickens is a possible adaptation to the

nutritional changes that occur after hatching. Additionally, Proszkowiec-Weglarz et al. (2018) demonstrated that Ca and P transporters showing increased expression in the short period immediately after hatching may be a result of the transporters corresponding to the change from yolk to feed, which is in line with the results of the present study. Miska and Fetterer (2019) revealed that the expression of diverse nutrient transporters including amino acids transporters, glucose, and fructose transporters in the intestinal segments were altered at an early age (by 14 d of age) in chickens. Furthermore, the expression of proteins connected to nutrient transporting, including fatty acid translocase and cytosolic fatty acid binding protein in birds, tends to change by the first week after hatching (Katongole and March, 1980; Xie et al., 2012). According to Gilbert et al. (2007), some amino acid transporters, including excitatory amino-acid transporter 3 (EAAT3) and neutral amino acid transporter ($B^0AT$), increased linearly, while large neutral amino acid transporter 1 (LAT1), heterodimeric amino acid transporters ($y^+LAT1$), cationic amino acid transporter 1 (CAT1), and CAT2 decreased linearly with age. Correspondingly, the abundance of $y^+LAT1$ changed cubically. These results indicate that the expression of amino acid transporters varies with age and among various organs. A study from Tang et al. (2018) demonstrated that antioxidant enzymes (superoxide dismutase, glutathione peroxidase 1, and glutathione peroxidase 7) and GSH in the small intestine were highly expressed in the first week after hatching, and decreased from 3 d of age. This suggests that the function of superoxide dismutase and glutathione peroxidase is maintaining redox balance after hatching, which helps the rapid development of the broiler chicken intestine, and further supports the results of the present study. Therefore, it can be seen that the developmental changes of chicken xCT in the intestine may be associated with the changes in redox status during development.

## 5. Conclusion

The full-length cDNA fragment encoding xCT was sequenced in our study, and it was found that the coding region cDNA sequence, protein sequence, and protein structure of chicken xCT are similar with other species. Furthermore, it was found that xCT is widely expressed in the different tissues of broiler chickens, and the colon has a higher level of xCT compared with other intestinal segments. We also discovered that xCT expression was altered dramatically in the intestine of broiler chickens, especially during the early stages of development. Further investigation is needed to characterize the functional relevance of xCT activity when oxidative stress and inflammation exists in the intestine of broiler chickens.

## Conflicts of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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