# The organic zinc with moderate chelation strength enhances the expression of related transporters in the jejunum and ileum of broilers

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**ABSTRACT** Our previous study demonstrated that the zinc (**Zn**) proteinate with moderate chelation strength (**Zn-Prot M**) enhanced the Zn absorption in the small intestine partially via increasing the expression of some Zn and amino acid transporters in the duodenum of broilers. However, it remains unknown whether the Zn-Prot M could also regulate the expression of related transporters in the jejunum and ileum of broilers in the above enhancement of Zn absorption. The present study was conducted to investigate the effect of the Zn-Prot M on the expression of related transporters in the jejunum and ileum of broilers compared to the Zn sulfate (**ZnS**). Zinc-deficient broilers (13-d-old) were fed with the Zn-unsupplemented basal diets (control) or the basal diets supplemented with 60 mg Zn/kg as ZnS or Zn-Prot M for 26 d. The results showed that in the jejunum, compared to the control, supplementation of the organic or inorganic Zn increased ($P < 0.05$) mRNA and protein expression of $b^{0,+}$-type amino acid transporter (**rBAT**), Zn transporter 10 (**ZnT10**), and peptide-transporter 1 (**PepT1**) mRNA expression and Zn transporter 7 (**ZnT7**) protein expression on d 28, while y+L-type amino transporter 2 (**y+LAT2**) mRNA and protein expression, and protein expression of ZnT7 and ZnT10 on 28 d and zrt-irt-like protein 3 (**ZIP3**) and zrt-irt-like protein 5 (**ZIP5**) on d 39 were higher ($P < 0.05$) for Zn-Prot M than for ZnS. In the ileum, Zn addition regardless of Zn source up-regulated ($P < 0.05$) mRNA expression of Zn transporter 9 (**ZnT9**) and ZIP3, ZIP5, and y+LAT2 protein expression on d 28, and PepT1 mRNA and protein expression, ZIP3 and y+LAT2 mRNA expression and ZnT10 protein expression on d 39. Furthermore, Zn transporter 4 (**ZnT4**) and ZnT9 mRNA expression and Zn transporter 1 (**ZnT1**) protein expression on d 28, and y+LAT2 mRNA expression and ZnT10 and PepT1 protein expression on d 39 were higher ($P < 0.05$) for Zn-Prot M than for ZnS. It was concluded that the Zn-Prot M enhanced the expression of the ZnT1, ZnT4, ZnT9, ZnT10, ZIP3, ZIP5, y+LAT2, and PepT1 in the jejunum or ileum of broilers compared to the ZnS.

**Key words:** the organic zinc with moderate chelation strength, transporters, jejunum, ileum, broiler

2023 Poultry Science 102:102477
https://doi.org/10.1016/j.psj.2023.102477

## INTRODUCTION

Zinc (**Zn**) is an essential trace element for humans and animals, and it is required for the biological activity of over 300 proteins and enzymes (Vallee and Auld, 1990; Vallee and Falchuk, 1993). Zinc deficiency can lead to growth retardation, hypogonadism, impaired skin-barrier function, delayed wound healing and immune system diseases (Apgar, 1985; Muhamed and Vadstrup, 2014; McClung, 2019). Because of these crucial functions, Zn additives are usually supplemented in diets to prevent Zn deficiencies or support optimal growth. In the poultry industry, the most commonly-used Zn supplements are Zn sulfate ($ZnSO_4.7H_2O$) and Zn oxide which are inorganic sources. However, the availabilities of these inorganic Zn sources are generally low due to its high hydroscopicity and excretion. In recent years, organic Zn sources for dietary supplementation have been popular in livestock and poultry production (Hill et al., 1987, 2014; Yu et al., 2017). Our previous studies have demonstrated that the organic Zn absorption was more effective than that of the inorganic Zn in three small intestinal segments of broilers, and



Received October 16, 2022.
Accepted January 1, 2023.

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increased with increasing chelation strength (defined as the quotient of formation ($Q_f$), which is a quantitative measurement of complex or chelation strength between metal and ligands) (Huang et al., 2009; Yu et al., 2010, 2017). It was also found that the organic Zn source with the moderate $Q_f$ was more bioavailable in broilers than the organic Zn sources with weak or strong $Q_f$ as assessed by the pancreatic metallothionein mRNA expression (Huang et al., 2009, 2013). In addition, possible molecular mechanisms of the absorption of the Zn proteinate with the moderate $Q_f$ (**Zn-Prot M**) in the duodenum of broilers have been elucidated in our previous study (Hu et al., 2022b). However, further studies are necessary to address possible molecular mechanisms of the absorption of the organic Zn-Prot M in the jejunum and ileum of broilers.

It is reported that the Zrt-irt-like proteins (**ZIP**s) and Zn transporters (**ZnT**s) are the proteins primarily involved in Zn transport in mammalian intestinal cells (Ford, 2004). The ZIP transporters mediate the influx of Zn from the intracellular organelles or vesicles into the cytosol, while the ZnT transporters facilitate the efflux of Zn from the cytosol into intracellular organelles or vesicles (Wessels et al., 2017). He et al. (2019) found that the organic Zn significantly increased Zn transporter 1 (**ZnT1**) mRNA expression in the jejunum of broilers challenged with coccidian and C. perfringensin. Our recent study indicated that the organic Zn-Prot M significantly enhanced the Zn absorption in the small intestine partially via up-regulating the expression of the mRNA and/or protein expression of Zn transporter 4 (**ZnT4**), Zn transporter 7 (**ZnT7**), Zn transporter 9 (**ZnT9**), Zrt-irt-like protein 3 (**ZIP3**), and Zrt-irt-like protein 5 (**ZIP5**) in the duodenum of broilers (Hu et al., 2022b). All of the above research efforts have shown that Zn transport proteins might participate in the absorption of Zn as organic Zn sources in the small intestine of broilers. Besides, Lu et al. (2018) found that amino acid transporters L-type amino transporter 1 (**LAT1**) and $B^0$-type amino acid transporter 1 (**$B^0$AT1**) might participate in the absorption of Fe as Fe amino acid chelates in the jejunum or ileum of broilers. Wen et al. (2022) reported that the peptide-transporter 1 (**PepT1**) might affect the transport of organic copper (copper proteinate and copper glycinate) in the jejunum of finishing pigs. Our recent study showed that the enhanced absorption of the organic Zn as the Zn-Prot M in the small intestine of broilers was associated with the increased mRNA and protein expression of y+L-type amino transporter 1 (**y+LAT1**) and $b^{0,+}$-type amino acid transporter (**rBAT**) in the duodenum, thereby suggesting that the y+LAT1 and rBAT in the duodenum of broilers might participate in the absorption of the organic Zn as the Zn-Prot M (Hu et al., 2022b). However, it remains unclear whether the organic Zn-Prot M could also up-regulate the expression of the above-mentioned Zn, amino acid and peptide transporters in the jejunum and ileum during the process of the above enhanced Zn absorption in the small intestine of broilers.

We hypothesized that the organic Zn-Prot M would enhance the expression of related transporters in the jejunum and ileum of broilers. Therefore, the aim of the study was to determine the effect of the supplemental organic Zn as the Zn-Prot M in comparison with the inorganic Zn sulfate (**ZnS**) on gene expression of Zn, amino acid and peptide transporters in the jejunum and ileum of broilers to test the above hypothesis.

## MATERIALS AND METHODS

### Animal Ethics

The use and care of animals complied with the guidelines of the Animal Use Committee of the Chinese Ministry of Agriculture (Beijing, China). The ethics application was approved by the Ethics Committee of the Department of Animal Science and Technology of Yangzhou University.

### Experimental Design and Treatments

A completely randomized design was used in the present study. A total of 3 dietary treatment groups were arranged, including the control with no Zn supplementation, the ZnS ($ZnSO_4.7H_2O$, reagent grade, 22.49% Zn by analysis; Sinopharm Chemical Reagent Co. LTD, Shanghai, China) and the Zn-Prot M (feed grade, $Q_f = 51.6$ and 17.09% Zn by analysis; Alltech Inc., Nicholasville, KY). The Zn-Prot M used in the present study is commercially available, and its Zn and AA composition and concentrations, and $Q_f$ value are all from our previous study (Hu et al., 2022b).

### Animals and Diets

A total of three hundred 1-d-old Arbor Acres commercial male chicks (Jinghai Broiler Livestock Industry Group, Haimen, China) were kept in a thermostatically controlled room equipped with stainless steel cages, feeders and plastic waterers. All broilers were allowed ad libitum access to the experimental diets and tap water containing no detectable Zn. During 1 to 13 d of age, the broilers were fed with a Zn-deficient starch-casein based semi-purified diet (containing 17.69 mg Zn/kg of diet by analysis on an as-fed basis, Table 1) to deplete the body Zn stores, and then fed a Zn-unsupplemented corn-soybean meal basal diets (containing 25.72 and 25.64 mg Zn/kg by analysis, Table 1) and the basal diets supplemented with 60 mg of Zn/kg of diet from one of the above two different Zn sources from d 14 to 39 according to our previous result (Huang et al., 2007b). At 14 d of age, a total of 240 broilers with similar body weights were randomly allotted to 1 of 3 treatment groups with 8 replicate cages (8 chickens/cage) for each treatment in a completely randomized design. The starch-casein diet and corn-soybean meal basal diets were formulated to meet or exceed the nutrient requirements for broilers (NRC, 1994) except for Zn (Table 1). Each Zn source was premixed with

**Table 1.** Composition and nutrient levels of the basal diets for 1- to 39-d-old broilers (as-fed basis).

| Item | D 1−21 | | D 22−39 |
|---|---|---|---|
| | Semi-purified diet (d 1−13) | Corn-soybean meal diet (d 14−21) | Corn-soybean meal diet |
| Ingredients (%) | | | |
| Corn | | 53.82 | 59.16 |
| Soybean meal | | 37.42 | 32.31 |
| Corn starch | 65.70 | | |
| Casein | 25.75 | | |
| Cellulose | 3.40 | | |
| Soybean oil | | 4.70 | 5.00 |
| DL-Met | 0.20 | 0.30 | 0.20 |
| $CaHPO_4{\cdot}2H_2O$[1] | 1.60 | 1.87 | 1.65 |
| $CaCO_3$[1] | 1.60 | 1.14 | 1.05 |
| NaCl[1] | 0.30 | 0.30 | 0.30 |
| Micronutrients[2] | 1.45(containing macrominerals) | 0.25 | 0.18 |
| Cornstarch + Zn[3] | | 0.20 | 0.15 |
| Total | 100.00 | 100.00 | 100.00 |
| Nutrient levels composition | | | |
| ME, Kcal/kg | 3140 | 3021 | 3096 |
| CP[4], % | 22.72 | 21.74 | 19.61 |
| Lys, % | 1.80 | 1.12 | 1.00 |
| Met, % | 0.86 | 0.59 | 0.48 |
| Met+cys, % | 0.96 | 0.90 | 0.76 |
| Ca[4], % | 0.99 | 1.02 | 0.89 |
| Nonphytate P, % | 0.48 | 0.45 | 0.40 |
| Zn[4], mg/kg | 17.69 | 25.72 | 25.64 |

[1]Reagent grade.

[2]Provided per kilogram of diet: for d 1-13 − vitamin A (all-trans retinol acetate), 12000 IU; vitamin $D_3$ (cholecalciferol), 4500 IU; vitamin E (all-rac-α-tocopherol acetate), 33 IU; vitamin $K_3$, 3 mg; vitamin $B_1$, 3 mg; vitamin $B_2$, 9.6 mg; vitamin $B_6$, 4.5 mg; vitamin $B_{12}$, 0.03 mg; pantothenic acid calcium, 15 mg; niacin, 54 mg; folic acid, 1.5 mg; biotin, 0.15 mg; choline, 700 mg; K (KCl), 3000 mg; Mg ($MgSO_4.7H_2O$), 600 mg; Cu ($CuSO_4{\cdot}5H_2O$), 8 mg; Mn ($MnSO_4{\cdot}H_2O$), 110 mg; Fe ($FeSO_4{\cdot}7H_2O$), 40 mg; I ($Ca(IO_3)_2{\cdot}H_2O$), 0.35 mg; Se($Na_2SeO_3$), 0.15mg; for d 14-21 − vitamin A (all-trans retinol acetate), 12000 IU; vitamin D3 (cholecalciferol), 4500 IU; vitamin E (all-rac-α-tocopherol acetate), 33 IU;vitamin $K_3$, 3 mg; vitamin $B_1$, 3 mg; vitamin $B_2$, 9.6 mg; vitamin $B_6$, 4.5 mg; vitamin $B_{12}$, 0.03 mg; Pantothenic acid calcium, 15 mg;Niacin, 54 mg; Folic acid, 1.5 mg; Biotin, 0.15 mg; Choline, 700 mg; Cu ($CuSO_4{\cdot}5H_2O$), 8 mg; Mn ($MnSO_4{\cdot}H_2O$), 110 mg; Fe ($FeSO_4{\cdot}7H_2O$), 40 mg; I ($Ca(IO_3)_2{\cdot}H_2O$), 0.35mg; Se ($Na_2SeO_3$), 0.15 mg; for d 22-39 − vitamin A (all-trans retinol acetate), 8000 IU; vitamin $D_3$ (cholecalciferol), 3000 IU; vitamin E (all-rac-α-tocopherol acetate), 22 IU; vitamin $K_3$, 2 mg; vitamin $B_1$, 2 mg; vitamin $B_2$, 6.4 mg; vitamin $B_6$, 3 mg; vitamin $B_{12}$, 0.02 mg; pantothenic acid calcium, 10 mg; niacin, 36 mg; folic acid, 1 mg; biotin, 0.1 mg; choline, 500 mg; Cu ($CuSO_4{\cdot}5H_2O$), 8 mg; Mn ($MnSO_4{\cdot}H_2O$), 80 mg; Fe ($FeSO_4{\cdot}7H_2O$), 30 mg; I ($Ca(IO_3)_2{\cdot}H_2O$), 0.35 mg; Se($Na_2SeO_3$), 0.15 mg.

[3]Zn supplements added in place of equivalent weights of cornstarch.

[4]Determined values.

corn starch to the same weight, and then was added to the respective experimental diet on the basis of its analyzed Zn concentration. Variable small amounts of DL-methionine or L-lysine monohydrochloride were added to respective experimental diets according to the amounts of methionine and lysine from the supplemental organic Zn proteinate so as to keep all 3 treatment diets containing equal levels of methionine and lysine. The analyzed Zn concentrations in diets are listed in Table 2. Mortality was recorded daily, and body weight and feed intake per cage were measured at 28 and 39 d of age to calculate the average daily feed intake (**ADFI**), average daily gain (**ADG**), feed conversion rate (**FCR**, feed/gain), and mortality during d 14 to 28 and 29 to 39.

**Table 2.** Analyzed Zn concentrations in diets of broilers from 14 to 39 d of age.[1]

| Zn source | Added Zn, mg/kg | Analyzed Zn contents (d 14−21), mg/kg | Analyzed Zn contents (d 22−39), mg/kg |
|---|---|---|---|
| Control | 0 | 25.72 | 25.64 |
| ZnS[2] | 60 | 84.91 | 85.27 |
| Zn-Prot M[3] | 60 | 86.93 | 84.29 |

[1]Values of analyzed Zn contents are based on triplicate determinations.

[2]ZnS = Zn sulfate.

[3]Zn-Prot M = Zn proteinate with moderate chelation strength ($Q_f = 51.6$).

## Sample Collections and Preparations

The feed ingredient and diet samples were taken for analyses of CP, Ca, and Zn contents. The tap water was collected for analysis of Zn content. At both 28 and 39 d of age, 3 birds per cage for each of the three treatments were selected according to average body weight of the cage and anaesthetized by intravenous injections of propofol (20 mg/kg body weight) via a wing vein. The birds were killed by cervical dislocation. Then, the mucosa of jejunum and ileum were scraped with an ice-cold microscope slide as described by Hu et al. (2022b), then immediately frozen in liquid nitrogen, and stored at −80°C for analyses of mRNA and protein expression of the Zn, amino acid and peptide transporters. The samples from 3 birds in each replicate cage were pooled into 1 sample in equal ratios before analyses.

## Measurements of Zn, Ca, CP Concentrations

The concentrations of Zn in Zn sources, water and diets, and Ca and CP in feed ingredients or diets were determined as described previously (Hu et al., 2022b).

## Quantitative Real-Time PCR

Total RNA in the jejunum or ileum mucosa was extracted using TRIzol reagent (Life Technologies,

**Table 3.** Primer sequences for real-time PCR amplification.

| Genes | GenBank ID | Primer sequences | Product length (bp) |
|---|---|---|---|
| *ZnT1* | XM_421021.5 | F: 5′-AGAGCCTGGGTTTGGATTCG-3′<br>R: 5′-AGCCCATGCATGAACACTGA-3′ | 229 |
| *ZnT4* | XM_423325 | F: 5′-GCCATCTTGACGGACGTAGT-3′<br>R: 5′-CAAGGTACACCAGGACACCC-3′ | 190 |
| *ZnT5* | NM_001031419.2 | F: 5′-ATGGAGGAAAAGTACAGCAGCC-3′<br>R: 5′-TCAGAAACTTGGCGAAGCAC-3′ | 118 |
| *ZnT7* | NM_001008788.1 | F: 5′-TGCTGCCCCTCTCCATTAAG-3′<br>R: 5′-AGAGGTTGCGGGATGTCTTG-3′ | 114 |
| *ZnT9* | XM_015285471.1 | F: 5′-ACATGTTTTTCCGTGCAGCC-3′<br>R: 5′-CGGAACACAACCTTTACCAGC-3′ | 265 |
| *ZnT10* | XM_015283897.1 | F: 5′-GAGCTGTAGAGATGGGTCGC-3′<br>R: 5′-ACACCGAGCAAACCGATGAT-3′ | 184 |
| *ZIP3* | XM_015299966.2 | F: 5′-CATACATCCAGGAGGCAGAGG-3′<br>R: 5′-CCTGGATGATCTTGACGGGG-3′ | 246 |
| *ZIP5* | XM_025145573.1 | F: 5′-CCAAGATGAAACGCACGCAA-3′<br>R: 5′-TAAGCTGCGACCAAGTCCTG-3′ | 284 |
| *$B^0AT1$* | XM_419056.6 | F: 5′-CTTGGGTGAGGTAGGTGGGA -3′<br>R: 5′-GATGCGGGTGCTCTCATGTA-3′ | 167 |
| *LAT1* | NM_001030579.2 | F: 5′-GGAAAGGCCCATCAAGGTGA-3′<br>R: 5′-ACGATTCTTGGGGAACCAC-3′ | 248 |
| *y+LAT2* | XM_001231336.4 | F: 5′-ATCTTGCGCCTGAGGGAAGG-3′<br>R: 5′-CGGCTCTGAACTCCAATCTGT-3′ | 296 |
| *rBAT* | XM_004935370.3 | F: 5′-CCTAGGAGGAGAGGCACGAA-3′<br>R: 5′-TCCTGCATAGGGCTGCAATG-3′ | 223 |
| *EAAT3* | XM_424930.6 | F: 5′-CGTCCAGGCCTGTTTTCAAC-3′<br>R: 5′-TCGGAATACATGCCCACGAT-3′ | 171 |
| *PepT1* | NM_204365.1 | F: 5′-AAAACAGGTTTCGGCATCGC-3′<br>R: 5′-CTGCTGGTCAAAAAGTGCCC-3′ | 167 |
| *β-actin* | NM_205518.1 | F: 5′-CGGTACCAATTACTGGTGTTA-GATG-3′<br>R: 5′-GCCTTCATTCACATCTATCACTGG-3′ | 163 |
| *GAPDH* | NM_204305.1 | F: 5′-CTTTGGCATTGTGGAGGGTC-3′<br>R: 5′-ACGCTGGGATGATGTTCTGG-3′ | 128 |

*ZnT1*, zinc transporter 1; *ZnT4*, zinc transporter 4; *ZnT5*, zinc transporter 5; *ZnT7*, zinc transporter 7; *ZnT9*, zinc transporter 9; *ZIP3*, Zrt-irt-like protein 3; *ZIP5*, Zrt-irt-like protein 5; *$B^0AT1$*, B-0-system neutral amino acid co-transporter; *LAT1*, L-type amino acid transporter 1; *y+LAT2*, y+L-type amino acid transporter 2; *rBAT*, $b^{0,+}$-type amino acid transporter; *EAAT3*, excitatory amino acid transporter 3; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; F, forward; R, reverse.

Carlsbad, CA) according to the manufacturer's instructions, then treated with RNase free DNase and reverse-transcribed to cDNA using HiScript II Q RT SuperMix (R223-01, Vazyme, Nanjing, Jiangsu, China) following the manufacturer's protocol. Real-time quantitative PCR was performed on applied biosystems quantstudio 3D digital PCR System (Applied Biosystems, Foster City, CA) using SYBR-Green PCR Master Mix. All primers (Table 3) were synthesized by Qingke Biotechnology Co., Ltd. (Nanjing, China). The mRNA expression levels of the target genes were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), and the geometric mean of internal reference genes (*β-actin* and *GAPDH*) was used to normalize the expression of the target genes.

## Total Protein Extraction and Western Blotting

Frozen jejunum or ileum mucosa samples were homogenized in ice-cold RIPA lysis buffer (R0010, Solarbio, Beijing, China) as previously described (Hu et al., 2022a). The homogenates were centrifuged at 8,000 × g for 5 min at 4°C, and the supernatants were collected for western blotting analysis. The primary antibodies and dilution rates were as follows: ZnT1, ab214356, 1:1000 (Abcam, Cambridge, UK); ZnT4, BA3484, 1:1000 (Boster, Wuhan, China); Zn transporter 5 (ZnT5), 25604-1-AP, 1:1000 (Proteintech, Chicago); ZnT7, 13966-1-AP, 1:1000 (Proteintech, Chicago, IL); ZnT9, BS62531, 1:1000 (Bioworld, Minneapolis, MN); Zn transporter 10 (**ZnT10**), PA5-49188, 1:1000 (Invitrogen, CA); ZIP3, ab254868, 1:500 (Abcam, Cambridge, UK); ZIP5, ab105194, 1:1000 (Abcam, Cambridge, UK); $B^0AT1$, ab180516, 1:1000 (Abcam, Cambridge, UK); LAT1, DF8065, 1:500 (Affinity Biosciences, Melbourne, Australia); y+LAT2, 13823-1-AP, 1:1000 (Proteintech, Chicago); rBAT, DF7379, 1:1000 (Affinity Biosciences, Melbourne, Australia) and PepT1, A03672-1, 1:500 (Boster, Wuhan, China). Images were captured by the chemiluminescence image scanner (Tanon, Shanghai, China) and the band density was analyzed with Tanon Gis 1D software (Tanon, Shanghai, China). The density of each protein band was normalized by β-tubulin, the internal control.

## Statistical Analyses

All data were analyzed by one-way ANOVA using the general linear model procedures of SAS 9.2 (SAS Institute Inc., Cary, NC) (Liu et al., 2020). Differences among means were tested by the LSD method. The replicate cage was the experimental unit, and the statistical significance was set at $P < 0.05$.

## RESULTS

### Growth Performance and Mortality

The Zn source did not affect ($P > 0.14$) the ADG, ADFI, FCR, and mortality of broilers during d 14 to 28 and d 29 to 39 of age as described in our previous study (Hu et al., 2022b).

### mRNA Expression Levels of Zn, Amino Acid and Peptide Transporters in the Jejunum

The Zn source did not affect ($P > 0.08$) the mRNA expression levels of ZnT1, ZnT4, ZnT5, ZnT7, ZnT9, ZIP3, ZIP5, $B^0AT1$, LAT1, and EAAT3, but influenced ($P < 0.05$) the mRNA expression levels of ZnT10, y+LAT2, rBAT, and PepT1 in the jejunum of broilers at 28 d of age (Table 4). Compared with the control, two Zn sources up-regulated ($P < 0.05$) the mRNA expression levels of ZnT10, rBAT, and PepT1 in the jejunum of broilers at 28 d of age with no difference ($P > 0.05$) between two Zn sources. Moreover, the y+LAT2 mRNA expression in the jejunum of broilers at 28 d of age was higher ($P < 0.05$) for the Zn-Prot M than for the control and ZnS with no difference ($P > 0.05$) between the control and ZnS.

The Zn source had no effect ($P > 0.13$) on the mRNA expression levels of all of ZnT1, ZnT4, ZnT5, ZnT7, ZnT9, ZnT10, ZIP3, ZIP5, $B^0AT1$, LAT1, y+LAT2, rBAT, EAAT3, and PepT1 in the jejunum of broilers at 39 d of age (Table 4).

### mRNA Expression Levels of Zn, Amino Acid and Peptide Transporters in the Ileum

The Zn source did not affect ($P > 0.13$) mRNA expression levels of ZnT1, ZnT5, ZnT7, ZnT10, ZIP5, $B^0AT1$, LAT1, y+LAT2, rBAT, EAAT3, and PepT1, but influenced ($P < 0.05$) mRNA expression levels of ZnT4, ZnT9, and ZIP3 in the ileum of broilers at 28 d of age (Table 5). Compared with the control, two Zn sources increased ($P < 0.05$) mRNA expression levels of ZnT9 and ZIP3 in the ileum of broilers on d 28. Compared with the ZnS, the Zn-Prot M enhanced ($P < 0.05$) ZnT4 and ZnT9 mRNA expression levels in the ileum of broilers on d 28.

The Zn source had no effect ($P > 0.13$) on mRNA expression levels of ZnT1, ZnT4, ZnT5, ZnT7, ZnT9, ZnT10, ZIP5, $B^0AT1$, LAT1, rBAT, and EAAT3 in the ileum of broilers at 39 d of age (Table 5), while mRNA expression levels of ZIP3, y+LAT2 and PepT1 in the ileum of broilers on d 39 were affected ($P < 0.05$) by Zn source. Compared with the control, two Zn sources up-regulated ($P < 0.05$) mRNA expression levels of ZIP3, y+LAT2, and PepT1 in the ileum of broilers on d 39. Compared with the ZnS, the Zn-Prot M increased ($P < 0.05$) the y+LAT2 mRNA expression. However, no differences ($P > 0.05$) were observed for ZIP3 and PepT1 mRNA expression levels between the Zn-Prot M and ZnS.

### Protein Expression Levels of Zn, Amino Acid and Peptide Transporters in the Jejunum

The Zn source had no effect ($P > 0.07$) on protein expression levels of ZnT1, ZnT4, ZnT5, ZnT9, ZIP3, ZIP5, $B^0AT1$, LAT1, and PepT1 in the jejunum of broilers on d 28 (Table 6 and Figure 1). However, protein expression levels of ZnT7, ZnT10, y+LAT2, and rBAT in the jejunum of broilers on d 28 were affected ($P < 0.05$) by Zn source. Compared with the control, two Zn sources increased ($P < 0.05$) protein expression levels of ZnT7 and rBAT in the jejunum of broilers at 28 d of

**Table 4.** Effect of dietary Zn source on the mRNA expression levels of Zn, amino acid and small peptide transporters in the jejunum of broilers at 28 and 39 d of age.[1]

| | D 28 | | | | | D 39 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Genes (RQ)[2] | Control | ZnS | Zn-Prot M | SEM | *P*-value | Control | ZnS | Zn-Prot M | SEM | *P*-value |
| ZnT1 mRNA | 1.04 | 1.04 | 0.85 | 0.05 | 0.253 | 1.02 | 1.04 | 0.94 | 0.04 | 0.617 |
| ZnT4 mRNA | 1.03 | 1.12 | 1.14 | 0.04 | 0.405 | 1.02 | 0.99 | 0.85 | 0.04 | 0.165 |
| ZnT5 mRNA | 1.01 | 1.14 | 1.04 | 0.03 | 0.204 | 1.06 | 0.96 | 0.93 | 0.07 | 0.739 |
| ZnT7 mRNA | 1.01 | 1.12 | 1.05 | 0.04 | 0.552 | 1.04 | 0.96 | 0.99 | 0.05 | 0.822 |
| ZnT9 mRNA | 1.01 | 1.08 | 1.02 | 0.07 | 0.912 | 1.04 | 0.87 | 1.03 | 0.07 | 0.516 |
| ZnT10 mRNA | 1.02[b] | 1.39[a] | 1.34[a] | 0.06 | 0.035 | 1.02 | 0.92 | 0.75 | 0.06 | 0.136 |
| ZIP3 mRNA | 1.03 | 1.02 | 0.89 | 0.05 | 0.477 | 1.02 | 1.00 | 1.14 | 0.04 | 0.366 |
| ZIP5 mRNA | 1.03 | 0.91 | 1.07 | 0.04 | 0.355 | 1.05 | 1.04 | 0.85 | 0.07 | 0.430 |
| $B^0AT1$ mRNA | 1.09 | 1.13 | 1.50 | 0.08 | 0.081 | 1.07 | 1.02 | 1.00 | 0.08 | 0.940 |
| LAT1 mRNA | 1.05 | 1.27 | 1.24 | 0.10 | 0.597 | 1.08 | 1.18 | 0.99 | 0.08 | 0.593 |
| y+LAT2 mRNA | 1.03[b] | 1.02[b] | 1.55[a] | 0.09 | 0.018 | 1.06 | 0.99 | 0.79 | 0.07 | 0.216 |
| rBAT mRNA | 1.02[b] | 1.26[a] | 1.23[a] | 0.04 | 0.022 | 1.01 | 1.11 | 1.30 | 0.11 | 0.559 |
| EAAT3 mRNA | 1.02 | 1.14 | 1.14 | 0.04 | 0.460 | 1.02 | 0.97 | 0.87 | 0.06 | 0.576 |
| PepT1 mRNA | 1.02[b] | 1.28[a] | 1.30[a] | 0.05 | 0.033 | 1.05 | 1.25 | 0.95 | 0.08 | 0.293 |

*ZnT1*, zinc transporter 1; *ZnT4*, zinc transporter 4; *ZnT5*, zinc transporter 5; *ZnT7*, zinc transporter 7; *ZnT9*, zinc transporter 9; *ZIP3*, Zrt-irt-like protein 3; *ZIP5*, Zrt-irt-like protein 5; *$B^0AT1$*, B-0-system neutral amino acid co-transporter; *LAT1*, L-type amino acid transporter 1; *y+LAT2*, y+ L-type amino acid transporter 2; *rBAT*, $b^{0,+}$-type amino acid transporter; *EAAT3*, excitatory amino acid transporter 3; *PepT1*, peptide-transporter 1; ZnS, Zn sulfate; Zn-Prot M, Zn proteinate with moderate chelation strength ($Q_f = 51.6$).

[a,b,c]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Values are the means of 7/8 replicate cages of 3 birds per replicate cage (n = 7/8).

[2]The mRNA levels were calculated as the relative quantities (RQ) of the target gene mRNA to the geometric mean of *β-actin* and *GAPDH* mRNA using the $2^{-\Delta\Delta CT}$ method.

**Table 5.** Effect of dietary Zn source on the mRNA expression levels of Zn, amino acid and small peptide transporters in the ileum of broilers at 28 and 39 d of age.[1]

| | D 28 | | | | | D 39 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Genes (RQ)[2] | Control | ZnS | Zn-Prot M | SEM | *P*-value | Control | ZnS | Zn-Prot M | SEM | *P*-value |
| ZnT1 mRNA | 1.02 | 1.01 | 1.11 | 0.07 | 0.823 | 1.03 | 1.21 | 0.97 | 0.06 | 0.222 |
| ZnT4 mRNA | 1.00[b] | 1.05[b] | 1.21[a] | 0.03 | 0.033 | 1.03 | 1.14 | 0.95 | 0.05 | 0.310 |
| ZnT5 mRNA | 1.02 | 1.10 | 0.98 | 0.05 | 0.622 | 1.01 | 1.05 | 0.90 | 0.04 | 0.361 |
| ZnT7 mRNA | 1.10 | 1.21 | 1.26 | 0.11 | 0.837 | 1.03 | 1.21 | 0.94 | 0.06 | 0.139 |
| ZnT9 mRNA | 1.06[c] | 1.65[b] | 2.29[a] | 0.16 | 0.002 | 1.04 | 1.21 | 0.95 | 0.07 | 0.353 |
| ZnT10 mRNA | 1.17 | 1.22 | 1.12 | 0.09 | 0.904 | 1.07 | 1.49 | 1.20 | 0.10 | 0.239 |
| ZIP3 mRNA | 1.01[b] | 1.42[a] | 1.37[a] | 0.06 | 0.009 | 1.07[b] | 1.81[a] | 1.93[a] | 0.13 | 0.005 |
| ZIP5 mRNA | 1.06 | 1.45 | 0.98 | 0.10 | 0.135 | 1.05 | 0.81 | 0.93 | 0.08 | 0.495 |
| $B^0AT1$ mRNA | 1.10 | 1.45 | 1.12 | 0.14 | 0.541 | 1.12 | 1.56 | 1.37 | 0.10 | 0.240 |
| LAT1 mRNA | 1.13 | 1.37 | 1.21 | 0.13 | 0.701 | 1.02 | 1.18 | 0.99 | 0.06 | 0.331 |
| y+LAT2 mRNA | 1.05 | 1.05 | 0.84 | 0.06 | 0.317 | 1.04[c] | 1.86[b] | 2.92[a] | 0.23 | 0.0006 |
| rBAT mRNA | 1.04 | 1.26 | 1.34 | 0.06 | 0.135 | 1.02 | 1.18 | 0.99 | 0.06 | 0.331 |
| EAAT3 mRNA | 1.06 | 1.12 | 1.03 | 0.06 | 0.846 | 1.02 | 1.13 | 0.96 | 0.05 | 0.366 |
| PepT1 mRNA | 1.05 | 1.33 | 1.23 | 0.07 | 0.244 | 1.05[b] | 1.61[a] | 1.48[a] | 0.09 | 0.026 |

*ZnT1*, zinc transporter 1; *ZnT4*, zinc transporter 4; *ZnT5*, zinc transporter 5; *ZnT7*, zinc transporter 7; *ZnT9*, zinc transporter 9; *ZIP3*, Zrt-irt-like protein 3; *ZIP5*, Zrt-irt-like protein 5; *$B^0AT1$*, B-0-system neutral amino acid co-transporter; *LAT1*, L-type amino acid transporter 1; *y+LAT2*, y+ L-type amino acid transporter 2; *rBAT*, $b^{0,+}$-type amino acid transporter; *EAAT3*, excitatory amino acid transporter 3; *PepT1*, peptide-transporter 1; ZnS, Zn sulfate; Zn-Prot M, Zn proteinate with moderate chelation strength ($Q_f = 51.6$).

[a,b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Values are the means of 7/8 replicate cages of 3 birds per replicate cage (n = 7/8).

[2]The mRNA levels were calculated as the relative quantities (RQ) of the target gene mRNA to the geometric mean of *β-actin* and *GAPDH* mRNA using the $2^{-\Delta\Delta CT}$ method.

**Table 6.** Effect of dietary Zn source on the protein expression levels of Zn, amino acid and small peptide transporters in the jejunum of broilers at 28 and 39 d of age.[1]

| | D 28 | | | | | D 39 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Genes (RQ)[2] | Control | ZnS | Zn-Prot M | SEM | *P*-value | Control | ZnS | Zn-Prot M | SEM | *P*-value |
| ZnT1 | 0.56 | 0.57 | 0.55 | 0.01 | 0.889 | 0.24 | 0.22 | 0.19 | 0.01 | 0.229 |
| ZnT4 | 0.29 | 0.28 | 0.28 | 0.01 | 0.907 | 0.42 | 0.37 | 0.34 | 0.02 | 0.279 |
| ZnT5 | 0.58 | 0.64 | 0.69 | 0.03 | 0.357 | 0.36 | 0.35 | 0.32 | 0.01 | 0.409 |
| ZnT7 | 0.26[c] | 0.35[b] | 0.43[a] | 0.02 | 0.0003 | 0.24 | 0.26 | 0.23 | 0.01 | 0.364 |
| ZnT9 | 0.32 | 0.38 | 0.33 | 0.01 | 0.074 | 0.47 | 0.44 | 0.49 | 0.01 | 0.320 |
| ZnT10 | 0.36[b] | 0.31[b] | 0.48[a] | 0.03 | 0.022 | 0.37 | 0.28 | 0.34 | 0.02 | 0.189 |
| ZIP3 | 0.64 | 0.52 | 0.65 | 0.04 | 0.379 | 0.53[b] | 0.51[b] | 0.68[a] | 0.03 | 0.024 |
| ZIP5 | 0.42 | 0.41 | 0.46 | 0.01 | 0.297 | 0.29[b] | 0.23[b] | 0.44[a] | 0.03 | 0.012 |
| $B^0AT1$ | 0.31 | 0.47 | 0.43 | 0.06 | 0.581 | 0.58 | 0.34 | 0.38 | 0.05 | 0.080 |
| LAT1 | 0.30 | 0.32 | 0.29 | 0.01 | 0.607 | 0.21 | 0.24 | 0.20 | 0.01 | 0.080 |
| y+LAT2 | 0.75[b] | 0.71[b] | 0.89[a] | 0.03 | 0.027 | 0.27 | 0.29 | 0.33 | 0.02 | 0.442 |
| rBAT | 0.26[b] | 0.30[a] | 0.30[a] | 0.01 | 0.040 | 0.54 | 0.64 | 0.58 | 0.03 | 0.371 |
| PepT1 | 0.50 | 0.43 | 0.41 | 0.02 | 0.083 | 0.42 | 0.43 | 0.40 | 0.01 | 0.602 |

ZnT1, zinc transporter 1; ZnT4, zinc transporter 4; ZnT5, zinc transporter 5; ZnT7, zinc transporter 7; ZnT9, zinc transporter 9; ZIP3, zrt-irt-like protein 3; ZIP5, zrt-irt-like protein 5; $B^0AT1$, B-0-system neutral amino acid co-transporter; LAT1, L-type amino acid transporter 1; y+LAT2, y+L-type amino acid transporter 2; rBAT, $b^{0,+}$-type amino acid transporter; PepT1, peptide-transporter 1; ZnS, Zn sulfate; Zn-Prot M, Zn proteinate with moderate chelation strength ($Q_f = 51.6$).

[a,b,c]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Values are the means of 7/8 replicate cages of 3 birds per replicate cage (n = 7/8).

[2]The protein expressions were calculated as the relative quantities (RQ) of the target gene protein to the β-tubulin protein.

age. Compared with the ZnS, the Zn-Prot M increased ($P < 0.05$) ZnT7, ZnT10, and y+LAT2 protein expression levels in the jejunum of broilers on d 28. And no differences ($P > 0.05$) were observed for ZnT10 and y +LAT2 protein expression levels between the control and ZnS.

The Zn source did not affect ($P > 0.07$) protein expression levels of ZnT1, ZnT4, ZnT5, ZnT7, ZnT9, ZnT10, $B^0AT1$, LAT1, y+LAT2, rBAT, and PepT1, but had an effect ($P < 0.05$) on protein expression levels of ZIP3 and ZIP5 in the jejunum of broilers on d 39 (Table 6 and Figure 1). Moreover, protein expression levels of ZIP3 and ZIP5 in the jejunum of broilers on d 39 were higher ($P < 0.05$) for the Zn-Prot M than for the control and ZnS with no differences ($P > 0.05$) between the control and ZnS.

## Protein Expression Levels of Zn, Amino Acid and Peptide Transporters in the Ileum

The Zn source did not affect ($P > 0.05$) protein expression levels of ZnT4, ZnT5, ZnT7, ZnT9, ZnT10, ZIP3, $B^0AT1$, LAT1, rBAT, and PepT1, but had an impact ($P < 0.05$) on protein expression levels of ZnT1, ZIP5 and y+LAT2 in the ileum of broilers on d 28 (Table 7 and Figure 2). Compared with the control, two Zn sources increased ($P < 0.05$) protein expression levels of ZIP5 and y+LAT2 in the ileum of broilers at 28 d of age with no differences ($P > 0.05$) between two Zn sources. Furthermore, the ZnT1 protein expression in the ileum of broilers on d 28 was higher ($P < 0.05$) for the Zn-Prot M than for the control and ZnS with no difference ($P > 0.05$) between the control and ZnS.

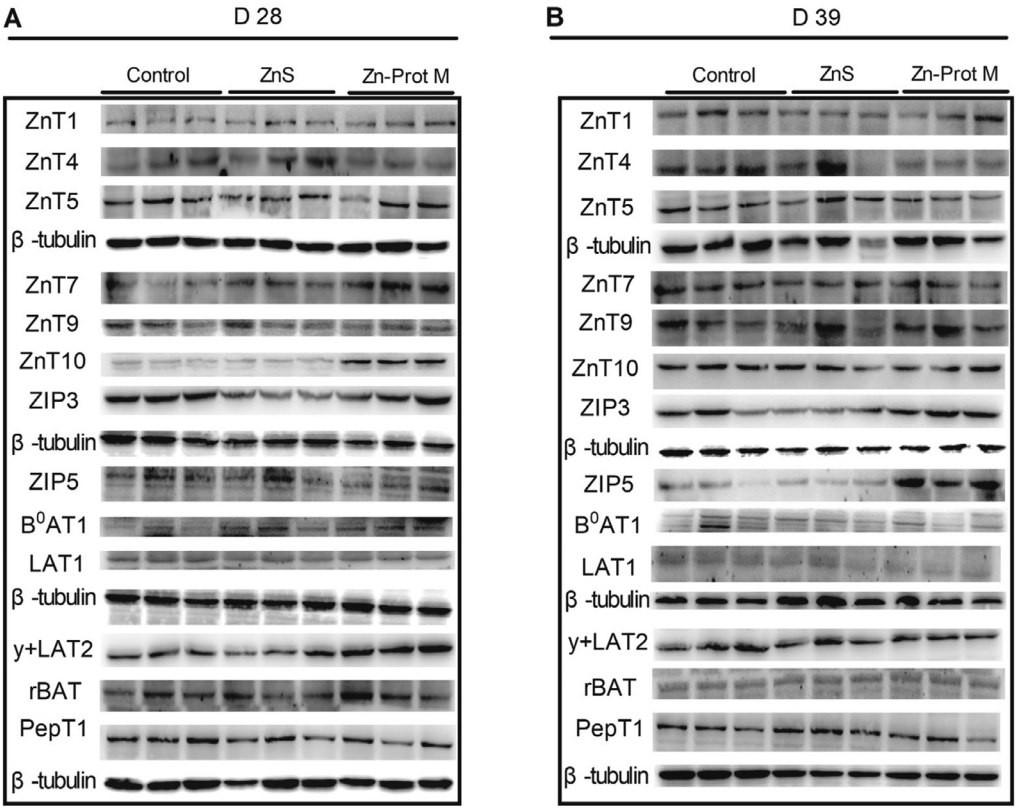


**Figure 1.** Representative immunoblots of ZnT1, ZnT4, ZnT5, ZnT7, ZnT9, ZIP3, ZIP5, $B^0AT1$, LAT1, y+LAT2, rBAT and PepT1 in the jejunum of broilers at 28 (A) and 39 d (B) of age. ZnT1, zinc transporter 1; ZnT4, zinc transporter 4; ZnT5, zinc transporter 5; ZnT7, zinc transporter 7; ZnT9, zinc transporter 9; ZIP3, Zrt-irt-like protein 3; ZIP5, Zrt-irt-like protein 5; $B^0AT1$, B-0-system neutral amino acid co-transporter; LAT1, L-type amino acid transporter 1; y+LAT2, y+L-type amino acid transporter 2; rBAT, $b^{0,+}$-type amino acid transporter; PepT1, peptide-transporter 1; ZnS, Zn sulfate; Zn-Prot M, Zn proteinate with moderate chelation strength ($Q_f = 51.6$).

The Zn source had no effect ($P > 0.07$) on protein expression levels of ZnT1, ZnT4, ZnT5, ZnT7, ZnT9, ZIP3, ZIP5, $B^0AT1$, LAT1, y+LAT2, and rBAT in the ileum of broilers on d 39 (Table 7 and Figure 2). However, protein expression levels of ZnT10 and PepT1 in the ileum of broilers on d 39 were affected ($P < 0.05$) by Zn source. Compared with the control, two Zn sources up-regulated ($P < 0.05$) ZnT10 and PepT1 protein expression levels in the ileum of broilers on d 39. Compared with the ZnS, the Zn-Prot M enhanced ($P < 0.05$) ZnT10 and PepT1 protein expression levels in the ileum of broilers on d 39.

**Table 7.** Effect of dietary Zn source on the protein expression levels of Zn, amino acid and small peptide transporters in the ileum of broilers at 28 and 39 d of age.[1]

| | D 28 | | | | | D 39 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Genes (RQ)[2] | Control | ZnS | Zn-Prot M | SEM | *P*-value | Control | ZnS | Zn-Prot M | SEM | *P*-value |
| ZnT1 | 0.34[b] | 0.41[b] | 0.62[a] | 0.04 | 0.003 | 0.52 | 0.39 | 0.42 | 0.03 | 0.095 |
| ZnT4 | 0.77 | 0.94 | 0.82 | 0.04 | 0.213 | 0.50 | 0.49 | 0.45 | 0.01 | 0.438 |
| ZnT5 | 0.41 | 0.49 | 0.48 | 0.02 | 0.299 | 0.46 | 0.49 | 0.46 | 0.02 | 0.867 |
| ZnT7 | 0.38 | 0.46 | 0.46 | 0.02 | 0.149 | 0.27 | 0.29 | 0.23 | 0.02 | 0.456 |
| ZnT9 | 0.41 | 0.46 | 0.50 | 0.02 | 0.056 | 0.30 | 0.28 | 0.26 | 0.01 | 0.255 |
| ZnT10 | 0.57 | 0.48 | 0.61 | 0.03 | 0.202 | 0.24[c] | 0.33[b] | 0.42[a] | 0.02 | 0.001 |
| ZIP3 | 0.74 | 0.61 | 0.80 | 0.04 | 0.182 | 0.70 | 0.77 | 0.66 | 0.03 | 0.233 |
| ZIP5 | 0.33[b] | 0.72[a] | 0.70[a] | 0.06 | 0.011 | 0.38 | 0.41 | 0.40 | 0.02 | 0.681 |
| $B^0AT1$ | 0.33 | 0.31 | 0.41 | 0.05 | 0.701 | 0.81 | 0.65 | 0.62 | 0.04 | 0.151 |
| LAT1 | 0.22 | 0.23 | 0.22 | 0.01 | 0.971 | 0.27 | 0.28 | 0.21 | 0.02 | 0.200 |
| y+LAT2 | 0.35[b] | 0.52[a] | 0.58[a] | 0.03 | 0.0006 | 0.48 | 0.66 | 0.58 | 0.03 | 0.075 |
| rBAT | 0.54 | 0.65 | 0.71 | 0.03 | 0.115 | 0.28 | 0.31 | 0.26 | 0.02 | 0.519 |
| PepT1 | 0.70 | 0.98 | 0.98 | 0.06 | 0.100 | 0.53[c] | 0.65[b] | 0.74[a] | 0.03 | 0.004 |

ZnT1, zinc transporter 1; ZnT4, zinc transporter 4; ZnT5, zinc transporter 5; ZnT7, zinc transporter 7; ZnT9, zinc transporter 9; ZIP3, zrt-irt-like protein 3; ZIP5, zrt-irt-like protein 5; $B^0AT1$, B-0-system neutral amino acid co-transporter; LAT1, L-type amino acid transporter 1; y+LAT2, y+L-type amino acid transporter 2; rBAT, $b^{0,+}$-type amino acid transporter; PepT1, peptide-transporter 1; ZnS, Zn sulfate; Zn-Prot M, Zn proteinate with moderate chelation strength ($Q_f = 51.6$).

[a,b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Values are the means of 7/8 replicate cages of 3 birds per replicate cage (n = 7/8).

[2]The protein expressions were calculated as the relative quantities (RQ) of the target gene protein to the $\beta$-tubulin protein.

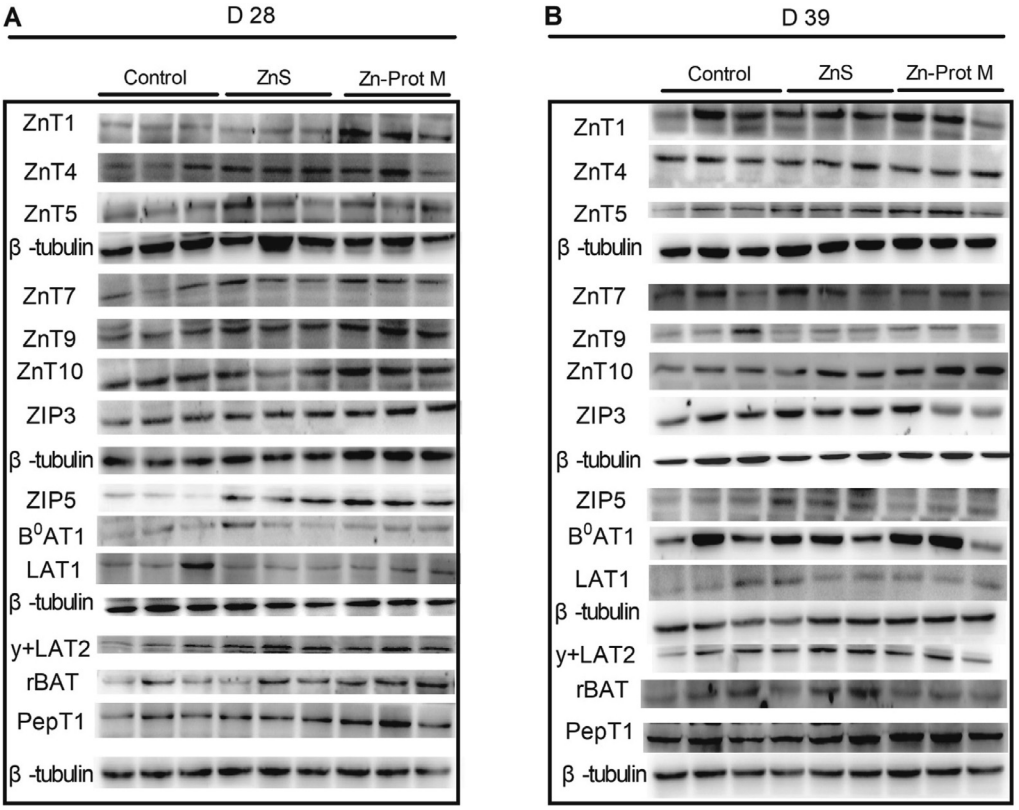


**Figure 2.** Representative immunoblots of ZnT1, ZnT4, ZnT5, ZnT7, ZnT9, ZIP3, ZIP5, $B^0AT1$, LAT1, y+LAT2, rBAT and PepT1 in the ileum of broilers at 28 (A) and 39 d (B) of age. ZnT1, zinc transporter 1; ZnT4, zinc transporter 4; ZnT5, zinc transporter 5; ZnT7, zinc transporter 7; ZnT9, zinc transporter 9; ZIP3, Zrt-irt-like protein 3; ZIP5, Zrt-irt-like protein 5; $B^0AT1$, B-0-system neutral amino acid co-transporter; LAT1, L-type amino acid transporter 1; y+LAT2, y+L-type amino acid transporter 2; rBAT, $b^{0,+}$-type amino acid transporter; PepT1, peptide-transporter 1; ZnS, Zn sulfate; Zn-Prot M, Zn proteinate with moderate chelation strength ($Q_f = 51.6$).

## DISCUSSION

Our previous study demonstrated that the Zn-Prot M could enhance the Zn absorption in the small intestine partially via up-regulating the expression of the ZnT4, ZnT7, ZnT9, ZIP3, ZIP5, y+LAT2, and rBAT in the duodenum of broilers (Hu et al., 2022b). In the present study, we revealed that the Zn-Prot M also significantly enhanced mRNA and/or protein expression of the ZnT7, ZnT10, ZIP3, ZIP5, and y+LAT2 in the jejunum and mRNA or protein expression of ZnT1, ZnT4, ZnT9, ZnT10, y+LAT2, and PepT1 in the ileum of broilers, suggesting that the above transporters might participate in the absorption of Zn as the organic Zn-Prot M in the jejunum and ileum of broilers, which has supported our hypothesis.

The possible reasons why the Zn-Prot M enhanced the expression of the above transporters in the jejunum or ileum of broilers compared to the ZnS might be that the Zn-Prot M could resist to interferences from some factors (such as low pH in stomach and phytate and fiber in the gut) in the gastrointestinal tract, and more Zn-Prot M arrive at the brush-border (the absorptive surface) of the small intestine and then be transported in the intact molecular form, which could promote the mRNA and protein expression levels of the above transporters. The Zn transporters, including ZIPs and ZnTs, are pivotal in maintaining Zn homeostasis by regulating the influx and efflux of Zn, respectively (Kambe et al., 2021). To date, 14 members of ZIPs (ZIP1−ZIP14) and 10 members of ZnTs (ZnT1−ZnT10) have been identified in mammals (Kambe et al., 2004). The ZIPs translocate Zn from the lumen of organelles or the extracellular space into the cytoplasm, whereas ZnTs export Zn into extracellular fluid/organelles/vesicles from the cytoplasm. He et al. (2019) reported that the organic Zn up-regulated the mRNA expression of ZnT1 in the jejunum during broiler challenge with Eimeria maximaand Clostridium perfringens. Our previous study revealed that the Zn-Prot M could enhance mRNA and/or protein expression of ZnT4, ZnT7, ZnT9, ZIP3, and ZIP5 in the duodenum of broilers (Hu et al., 2022b). Similarly, in the present study, the Zn-Prot M also significantly up-regulated ZnT7 protein expression on d 28 and ZIP3 and ZIP5 protein expression on d 39 in the jejunum of broilers, and ZnT4 and ZnT9 mRNA expression and ZnT1 protein expression on d 28 in the ileum of broilers. In mammalian intestinal epithelial cells, the ZnT1 is located in the baso-lateral membrane and involved in releasing Zn into the portal vein (Jou et al., 2009). Andrews et al. (2004) reported that the ZnT1 knockout mice showed early embryonic lethality, indicating that the ZnT1 is essential for the absorption of Zn in the early fetus development. The ZnT7 is predominantly

expressed in the absorptive epithelium of the small intestine and plays an important role in transporting the cytoplasmic Zn into the Golgi apparatus of the cell for Zn storage (Kirschke and Huang, 2003). Huang et al. (2007a) demonstrated that the ZnT7-deficient mice had reduced Zn absorption in the intestine, indicating that the ZnT7 has essential functions in dietary Zn absorption. The ZIP3 is localized in the apical cell membrane and involved in Zn acquisition by cells of the body (Dufner-Beattie et al., 2006). Dufner-Beattie et al. (2006) observed that the ZIP3 knockout mice were more sensitive to the teratogenic effects of dietary Zn deficiency during pregnancy, suggesting that the ZIP3 play a role in Zn uptake. The ZIP5 is localized in the baso-lateral membrane of epithelial cells and involved in enterocyte sensing of body Zn status through serosal-tomucosal transport of Zn (Geiser et al., 2013).

Therefore, our findings along with those of others have clearly indicated that ZnT1, ZnT7, ZIP3, and ZIP5 might play an important role in the Zn absorption in the jejunum and ileum of broilers and other animals. In addition, in the present study, Zn addition regardless of Zn source significantly increased ZIP10 mRNA and protein expression in the jejunum of broilers at 28 d of age. Moreover, ZnT10 protein expression in the jejunum of broilers at 28 d of age were significantly higher for the organic Zn than for the inorganic Zn, which is in line with a previous report that the organic Zn could increase the ZnT10 expression in the cecal tonsil of broilers (He et al., 2019). As the last member of the ZnT family, ZnT10 was highly expressed in the small intestine and involved in the transport of Zn into vesicles (Patrushev et al., 2012). Therefore, based on our present findings along with our previous report (Hu et al., 2022b), it could be elucidated why the Zn-Prot M exhibited higher Zn absorption in the small intestine of broilers compared to the ZnS.

Previous studies showed that amino acid transporters might participate in the absorption of organic mineral complex in mammals (Wapnir et al., 1983; Gao et al., 2014). Our recent study demonstrated that the amino acid transporters y+LAT2 and rBAT might participate in the absorption of Zn as the Zn-Prot M in the duodenum of broilers (Hu et al., 2022b). Lu et al. (2018) reported that the amino acid transporters LAT1 and $B^0AT1$ might be involved in the absorption of Fe as Fe amino acid chelates in in situ ligated jejunum or ileum loops of broilers. Consistent with previous results, in the present study, the y+LAT2 protein expression on d 28 in the jejunum of broilers was significantly higher for the Zn-Prot M than for the ZnS. In addition, we also found that the protein expression of peptide transporter PepT1 in the ileum of broilers at 28 d of age was significantly higher for the Zn-Prot M than for the ZnS. Therefore, the above results suggested that the above amino acid and peptide transporters might be involved in the absorption of Zn from the organic Zn-Prot M in the jejunum or ileum of broilers.

It is noted that the discordant mRNA and protein expression of some of Zn, amino acid and peptide transporters in the jejunum or ileum were observed in the present study. For instance, the mRNA expression levels of the ZnT4 and ZnT9 on d 28, and the y+LAT2 on d 39 were significantly increased in the ileum of broilers fed with the diet supplemented with the organic Zn-Prot M compared to the inorganic ZnS, whereas no alterations were detected for their corresponding protein expression levels. The protein expression levels of the ZnT7 and ZnT10 on d 28 and the ZIP3 and ZIP5 in the jejunum on d 39, and the ZnT1 on d 28 and the ZnT10 and PepT1 in the ileum on d 39 were significantly up-regulated by the addition of the organic Zn-Prot M compared to the inorganic ZnS, yet no alterations were detected for their mRNA abundances. In fact, the post-transcriptional processes may play an important role in these complex responses. The mismatches between the mRNA and protein expression of the above transporters might be due to protein translation, post-translational modification and degradation. Furthermore, the exact reasons on the inconsistent mRNA or protein expression levels of the above transporters between two Zn sources at the two time points remain unclear and might be related to the time-specific expression of these transporters. Theoretically, it would be possible that if the transporters are involved in Zn uptake, then the up-regulation of transporters should be enhanced during a Zn deficiency, and alternatively are a lagged response from the pretrial period during which diets with no supplemental Zn were fed. However, in our present study, all of these three treatments were compared with each other at the same time and under the same conditions. If the above effect existed, then it should have existed in all of these three treatments, and especially as for the control treatment of the Zn deficiency, it should have been contained in its results. Therefore, the differential responses of transporters at the various growth stages could reflect the action of either ZnS or Zn-Prot M, but not a recovery from a Zn deficiency.

In conclusion, the Zn-Prot M enhanced the expression of the ZnT1, ZnT4, ZnT9, ZnT10, ZIP3, ZIP5, y+LAT2, and PepT1 in the jejunum or ileum of broilers compared to the ZnS, suggesting that the above transporters might participate in the absorption of Zn as the organic Zn-Prot M in the jejunum and ileum of broilers. Further studies need be conducted using the primary cultured small intestinal epithelial cell model of broilers as well as gene over-expression and RNA silencing tools to verify the roles of the above-mentioned transporters in enhancing the absorption of the organic Zn as the Zn-Prot M in the small intestine of broilers.

## ACKNOWLEDGMENTS

This work was supported by the Jiangsu Shuang Chuang Ren Cai Program (JSSCRC2021541), the

National Natural Science Foundation of China (31972583), the Key International Cooperation Program of the National Natural Science Foundation of China (32120103011), the Jiangsu Shuang Chuang Tuan Dui Program (JSSCTD202147), the Initiation Funds of Yangzhou University for Distinguished Scientists, the General Project of Natural Science Research in Colleges and Universities of Jiangsu Province (20KJB230002) and the Innovation and Entrepreneurship Training Program for College Students (X20210653).

## DISCLOSURES

None of the authors have any conflicts of interest to declare.

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