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# Kinetics Absorption Characteristics of Ferrous Glycinate in SD Rats and Its Impact on the Relevant Transport Protein

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**Abstract** Ferrous glycinate (Fe-Gly) maintains high bioavailability in animals, but its exact absorption mechanism is still unknown. Here, we studied on the absorption kinetics of ferrous glycinate and its impact on the relevant transport protein in Sprague-Dawley (SD) rats. A total of 72 SD rats (male, BW  $100 \pm 6.25$  g) were randomly allotted to three treatments. These treatments were perfused with 1 mL of normal saline, ferrous sulfate ( $\text{FeSO}_4$ ), and ferrous glycinate (71.35 mg/L as iron) separately. Four rats were selected from each treatment for collection of blood from the tails at certain times (15, 30, 45, 60, 75, 90, 120, 240, and 360 min) after gavage. Moreover, other six rats selected from each treatment were slaughtered for sampling after gavage at 2, 4, and 6 h to evaluate the expression of intestinal transport protein. Pharmacokinetic parameters of iron were determined by one-compartmental analysis. Compared with  $\text{FeSO}_4$ , the peak plasma concentration of iron ( $C_{\max}$ ) is higher in the rats given gavage with Fe-Gly ( $P < 0.05$ ). Four hours after gavage with Fe-Gly, the expression of divalent metal transporter 1 (DMT1) in the duodenum is significantly decreased ( $P < 0.05$ ), but the expression of ferroportin 1 (Fpn1) is significantly increased ( $P < 0.05$ ). This study indicates that Fe-Gly as iron sources can be absorbed more and utilized faster than  $\text{FeSO}_4$ , and they had different effects on the expression of intestinal transport protein.

**Keywords** Ferrous glycinate · Absorption kinetics · Transport protein

## Introduction

Iron is an essential trace element for animal growth and health, which is involved in numerous vital biologic processes, including oxygen transport, DNA biosynthesis, energy metabolism, and oxidative phosphorylation [1, 2]. For lack of a controlled excretion mechanism, iron homeostasis in the body is mainly regulated by its absorption and transport through duodenum epithelia [3]. However, iron deficiency is a prevalent nutritional problem for humans and animals [4]. It has a long history of supplementation iron to diets to prevent and treat iron deficiency in animals [5–7], but different iron sources varied in their bioavailability. It was reported that metal chelated with amino acid or protein has good bioavailability in animals [8–10]. Studies have showed that ferrous glycinate (Fe-Gly) was more effective in animal production compared with ferrous sulfate ( $\text{FeSO}_4$ ) [11, 12], and the fact that Fe-Gly could be easily absorbed and maintained high bioavailability in humans in spite of the presence of iron absorption inhibitory factors such as phytic acid [13, 14] also made it an effective iron reinforcing agent.

However, the data as to the absorption process of Fe-Gly and studies about the mechanism of its high bioavailability is limited. Therefore, the main objective of the present research was to study on the kinetics absorption characteristics of Fe-Gly and its impact on the relevant intestinal transport proteins in Sprague-Dawley (SD) rats.

## Materials and Methods

### Animals and Experimental Design

The experiment was conducted in the Laboratory Animal Center of Zhejiang University. Seventy-two SD rats (male,

**Table 1** Composition and nutrient levels of basal diets

Ingredients	Percentage
Corn starch	39.75
Casein lactic	20.00
Granular sugar	10.00
Dextrin	13.20
Solka Floc-40	5.00
Mineral mix	3.50
Vitamin mix	1.00
L-cystine	0.30
Choline bitartrate	0.25
Soy oil	7.00

Supplied mineral mix contained (based on total diet) calcium, 0.52 %; phosphorus, 0.20 %; potassium, 0.38 %; sodium, 0.11 %; magnesium, 0.05 %; iron, 52.02 ppm; zinc, 36.76 ppm; manganese, 11.33 ppm; copper, 6.73 ppm; cobalt, 0.02 ppm; and iodine, 0.21 ppm. Supplied vitamin mix contained (based on total diet) vitamin A, 4.00 IU/g; vitamin D<sub>3</sub>, 1.00 IU/g; alpha-tocopherol, 75.00 IU/kg; thiamine, 5.00 ppm; riboflavin, 6.00 ppm; niacin, 30.00 ppm; pantothenic acid, 15.00 ppm; choline, 1,000.00 ppm; pyridoxine, 6.00 ppm; folic acid, 2.00 ppm; biotin, 0.20 ppm; vitamin B<sub>12</sub>, 25.00 µg/kg; and vitamin K, 0.90 ppm

After 3 days of pre-feeding, all rats were fasted for 12 h before the experiment. Then, these treatments were perfused with 1 mL of normal saline, FeSO<sub>4</sub>, and Fe-Gly (71.35 mg/L as iron), respectively. All the animal studies were approved by the Animal Ethics Committee of Zhejiang University.

SD rats were reared in a clean standard room. Their diet (Table 1) was formulated according to the international standards of experimental animals, AIG-93G (purchased from Slack Experimental Animals LLC, Shanghai). The room temperature was maintained at about 23–25 °C, and the relative humidity was 40–60 %. Twelve hours of alternating lighting was used for the SD rats. All rats were housed in a stainless steel cage and given deionized water to avoid uptaking extra iron except from the diet.

### Sample Collection

After gavage, four rats were selected from each treatment for collection of blood from the tails at certain times (15, 30, 45, 60, 75, 90, 120, 240, and 360 min). Serum was separated by

centrifugation at 3,000×g for 10 min at 4 °C and stored at –80 °C until kinetics absorption study.

Moreover, other six rats selected from each treatment were slaughtered for sampling after gavage at 2, 4, and 6 h respectively. The rats were sacrificed by cervical dislocation, and about 3 cm of the duodenum just proximal to the pyloric sphincter was removed, washed with normal saline, packed with sterile and RNase-free silver paper, and after, rapidly frozen in liquid nitrogen and stored at –80 °C for later analyses of divalent metal transporter 1 (DMT1) and ferroportin 1 (Fpn1).

### Pharmacokinetic Analysis

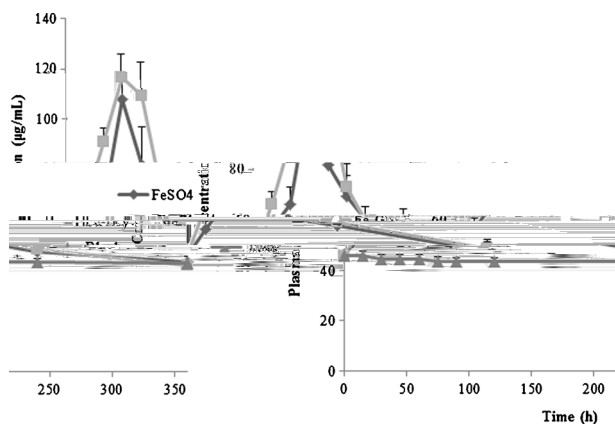
Concentration of iron in the serum was analyzed with an atomic absorption spectrophotometer (AA6501, Shimadzu Ltd., Kyoto, Japan) [15]. Plasma concentration-time plots according to the data of serum iron in different time points were made. Pharmacokinetic parameters of iron were calculated using PKSolver 1.0 [16] by one-compartmental analysis. The involved pharmacokinetic parameters are listed as follows: *t*<sub>1/2</sub>, terminal half-life; V/F, apparent volume of distribution; CL/F, clearance; *C*<sub>max</sub>, the observed peak plasma concentration; *T*<sub>max</sub>, observed time to achieve *C*<sub>max</sub>; AUC, the area under the plasma concentration-time curve; and MRT, the mean residence time.

### Quantification Real-time PCR Analysis

Expression of duodenum-specific transcripts was analyzed by RT-PCR [17, 18]. Primers for DMT1, Fpn1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed with Primer Express 2.0, Beacon designer, and GenBank (Sangon Biotech, Table 2). Total RNA from mouse duodenum was extracted by Trizol Reagent kits (Jiancheng Bioengineering Institute, Nanjing, China) [19]. RNA was then reverse transcribed into cDNA with the M-MLV Reverse Transcriptase RNase H- (Promega). Real-time PCR was conducted using the iQ<sup>TM</sup>5 real-time multiplexing system (Bio-Rad Inc., Hercules, CA, USA). The fluorescence quantitative PCR mixtures contained 1 µL of cDNA, 12.5 µL 2× SYBR green PCR master mix, 1 µL each of the forward and reverse

**Table 2** Choosing primers for quantitative PCR

Item	No. in GenBank	Sequences	Length	Annealing temperature
GAPDH	NM_017008	5'-GGTGGACCTCATGGCCTACAT-3' 5'-GCCTCTCTTGCTCTCAGTATCCT-3'	88	61
DMT1	AY336075	5'-GTGGTGGCTGGGGCTTCATTG-3' 5'-GGGTCTTGGCCTGTCCGTTCA-3'	88	63
Fpn1	AF394785	5'-CTGGTGGAACTCTACGGAAACG-3' 5'-GGGACGTCTGGGCCACTTTAA-3'	139	61



**Fig. 1** Mean plasma iron concentration versus time after gavage with different iron sources to SD rats. Error bars represent standard deviation of the mean ( $n=4$ )

primers, and 9.5  $\mu$ L of RNase-free deionized water in a final volume of 25  $\mu$ L. This standard PCR protocol was carried out at 15 s at 95  $^{\circ}$ C and 30 s at 60  $^{\circ}$ C for 40 cycles, and then thermal denaturing step was done in order to generate the dissociation curves to verify amplification specificity. Results were normalized to GAPDH. The relative quantification was performed using Relative Expression Software Tool-384 (Bio-Rad Inc., Hercules, CA, USA) which is based on the calculation of  $2^{-\Delta\Delta Ct}$  value.

**Western Blotting Analysis**

The proteins were extracted by T-PER Tissue Extraction Reagent (Pierce, USA) from rat intestinal tissues, and then proteins were quantitated by BCA Quantification Kit (Beyotime Institute of Biotechnology, Shanghai, China). Tissue lysates (total protein of 60  $\mu$ g) were separated by 10 % SDS-PAGE and transferred to a PVAF membrane. Membranes were

**Table 3** Pharmacokinetic parameters after gavage with different iron sources to the rats

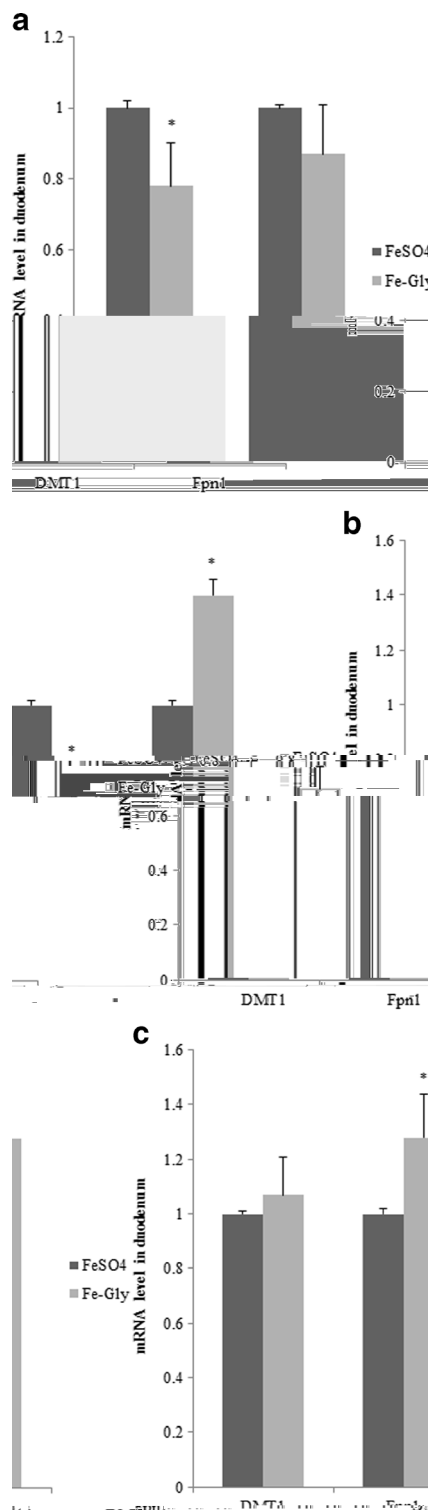
Pharmacokinetic parameters	Unit	FeSO <sub>4</sub>	Fe-Gly
$t_{1/2}$	h	0.493	0.426
V/F	L	4.24	2.86
CL/F	L/h	5.68	4.42
$C_{max}$	mg/L	35.54	52.76 <sup>a</sup>
$T_{max}$	h	0.73	0.63
AUC	mg/(L h)	70.25	89.27
MRT	h	1.458	1.261

The values are the mean

$t_{1/2}$  terminal half-life, V/F apparent volume of distribution, CL/F clearance,  $C_{ma}$  the observed peak plasma concentration,  $T_{ma}$  observed time to achieve  $C_{max}$ , AUC the area under the plasma concentration-time curve, MRT the mean residence time

<sup>a</sup> Significantly different from FeSO<sub>4</sub>

blocked with 5 % nonfat milk in T-TBS buffer at room temperature for 1 h. Thereafter, the membranes were immunoblotted with primary antibodies (Santa Cruz



**Fig. 2** The mRNA expression of DMT1 and Fpn1 in the duodenum. a–c Representations of 2, 4, and 6 h after gavage, respectively. Values are presented as mean $\pm$ SD ( $n=6$ ). \* $P<0.05$ , significant difference of mean values between the two groups

Biotechnology, Inc., USA) overnight at 4 °C, followed by incubation with goat anti-mouse IgG (Pierce, USA) for 1 h at room temperature. Blots were visualized by SuperSignal® West Dura Extended Duration Substrate (Pierce, USA), and the protein expression levels were quantified by densitometric scan.

### Statistical Analysis

The data were analyzed by one-way ANOVA of SPSS 16.0. Multiple comparisons were made by least significant difference (LSD). Values were expressed as means ± standard deviation (SD).  $P$  value < 0.05 was considered to be statistically significant.

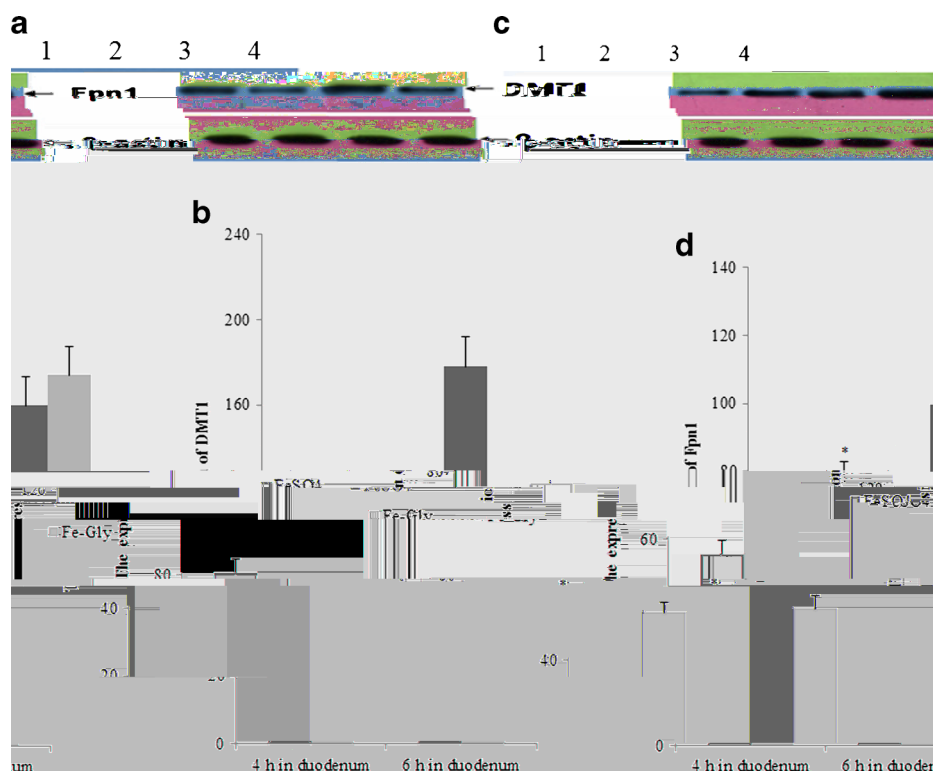
### Results

As shown in Fig. 1, the plasma concentration-time profiles are very similar between two iron source treatments; both curves reached the peak in 50 min, but the Fe-Gly group maintained higher iron levels on each time point especially in the 0- to 100-min period compared with the FeSO<sub>4</sub> group. Absorption

was rapid as indicated by the occurrence of mean peak plasma concentrations in less than 0.8 h within different iron source treatments (Table 3). There was no significant difference in the AUC values between the two iron sources, but the AUC of Fe-Gly was enhanced by 27.07 % compared with that of FeSO<sub>4</sub>. The V/F values were 32.55 % lower and the CL/F values was 22.18 % lower in the Fe-Gly group. Compared with that of FeSO<sub>4</sub>, the  $C_{max}$  of Fe-Gly was increased ( $P < 0.05$ ), but the MRT values of Fe-Gly was 13.51 % lower than that of FeSO<sub>4</sub>.

Different iron sources have different effects on the mRNA expression of transport proteins in the duodenum (Fig. 2). Compared with that in the FeSO<sub>4</sub> group, the mRNA level of DMT1 in the Fe-Gly group was lower in 2 and 4 h ( $P < 0.05$ ), but the difference vanished in 6 h. The Fpn1 mRNA of Fe-Gly had a slight decrease in 2 h after gavage, but higher Fpn1 mRNA was observed in the next 4 h compared with that of FeSO<sub>4</sub> ( $P < 0.05$ ).

The protein expressions of DMT1 and Fpn1 in the duodenum were shown in Fig. 3. After gavage, the expression of DMT1 in the Fe-Gly group was lower than that in the FeSO<sub>4</sub> group in 4 and 6 h ( $P < 0.05$ ). The expression of Fpn1 in the Fe-Gly group was enhanced in 4 h compared with that in the FeSO<sub>4</sub> ( $P < 0.05$ ), whereas the expression level of Fpn1 between the two groups is similar in 6 h ( $P > 0.05$ ).



**Fig. 3** a Western blotting analysis of DMT1 expression in the duodenum: lane 1, DMT1 expression of the FeSO<sub>4</sub> group in the duodenum, 4 h after gavage; lane 2, DMT1 expression of the Fe-Gly group in the duodenum, 4 h after gavage; lane 3, DMT1 expression of the FeSO<sub>4</sub> group in the duodenum, 6 h after gavage; lane 4, DMT1 expression of the Fe-Gly group in the duodenum, 6 h after gavage. b The results of DMT1 expression by densitometric scan. c Western blotting analysis of Fpn1

expression in the duodenum: lane 1, Fpn1 expression of the FeSO<sub>4</sub> group in the duodenum, 4 h after gavage; lane 2, Fpn1 expression of the Fe-Gly group in the duodenum, 4 h after gavage; lane 3, Fpn1 expression of the FeSO<sub>4</sub> group in the duodenum, 6 h after gavage; lane 4, Fpn1 expression of the Fe-Gly group in the duodenum, 6 h after gavage. d The results of Fpn1 expression by densitometric scan

## Discussion

Many studies have shown that amino acid-chelated iron was better absorbed than inorganic iron such as ferrous sulfate [20–22]. In the present research, we established rat intragastric administration model to compare the intestinal absorption characteristics of ferrous sulfate and ferrous glycinate. The assay was liner in the concentration range of 0–140  $\mu\text{g/mL}$  with the correlation coefficients  $R^2 > 0.999$ , and it was suitable for the later plasma pharmacokinetics study on iron [23–25]. The absorption curves of two iron sources were similar, but higher plasma iron levels were observed on each time point especially from the 0- to 100-min period in the Fe-Gly group compared with those in the  $\text{FeSO}_4$  group. It can be inferred that ferrous glycinate indeed maintained better absorption efficiency than ferrous sulfate, which was confirmed by the pharmacokinetic parameters. The area under the plasma concentration-time curve (AUC) was used to evaluate the total absorption of drugs in the body, and the mean residence time (MRT) reflected the utilization of drugs in the body. The enhanced AUC values and decreased MRT values in the Fe-Gly group indicated that ferrous glycinate was better absorbed and utilized faster than ferrous sulfate. Thus, iron sources from ferrous glycinate exhibited better bioavailability compared with ferrous sulfate.

DMT1 and Fpn1 were very important intestinal membrane transport proteins for iron absorption. Distributed in the brush border of duodenal villi and crypts, DMT1 is responsible for transporting iron across the apical membrane into the enterocyte [26, 27]. As for Fpn1, which are abundantly expressed on the basolateral membrane of polarized enterocytes in the duodenum, is the only known iron exporter acting as a basolateral iron pump [28, 29]. An increased expression level of DMT1 and Fpn1 has been observed in patients with iron deficiency [30, 31], whereas the expression of DMT1 is decreased after high oral iron intakes [32]. Similar results were reported in various cell culture models [33, 34]. Duodenum is the main iron absorption site in the body [35], and the expression levels of DMT1 and Fpn1 in the duodenum have been shown to be regulated in response to iron status [3, 36]. Therefore, the paper focused on the expression of DMT1 and Fpn1 in the duodenum. In the present study, lower mRNA abundance of DMT1 was observed in the Fe-Gly group during 2 and 4 h, which was consistent with the protein expression results by Western blotting analysis. It indicated that Fe-Gly is easier to be absorbed, for the increased cell iron can reduce DMT1 levels [37]. There were no significant differences in Fpn1 mRNA level in 2 h after gavage, but Fpn1 mRNA level of Fe-Gly was increased in 4 and 6 h compared with that of  $\text{FeSO}_4$ . The protein expression of Fpn1 in Fe-Gly was significantly

increased in 4 h, but the high expression levels were not sustained in 6 h after gavage. The Fpn1 protein expression was not consistent with the mRNA expression; a part of the reason may be the regulation of Fpn1 at a transcriptional level [26]. The results also revealed that ferrous glycinate is more effective in the transportation because of the relatively high Fpn1 levels. The reasons for the different effects of two iron sources on the relevant transporters warrant further study.

## Conclusions

In summary, the present study indicated that ferrous glycinate is more effective in absorption and utilization than ferrous sulfate in SD rats. The expression of iron-related transporters also differed between the  $\text{FeSO}_4$  and Fe-Gly groups. As for the relationship of the absorption rate and different intestinal transporters' expression between two iron sources, it remains to be further researched.

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## References

1. Hentze MW, Muckenthaler MU, Andrews NC (2004) Balancing acts: molecular control of mammalian iron metabolism. *Cell* 117(3):285–297
2. Andrews NC (2000) Intestinal iron absorption: current concepts circa 2000. *Dig Liver Dis* 32(1):56–61
3. Núñez MT, Tapia V, Rojas A, Aguirre P, Gómez F, Nualart F (2010) Iron supply determines apical/basolateral membrane distribution of intestinal iron transporters DMT1 and ferroportin 1. *Am J Physiol Cell Physiol* 298(3):C477–C485
4. World Health Organization (1999) Nutrition for health and development: progress and prospects on the eve of the 21st century. WHO, Geneva
5. Veum TL, Bollinger DW, Ellersieck M, Halley JT (1995) Proteinated trace minerals and condensed fish protein digest in weanling pig diets. *J Anim Sci* 73(Suppl 1):308
6. Bovell-Benjamin AC, Viteri FE, Allen LH (2000) Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. *Am J Clin Nutr* 71(6):1563–1569
7. Kegley EB, Spears JW, Flowers WL, Schoenherr WD (2002) Iron methionine as a source of iron for the neonatal pig. *Nutr Res* 22(10):1209–1217
8. Creech BL, Spears JW, Flowers WL, Hill GM, Lloyd KE, Armstrong TA, Engle TE (2004) Effect of dietary trace mineral concentration and source (inorganic vs. chelated) on performance, mineral status, and fecal mineral excretion in pigs from weaning through finishing. *J Anim Sci* 82(7):2140–2147

9. Feng J, Ma WQ, Niu HH, Wu XM, Wang Y, Feng J (2010) Effects of zinc glycine chelate on growth, hematological, and immunological characteristics in broilers. *Biol Trace Elem Res* 133(2):203–211
10. Ma WQ, Niu HH, Feng J, Wang Y, Feng J (2011) Effects of zinc glycine chelate on oxidative stress, contents of trace elements, and intestinal morphology in broilers. *Biol Trace Elem Res* 142(3):546–556
11. Ma WQ, Sun H, Zhou Y, Wu J, Feng J (2012) Effects of iron glycine chelate on growth, tissue mineral concentrations, fecal mineral excretion, and liver antioxidant enzyme activities in broilers. *Biol Trace Elem Res* 149(2):204–211
12. Fang CL, Zhuo Z, Fang SL, Yue M, Feng J (2013) Iron sources on iron status and gene expression of iron related transporters in iron-deficient piglets. *Anim Feed Sci Technol* 182:121–125
13. Layrisse M, Garcia-Casal MN, Solano L, Barón MA, Arguello F, Llovera D et al (2000) Iron bioavailability in humans from breakfasts enriched with iron bis-glycine chelate, phytates and polyphenols. *J Nutr* 130(9):2195–2199
14. Pineda O, Ashmead HD (2001) Effectiveness of treatment of iron-deficiency anemia in infants and young children with ferrous bis-glycinate chelate. *Nutr* 17(5):381–384
15. Rodgerson DO, Helfer RE (1966) Determination of iron in serum or plasma by atomic absorption spectrophotometry. *Clin Chem* 12(6):338–349
16. Yong Z (2007) A data analysis program in pharmacokinetics base on Microsoft Excel—development and validation of PKSllover 1.0. *J Math Med* 1, 026
17. Design RTPP (2005) Facile means for quantifying microRNA expression by real-time PCR. *Biotechniques* 39(4):519–525
18. Dupic F, Fruchon S, Bensaid M, Loreal O, Brissot P, Borot N et al (2002) Duodenal mRNA expression of iron related genes in response to iron loading and iron deficiency in four strains of mice. *Gut* 51(5):648–653
19. Rio DC, Ares M, Hannon GJ, Nilsen TW (2010). Purification of RNA using TRIzol (TRI reagent). *Cold Spring Harb Protoc*, 2010(6):pdb-prot5439 doi: [10.1101/pdb.prot5439](https://doi.org/10.1101/pdb.prot5439)
20. Ammerman CB, Baker DH, Lewis AJ (1995) Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. Academic, San Diego
21. Allen LH, Bovell-Benjamin AC, Viteri F (1998) Ferrous bis- and ferric tris-glycinates as iron fortificants for whole maize: bioavailability and regulation by iron status. *FASEB J* 12:A821
22. Langini S, Carbone N, Galdi M et al (1988) Ferric glycinate iron bioavailability for rats, as determined by extrinsic radioisotopic labeling of infant formulas. *Nutr Rep Int* 38:729–735
23. Aghazadeh-Habashi A, Sattari S, Pasutto F, Jamali F (2002) Single dose pharmacokinetics and bioavailability of glucosamine in the rat. *J Pharm Pharm Sci* 5(2):181–184
24. Daud AI, Xu C, Hwu WJ, Urbas P, Andrews S, Papadopoulos NE et al (2011) Pharmacokinetic/pharmacodynamic analysis of adjuvant pegylated interferon  $\alpha$ -2b in patients with resected high-risk melanoma. *Cancer Chemother Pharmacol* 67(3):657–666
25. Zhang Y, Zhao D, Xu J, Xu C, Dong C, Liu Q et al (2013) Effects of dietary factors on the pharmacokinetics of  $^{58}\text{Fe}$ -labeled Hemin after oral administration in normal rats and the iron-deficient rats. *Biol Trace Elem Res* 1–8
26. Zoller H, Theurl I, Koch R, Kaser A, Weiss G (2002) Mechanisms of iron mediated regulation of the duodenal iron transporters divalent metal transporter 1 and ferroportin 1. *Blood Cells Mol Dis* 29(3):488–497
27. Foot NJ, Dalton HE, Shearwin-Whyatt LM, Dorstyn L, Tan SS, Yang B, Kumar S (2008) Regulation of the divalent metal ion transporter DMT1 and iron homeostasis by a ubiquitin-dependent mechanism involving Ndfips and WWP2. *Blood* 112(10):4268–4275
28. Abboud S, Haile DJ (2000) A novel mammalian iron-regulated protein involved in intracellular iron metabolism. *J Biol Chem* 275(26):19906–19912
29. Mackenzie EL, Iwasaki K, Tsuji Y (2008) Intracellular iron transport and storage: from molecular mechanisms to health implications. *Antioxid Redox Signal* 10(6):997–1030
30. Rolfs A, Bonkovsky HL, Kohlroser JG, McNeal K, Sharma A, Berger UV, Hediger MA (2002) Intestinal expression of genes involved in iron absorption in humans. *Am J Physiol Gastrointest Liver Physiol* 282(4):G598–G607
31. Zoller H, Koch RO, Theurl I, Obrist P, Pietrangelo A, Montosi G et al (2001) Expression of the duodenal iron transporters divalent-metal transporter 1 and ferroportin 1 in iron deficiency and iron overload. *Gastroenterology* 120(6):1412–1419
32. Oates PS, Trinder D, Morgan EH (2000) Gastrointestinal function, divalent metal transporter-1 expression and intestinal iron absorption. *Pflugers Arch* 440(3):496–502
33. Tandy S, Williams M, Leggett A, Lopez-Jimenez M, Dedes M, Ramesh B et al (2000) Nramp2 expression is associated with pH-dependent iron uptake across the apical membrane of human intestinal Caco-2 cells. *J Biol Chem* 275(2):1023–1029
34. Arredondo M, Orellana A, Garate MA, Nunez MT (1997) Intracellular iron regulates iron absorption and IRP activity in intestinal epithelial (Caco-2) cells. *Am J Physiol Gastrointest Liver Physiol* 273(2):G275–G280
35. van Campen DR, Mitchell EA (1965) Absorption of  $\text{Cu}^{64}$ ,  $\text{Zn}^{65}$ ,  $\text{Mo}^{99}$ , and  $\text{Fe}^{59}$  from ligated segments of the rat gastrointestinal tract. *J Nutr* 86(2):120–124
36. Pantopoulos K, Porwal SK, Tartakoff A, Devireddy L (2012) Mechanisms of mammalian iron homeostasis. *Biochem* 51(29):5705–5724
37. Mena NP, Esparza A, Tapia V, Valdés P, Núñez MT (2008) Hepcidin inhibits apical iron uptake in intestinal cells. *Am J Physiol Gastrointest Liver Physiol* 294(1):G192–G198