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Original Research Article

Effect of different sources and levels of iron in the diet of sows on iron status in neonatal pigs

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ABSTRACT

This study was conducted to determine the effects of maternal dietary supplementation of ferrous glycine chelate (Fe-Gly) and ferrous sulfate monohydrate ($FeSO_4 \cdot H_2O$) on the relative organ weight, tissue iron contents, red blood cells (RBC), hemoglobin concentration (HGB) and hematocrit (HCT) in blood, as well as ferritin (Fn), serum iron (SI), and total iron binding capacity (TIBC) in serum of newborn piglets. Forty-five sows (Landrace × Large white, mean parity 3 to 4, no significant differences in BW) were randomly allotted to 9 treatments ($n = 5$ sows/treatment): control (basal diet with no Fe supplementation), the basal diet supplemented with 50, 80, 110 or 140 mg Fe/kg as Fe-Gly, and the basal diet supplemented with 50, 80, 110 or 140 mg Fe/kg as $FeSO_4 \cdot H_2O$. The neonatal piglets ($n = 45$) were used to determine the relative organ weight, tissue iron contents and blood biochemical indices. Compared with the control, the relative weight of spleen and kidney were significantly increased ($P < 0.05$) in the Fe-Gly groups. The iron contents in liver, spleen, kidney and femur were also found increased ($P < 0.05$) in the Fe-Gly groups. The RBC (d 1 and 21), HGB (d 1 and 21) and HCT (d 1 and 21) in blood and Fn (d 1) and SI (d 1 and 21) significantly increased ($P < 0.05$), but the TIBC (d 1 and 21) in serum decreased ($P < 0.05$) in the Fe-Gly groups. Moreover, the kidney relative weight, iron content in liver, spleen, kidney and femur, RBC (d 1) and HGB (d 21) in blood, and SI (d 1) in the Fe-Gly groups increased ($P < 0.05$) compared with the $FeSO_4 \cdot H_2O$ treatment. Linear and quadratic responses of the kidney relative weight, the iron content in liver, spleen, kidney and femur, RBC (d 1 and 21), HGB (d 1 and 21) and HCT (d 1 and 21) in whole blood, SI (d 1) and TIBC (d 1 and 21) in the Fe-Gly groups were observed ($P < 0.05$). Linear responses of Fn (d 1 and 21) and SI (d 21) in the Fe-Gly groups, and spleen relative weight, HCT (d 1), Fn (d 1) and TIBC (d 1 and 21) in the $FeSO_4 \cdot H_2O$ groups were observed ($P < 0.05$). These finding suggest that Fe-Gly supplemented at the level of 110 mg/kg in the diet of sows in this experiment is superior to other forms of supplementation, based on HGB concentration, the relative organ weight, tissue iron contents and blood biochemical indices of piglets.

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1. Introduction

Iron is recognized as one of the most important trace elements for animal growth and health. Iron deficiency anemia is prevalent in piglets, because their body iron stores at birth are low and the amount of iron in sows' milk is insufficient to meet the demands of fast growing (Svoboda and Drabek, 2005). Piglets would have low feed intake, low growth rate and increased chances of iron deficiency anemia and diarrhea, if the iron supplementation is not timely and effective. Supplementation of inorganic iron to pregnant and lactating sows is a common practice in the swine industry to prevent iron deficiency anemia in piglets. However, the absorption and activity of inorganic iron are impaired by the antagonism

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among trace elements and macroelements (Umbreit, 2005). Studies suggested that amino acid chelated irons had the advantages of stability, high biological efficacy, nutritional benefits, anti-stress effects, and reducing excretion compared with inorganic iron (Ettle et al., 2008; Ma et al., 2012; Wu et al., 2013). Henry and Miller (1995) reported that chelated or protein sources of iron has a 125% to 185% relative biological efficiency compared with ferrous sulfate ($FeSO_4$). Ferrous glycine chelate (Fe-Gly) is an efficient iron preparation in human, and especially in infant food (Fox et al., 1998). Layrisse et al. (2000) showed that food supplemented with Fe-Gly had twice the iron absorption rate compared with food that had $FeSO_4 \cdot H_2O$ added. However, there is little information on the effects of supplementing the diets of sows with different levels of Fe-Gly and $FeSO_4 \cdot H_2O$ on the iron nutritional status of newborn piglets. Hence, this study aimed to determine the reasonable addition of organic or inorganic iron in sow diets by investigating the effects of maternal dietary supplementing with varying levels of Fe-Gly and $FeSO_4 \cdot H_2O$ from d 86 of gestation to d 21 of lactation on the iron nutritional status of neonatal pigs.

2. Materials and methods

2.1. Care and use of animals

Animals were cared and handled in accordance with the guidelines for the care and use of laboratory animals of the Animal Nutrition Research Institute of Shandong Agricultural University and the Ministry of Agriculture of China.

2.2. Iron additives

The feed-grade Fe-Gly (99.0%, Tanke International Group, Guangzhou, China) was composed of 2 Gly molecules bound to a ferrous cation to form a double heterocyclic ring compound. The iron content of the Fe-Gly was 14.75%. The iron content of ferrous sulfate monohydrate ($FeSO_4 \cdot H_2O$, 98.5%, Zeweier Feed Co., Ltd, Nanning, China) was 29.84%.

2.3. Animals, experimental design and diets

Forty-five Landrace × Large white sows (3 to 4 parities) were used from d 86 of gestation to d 21 of lactation. The treatments were arranged as a complete randomized design, and sows were randomly divided into 9 treatments (5 sows/treatment): control (basal diet only, no Fe supplementation), the basal diet supplemented with 50, 80, 110, or 140 mg Fe/kg diet as Fe-Gly, and the basal diet supplemented with 50, 80, 110, or 140 mg Fe/kg as $FeSO_4 \cdot H_2O$.

The basal diet consisted of corn-wheat-soybean meal that was formulated to meet or exceed the NRC (1998) nutrient requirements for gestating and lactating sows without iron supplementation (Table 1). A composite sample of basal diet was sampled to analyze the iron content using flame atomic absorption spectrophotometry (TAS-990, Purkinje General Instrument Co., Ltd. Beijing, China). The actual iron concentration (analyzed) were 68.35 ± 1.32 and 63.54 ± 2.01 (means ± SD) mg/kg for the gestation and lactation diets, respectively.

Feed-grade Fe-Gly and $FeSO_4 \cdot H_2O$ crystalline powders were mixed with 100 kg of ground corn, respectively. The premix was then combined at the appropriate levels with the previously described basal diets to make the experimental diets.

2.4. Housing, feeding and management

At the start of the experiment, gestating sows were fed individually in pens (180 cm × 120 cm) that had a concrete floor, and was equipped with cement trough and bowl type drinker. Sows were fed twice daily (06:00 and 16:00) during the gestation period from 86 d of gestation to expected farrowing, for a total of 28 d. Each sow was given 2.8 kg of diets on d 1 of the experiment, with the feeding quantity increased by 0.2 kg every 5 d until the feed allowance of the sows was 3.6 kg/d on d 4 before expected farrowing. During 3 days before expected farrowing, each sow was fed 3.4 kg daily. Approximately 1 wk (d 107 of gestation) before expected farrowing, sows were moved from the gestation barn to the farrowing room, where they were also fed individually. Sows had free access to feed and remained with their litters during the lactation period. Throughout the experimental period, the sows had free access to water.

Piglets had free access to water and the breast milk of their mothers. An i.m. injection of Fe-dextran (100 mg Fe/piglet) was administered on d 3.

Table 1
Ingredient and nutrient composition of basal diets (air-dry basis).

Item	Gestation	Lactation
Ingredients, %		
Corn	35.60	35.09
Wheat	30.00	34.00
Soybean meal	14.00	17.00
Wheat bran	14.26	4.00
Soy oil	1.00	2.00
Fish meal	0.00	2.00
Limestone	1.00	1.00
Dicalcium phosphate	1.00	1.00
Salt	0.40	0.40
Glucose	0.00	1.00
L-Lysine-HCl	0.40	0.27
DL-methionine	0.15	0.11
L-threonine	0.19	0.13
Premix[1]	2.00	2.00
Total	100.00	100.00
Nutrient levels, %		
DE[2], MJ/kg	13.14	13.73
CP[2]	15.31	16.82
Ca[2]	0.76	0.86
Total P[2]	0.62	0.61
Lysine[2]	1.10	1.10
Methionine + Cysteine[2]	0.70	0.70
Threonine[2]	0.73	0.73
Thyptophan[2]	0.21	0.21
Fe[3], mg/kg	68.35	63.54

[1] Premix supplied per kg of diet: vitamin A, 7,000 IU; vitamin D, 32,100 IU, vitamin E, 36 IU; vitamin K_3, 1.70 mg; vitamin B_1, 5.00 mg; vitamin B_2, 2.00 mg; pantothenic acid, 10.00 mg; niacin, 20.00 mg; vitamin B_6, 1.80 mg; vitamin B_{12}, 0.02 mg; choline (choline chloride), 800 mg; D-biotin, 0.08 mg; folic acid, 1.0 mg; 5.0 mg of Cu as $CuSO_4 \cdot 5H_2O$; 50 mg of Zn as $ZnSO_4 \cdot H_2O$; 25 mg of Mn as $MnSO_4 \cdot H_2O$; 0.14 mg of I as KI; 0.23 mg Se as Na_2SeO_3.
[2] Calculated values according to the tables of feed composition and nutritive values in China (Xiong et al., 2011).
[3] Analyzed values.

2.5. Sample collection and processing

Piglets per litter were weighed immediately after birth. One piglet (whose birth weight was close to litter average weight) per litter was selected and euthanized at birth after being weighed. In every treatment, the selected piglets were 3 female piglets and 2 male piglets. The chest and abdominal cavities were immediately opened after confirming of the death, and the heart, liver, spleen and kidneys were removed and weighed. Relative organ weight (organ weight [g]/live body weight [kg]) was determined according to Lu et al. (1996). The heart, liver, kidney, spleen and femur were sampled and immediately stored at −20 °C until analysis of iron concentrations.

Blood samples (5 mL) were collected in a test tube containing an anticoagulant (EDTA) from the superior vena cava of one piglet per

litter at birth and on d 21 (collected blood samples at birth and on d 21 were in the same) for immediate analysis of red blood cells (RBC), hemoglobin (HGB) concentration and hematocrit (HCT). Approximately 10 mL of blood was collected in non-heparinized tubes, centrifuged (D-37520, Kendro, Germany) at 1,500 × *g* for 10 min and the resultant plasma was immediately stored at −20 °C until analysis.

2.6. Determination of iron content of piglet organs

All organ samples were dried at 65 °C according to AOAC (2005) and ground uniformly using a mortar, and then samples of organs were digested using a microwave digestion system (Model MDS-81D; CEM Corp., Matthews, NC, USA) according to the method described by Armstrong et al. (2004). Ultra-pure water and iron standard solution were used as a blank and reference standards, respectively. Iron contents were analyzed with flame atomic absorption spectrophotometry.

2.7. Determination of blood biochemical indices

Whole blood indices (RBC, HGB and HCT) were measured using automatic hematology analyzer (KX-21, Sysmex, Japan) according to the instructions of the manufacturer. Serum iron (SI) and total iron binding capacity (TIBC) were determined using assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China), and serum ferritin (Fn) was analyzed using ELISA kits (Bioleaf Biotech Co., Ltd, Shanghai, China).

2.8. Statistical analyses

Data was analyzed by a one-way analysis of variance (ANOVA) using SAS 9.2. The effects of supplemental Fe-Gly or $FeSO_4 \cdot H_2O$ were determined using a contrast test between control and Fe-Gly, control and $FeSO_4 \cdot H_2O$, and $FeSO_4 \cdot H_2O$ and Fe-Gly treatments. Orthogonal polynomial contrasts were used to determine linear and quadratic responses to Fe levels for the Fe-Gly or $FeSO_4 \cdot H_2O$ treatments. The significance of differences among treatments was tested using Duncan's multiple range tests. All statements of significance were based on the probability of $P < 0.05$.

3. Results

3.1. Relative organ weight

The effects of maternal supplementation of different sources and levels of iron on newborn piglets' relative organ weight are shown in Table 2. Compared with the control, the experimental diets with Fe-Gly significantly increased the spleen relative weight ($P = 0.035$) and kidney relative weight ($P = 0.037$) of newborn piglets. Moreover, piglets in the Fe-Gly groups had a significantly higher kidney relative weight ($P = 0.046$) than those in the $FeSO_4 \cdot H_2O$ groups. The spleen relative weight showed a linear ($P = 0.016$) increase and the kidney relative weight demonstrated both linear ($P = 0.009$) and quadratic ($P = 0.033$) increases in piglets with rising levels of Fe-Gly in the diets. In addition, the spleen relative weight of piglets increased linearly ($P = 0.029$) as the amount of $FeSO_4 \cdot H_2O$ in the diets rose. There was no significant difference ($P > 0.05$) between 140 mg/kg Fe-Gly group and 110 mg/kg Fe-Gly group in relative organ weight.

3.2. Iron content in piglets' organs

Table 3 shows the effects of newborn piglets' tissue iron content when sows were fed different sources and levels of iron. As the Fe-Gly content of the diets increased, there was a significant increase of iron contents in liver ($P < 0.001$), spleen ($P = 0.002$), kidneys ($P = 0.014$) and femur ($P = 0.001$) in piglets compared with the control. Compared with the $FeSO_4 \cdot H_2O$ groups, the piglets from the Fe-Gly groups had a significant increased iron content in the liver ($P < 0.001$), kidneys ($P < 0.001$), spleen ($P < 0.001$) and femur ($P = 0.002$). However, there was no significant difference in terms of organ iron content between the $FeSO_4 \cdot H_2O$ groups and the control group. Increasing the supplement amount of Fe-Gly in the diet of sows enhanced the heart ($P = 0.030$), liver ($P < 0.001$), spleen ($P < 0.001$), kidneys ($P < 0.001$) and femur ($P < 0.001$) iron content of piglets linearly, and as well as the heart ($P = 0.010$), liver

Table 2
Changes in newborn piglets' relative organ weight when sows were fed diets with different sources and levels of Fe ($n = 5$).

Item	Fe levels addition, mg/kg	Relative organ weight[1], g/kg				Piglet birth weight, kg
		Heart	Liver	Spleen	Kidney	
Control	0	6.91	24.09	1.06^{b}	7.36^{b}	1.31^{a}
Fe-Gly[2]	50	6.98	24.91	1.16^{ab}	7.57^{ab}	1.39^{a}
	80	7.01	24.98	1.20^{ab}	7.72^{ab}	1.44^{a}
	110	7.00	25.51	1.25^{a}	7.90^{a}	1.48^{b}
	140	6.98	25.53	1.26^{a}	7.90^{a}	1.46^{ab}
$FeSO_4 \cdot H_2O$[2]	50	6.91	24.48	1.12^{ab}	7.46^{ab}	1.34^{a}
	80	6.88	24.65	1.16^{ab}	7.57^{ab}	1.39^{a}
	110	6.94	24.91	1.19^{ab}	7.57^{ab}	1.40^{a}
	140	6.96	24.95	1.21^{ab}	7.60^{ab}	1.38^{a}
SEM		0.239	0.845	0.069	0.259	0.053
P-value						
Control vs. Fe-Gly		0.581	0.222	0.035	0.037	0.026
Control vs. $FeSO_4 \cdot H_2O$		0.914	0.403	0.065	0.238	0.193
Fe-Gly vs. $FeSO_4 \cdot H_2O$		0.474	0.376	0.225	0.046	0.047
Fe-Gly	Linear	0.683	0.175	0.016	0.009	0.028
	Quadratic	0.846	0.394	0.051	0.033	0.075
$FeSO_4 \cdot H_2O$	Linear	0.741	0.346	0.029	0.180	0.175
	Quadratic	0.908	0.643	0.092	0.397	0.355

Fe-Gly = ferrous glycine chelate.
[a,b] Mean values within a column without a common superscript differ significantly ($P < 0.05$).
[1] Determined according to: Relative organ weight (g/kg) = Organ weight (g)/Live body weight (kg), Lu et al. (1996).
[2] Fe sources.

Table 3
Differences in newborn piglets' organ Fe content when sows were fed diets with different sources and levels of Fe ($n = 5$).

Item	Fe level addition, mg/kg	Heart, mg/kg	Liver, mg/kg	Spleen, mg/kg	Kidney, mg/kg	Femur, mg/kg
Control	0	100.04	373.12^{c}	605.37^{c}	112.51^{c}	40.35^{c}
Fe-Gly[1]	50	104.70	394.63^{bc}	632.91^{b}	120.40^{c}	43.14^{ab}
	80	108.15	419.27^{b}	656.03^{ab}	132.71^{bc}	45.26^{a}
	110	112.78	447.22^{ab}	672.43^{a}	147.70^{ab}	46.13^{a}
	140	111.77	464.36^{a}	694.01^{a}	156.23^{a}	46.25^{a}
$FeSO_4 \cdot H_2O$[1]	50	102.35	377.36^{c}	607.44^{c}	114.42^{c}	41.63^{c}
	80	101.71	372.82^{c}	613.89^{c}	117.90^{c}	42.48^{ab}
	110	102.61	376.85^{c}	611.30^{c}	116.74^{c}	42.02^{ab}
	140	100.46	374.76^{c}	614.24^{c}	117.17^{c}	42.95^{ab}
SEM		6.567	8.678	11.601	7.671	2.277
Control vs. Fe-Gly		0.115	<0.001	0.002	0.014	0.001
Control vs. $FeSO_4 \cdot H_2O$		0.786	0.759	0.397	0.493	0.207
Fe-Gly vs. $FeSO_4 \cdot H_2O$		0.064	<0.001	<0.001	<0.001	0.002
Fe-Gly	Linear	0.030	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.010	<0.001	<0.001	<0.001	<0.001
$FeSO_4 \cdot H_2O$	Linear	0.922	0.870	0.299	0.469	0.177
	Quadratic	0.940	0.968	0.584	0.743	0.392

Fe-Gly = ferrous glycine chelate.
[a,b,c] Mean values within a column without a common superscript differ significantly ($P < 0.05$).
[1] Fe sources.

($P < 0.001$), spleen ($P < 0.001$), kidneys ($P < 0.001$) and femur ($P < 0.001$) iron content of piglets quadratically. No significant difference ($P > 0.05$) could be found between 110 mg/kg Fe-Gly and 140 mg/kg Fe-Gly groups.

3.3. Blood physiological parameters of piglets

The effects of newborn piglets' blood physiological parameters when sows were fed different sources and levels of iron are presented in Table 4. Compared with the control, the piglets in Fe-Gly groups had higher RBC ($P = 0.005$, d 1; $P = 0.005$, d 21), HGB concentration ($P = 0.023$, d 1; $P = 0.009$, d 21) and HTC ($P = 0.001$, d 1; $P = 0.032$, d 21). Compared with $FeSO_4 \cdot H_2O$ groups, the Fe-Gly groups had significant increases in RBC ($P = 0.015$, d 1) and HGB concentration ($P = 0.021$, d 21). In terms of blood physiological parameters, there was no significant difference between the $FeSO_4 \cdot H_2O$ groups and the control. As the Fe-Gly levels increased, there was a linear increase in RBC ($P < 0.001$, d 1; $P = 0.001$, d 21), HGB concentration ($P < 0.001$, d 1; $P = 0.001$, d 21) and HTC ($P < 0.001$, d 1; $P = 0.002$, d 21). Similarly, increasing the supplement amount of Fe-Gly in diets increased RBC ($P = 0.003$, d 1; $P = 0.004$, d 21) and HGB concentrations ($P < 0.001$, d 1; $P = 0.005$, d 21), and HTC ($P < 0.001$, d 1; $P = 0.010$, d 21) of piglets quadratically.

3.4. Serum biochemical indices of piglets

Analyzed serum biochemical indices when sows were fed different sources and levels of iron are shown in Table 5. Compared with the control, serum Fn in piglets ($P = 0.047$, d 1) and SI ($P = 0.014$, d 1; $P = 0.041$, d 21) significantly increased; however, TIBC ($P = 0.041$, d 1; $P = 0.044$, d 21) significantly decreased in the Fe-Gly groups. Compared with the $FeSO_4 \cdot H_2O$ groups, the increased serum SI ($P = 0.047$, d 1) of piglets was significant in the Fe-Gly groups. With increasing Fe-Gly levels in experimental diets, the serum Fn of piglets ($P = 0.018$, d 1; $P = 0.015$, d 21), SI ($P = 0.005$, d 1; $P = 0.015$, d 21) increased linearly, but a linear decrease ($P < 0.05$) in the serum TIBC of piglets at d 1 and 21 was observed. Furthermore, increasing the supplemental amount of Fe-Gly in the diets quadratically increased SI ($P = 0.015$, d 1), but serum TIBC at d 1 ($P = 0.027$) and 21 ($P = 0.047$) decreased quadratically. In Fe-Gly groups, the Fe-Gly additive amount of 110 and 140 mg/kg had similar results. In the $FeSO_4 \cdot H_2O$ groups, there was a linear increase in the Fn of piglets ($P = 0.027$, d 21) and a linear decrease ($P < 0.05$) in serum TIBC on d 1 and 21 of piglets.

4. Discussion

4.1. Relative organ weight of piglets

The relative organ weight has been used as an indicator of organ function (Lu et al., 1996). In addition, fetal growth and development rely entirely on the maternal supply of nutrients, including trace mineral elements (Van Saun, 2008). Previous research had shown that maternal nutrient levels have significant effects on the liver and kidney weight of progeny (Pond et al., 1986). Condeaguilera et al., 2011 had reported that an increased relative weight of visceral tissues might improve the nutrition intake required because visceral tissues were metabolically more active than carass tissues. In the present study, the spleen and kidney indices of piglets were significantly increased in the Fe-Gly group, which might indicate that the pregnancy sows could provide higher nutrition for piglets' visceral tissues development in the Fe-Gly groups than in the $FeSO_4 \cdot H_2O$ groups.

4.2. Iron contents in tissues of piglets

Yu et al. (1994) found that iron concentrations in the liver and spleen had a significant and positive correlation with iron levels in the diet of rats. Tissue iron content of piglets increased significantly with rising levels of Fe-Gly in the sows' diets in the present study. However, as the $FeSO_4 \cdot H_2O$ content of the sows' diets increased at the same level, there was no significant increase of the iron content of tissues. This result may provide evidence that Fe-Gly has higher biological efficacy than $FeSO_4 \cdot H_2O$. In addition, reports have indicated that feeding an organic iron source to pregnant sows could increase fetal iron stores (Close, 1999). Spruill et al. (1971) showed that gestation diets with high iron levels resulted in a slight but non-significant increase in the placental transfer of iron as measured by the liver iron content of newborn pigs. In the research, the iron content in the liver, kidney, spleen and femur also significantly increased in the Fe-Gly piglet groups as compared with the $FeSO_4 \cdot H_2O$ piglet groups. This finding is consistent with previous studies (Yu et al., 2000; Feng et al., 2007, 2009). These results may be attributed to the fact that Gly has a similar structure to HGB and plays

Table 4
Changes in blood physiological indices of newborn piglets when sows were fed diets with different sources and levels of Fe ($n = 5$).

Item	Fe level addition, mg/kg	d 1			d 21		
		RBC, 10^{12}/L	HGB, g/L	HCT, %	RBC, 10^{12}/L	HGB, g/L	HCT, %
Control	0	3.75^{c}	80.80^{c}	32.78^{c}	3.83	85.00^{b}	34.56^{b}
Fe-Gly[1]	50	3.99^{bc}	90.20^{bc}	34.06^{bc}	4.02	94.40^{ab}	35.52^{ab}
	80	4.16^{ab}	95.60^{ab}	34.98^{bc}	4.13	100.80^{ab}	36.58^{ab}
	110	4.29^{a}	104.60^{ab}	36.60^{a}	4.22	106.60^{a}	38.18^{a}
	140	4.30^{a}	106.40^{a}	36.74^{a}	4.23	107.40^{a}	38.20^{a}
$FeSO_4 \cdot H_2O$[1]	50	3.89^{bc}	89.20^{bc}	33.72^{bc}	3.96	91.00^{ab}	35.20^{ab}
	80	3.95^{bc}	92.40^{ab}	34.18^{bc}	4.07	92.00^{ab}	35.66^{ab}
	110	4.00^{bc}	93.80^{ab}	35.04^{ab}	4.10	94.60^{ab}	36.52^{ab}
	140	4.03^{bc}	95.80^{ab}	35.36^{b}	4.13	96.20^{ab}	36.64^{ab}
SEM		0.218	5.763	0.850	0.248	5.254	1.018
Control vs. Fe-Gly		0.005	0.023	0.001	0.005	0.009	0.032
Control vs. $FeSO_4 \cdot H_2O$		0.081	0.282	0.080	0.228	0.148	0.215
Fe-Gly vs. $FeSO_4 \cdot H_2O$		0.015	0.086	0.089	0.415	0.021	0.137
Fe-Gly	Linear	<0.001	<0.001	<0.001	0.001	0.001	0.002
	Quadratic	0.003	<0.001	<0.001	0.004	0.005	0.010
$FeSO_4 \cdot H_2O$	Linear	0.050	0.148	0.021	0.165	0.103	0.092
	Quadratic	0.141	0.359	0.075	0.375	0.266	0.249

Fe-Gly = ferrous glycine chelate; RBC = red blood cells; HGB = hemoglobin concentration; HCT = hematocrit.
[a,b,c] Mean values within a column without a common superscript differ significantly ($P < 0.05$).
[1] Fe sources.

Table 5
Changes in serum biochemical indices of newborn piglets when sows were fed diets with different sources and levels of Fe (n = 5).

Item	Fe level addition, mg/kg	d 1			d 21		
		Fn, ng/mL	SI, mg/L	TIBC, μmol/L	Fn, ng/mL	SI, mg/L	TIBC, μmol/L
Control	0	106.83	110.06^{b}	321.23^{a}	113.30	121.49	311.38^{a}
Fe-Gly[1]	50	113.91	119.84^{ab}	312.36^{ab}	120.58	129.07	301.53^{ab}
	80	117.28	124.49^{a}	304.83^{ab}	125.35	134.52	295.80^{ab}
	110	121.23	128.81^{a}	296.25^{bc}	131.50	138.04	289.42^{a}
	140	122.47	129.82^{a}	294.79^{c}	133.54	139.25	287.36^{b}
$FeSO_4 \cdot H_2O$[1]	50	110.81	113.51^{ab}	317.90^{ab}	118.48	124.34	306.67^{ab}
	80	114.41	116.18^{ab}	312.39^{ab}	123.48	127.81	302.36^{ab}
	110	117.05	120.47^{ab}	307.26^{bc}	124.52	130.70	299.43^{ab}
	140	118.02	122.88^{a}	303.29^{bc}	129.09	134.51	296.66^{ab}
SEM		6.447	5.500	7.886	6.304	5.779	6.657
Control vs. Fe-Gly		0.047	0.014	0.041	0.072	0.041	0.044
Control vs. $FeSO_4 \cdot H_2O$		0.159	0.218	0.111	0.081	0.238	0.112
Fe-Gly vs. $FeSO_4 \cdot H_2O$		0.346	0.047	0.103	0.399	0.136	0.096
Fe-Gly	Linear	0.018	0.005	0.007	0.015	0.015	0.013
	Quadratic	0.061	0.017	0.027	0.051	0.051	0.047
$FeSO_4 \cdot H_2O$	Linear	0.076	0.080	0.015	0.027	0.083	0.036
	Quadratic	0.213	0.220	0.051	0.091	0.223	0.117

Fe-Gly = ferrous glycine chelate; Fn = serum ferritin; SI = serum Fe; TIBC = total Fe binding capacity.
[a,b,c] Mean values within a column without a common superscript differ significantly ($P < 0.05$).
[1] Fe sources.

an important role in heme synthesis. Besides, the Gly, as a carrier of iron transport to the target cell, has specificity in theory (Wei et al., 2005). When the Fe-Gly additive amount exceeded 110 mg/kg, there was no significant increase in iron content of piglet's organs. That may mean iron content in piglets was enough in 110 mg/kg Fe-Gly group, providing more iron could not achieve better results.

4.3. Blood physiological parameters of piglets

Low HCT and HGB levels are associated with anemia (Kals et al., 2016). In the present study, adding Fe-Gly to the diet of sows significantly increased RBC, HGB concentrations and HTC of newborn piglets, this finding is in agreement with the results of previous studies (Peters and Mahan, 2008; Wang et al., 2014). The only exception was the high-dose groups (110 and 140 mg/kg). This finding may be explained by the homeostatic iron regulation mechanisms that organisms have evolved, which include complex homeostatic circuits and specialized molecules to regulate iron levels (Yu et al., 2000). The HGB concentration of piglets can be increased by both providing high-dose iron supplementation to pregnant sows, which increases fetal iron stores, and increasing the iron level of diets fed to sows during lactation, which increases the iron content of the milk. In addition, piglets may acquire iron from consuming sow feces (Brady et al., 1978). A HGB concentration of 100 g/L indicates an adequate iron content in an organism; 80 g/L is considered the borderline of anemia edge; and 60 g/L indicates severe anemia (McDowell, 1992). When the Fe-Gly dosage in experimental diets was 110 mg/kg, the HGB of the newborn piglets exceeded 100 g/L in the present study, which indicated an adequate HGB concentration.

4.4. Biochemical indices of piglet serum

Ferritin, SI, and TIBC are important biochemical indicators that reflect the iron status of piglets (Gottschalk et al., 2000; Ferreira da Silva et al., 2004; Zhang et al., 2017). The major iron storage protein is Fn, which decreases in animals with anemia (Bradley et al., 2004). Serum iron, the iron combined with transferrin in serum, has been used for qualitative measurement of bioavailability of iron supplements (Ferreira da Silva et al., 2004). Total iron binding capacity is the maximum amount of iron that transferrin may combine with in 100 mL of serum, a value that increases with anemia (Smith et al., 1984). When Fe-Gly was added to the diets of sows, piglet serum Fn and SI increased significantly and TIBC decreased significantly. However, the beneficial effects of supplementation with $FeSO_4 \cdot H_2O$ were not significant. Spruill et al. (1971) reported that feeding high iron diets during gestation could increase SI, and Yu et al. (2000) found that iron from an amino acid complex could increase SI in the blood. Both of these findings are in agreement with the results of the present study, and can be explained by the fact that inorganic iron is absorbed when its post-coenzyme combines with amino acid or other substances to form chelate salts. Amino acids chelated iron, which is the main form of iron absorbed by the body can be taken up directly, thereby avoiding competition with other minerals (Close, 1999). Therefore, amino acids chelated iron has a higher biological value. The present study also found that serum Fn, SI and TIBC of piglets varied linearly as the amount of Fe-Gly supplementation changed, but the differences between the 110 and 140 mg/kg groups were not significant. This finding may be explained by the fact that the absorption of iron in the body is adjusted according to the demands of the body (Morgan and Oates, 2002). The sample size was small in the present study, though. Therefore, further studies are needed to confirm the current results.

5. Conclusion

According to the HGB concentration of piglets, and taking relative organ weight, tissue iron contents and blood biochemical indices into consideration, the appropriate dosage of Fe-Gly in the diet of sows was 110 mg/kg in the present study. However, when $FeSO_4 \cdot H_2O$ was supplemented at 140 mg/kg, the HGB concentration of piglets could not be higher than 100 g/L. Further studies are needed to determine the appropriate amount of $FeSO_4 \cdot H_2O$ in the diet of sows that could meet the iron nutritional requirements of neonatal piglets. As iron anemia is a common and detrimental problem for piglets, these findings can be used for improving management practices in the swine industry.

Conflicts of interest

The authors declare that there are no competing financial interests in the work described.

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