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**EFFECTS OF DIETARY IRON LEVELS ON GROWTH PERFORMANCE AND IRON METABOLISM-RELATED GENES EXPRESSION IN GROWING REX RABBITS**

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# EFFECTS OF DIETARY IRON LEVELS ON GROWTH PERFORMANCE AND IRON METABOLISM-RELATED GENES EXPRESSION IN GROWING *REX* RABBITS

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## ABSTRACT

This study was conducted to evaluate the effects of dietary iron levels on growth performance and the expression levels of iron metabolism-related genes in growth *Rex rabbits*. Two hundred healthy rabbits were randomly assigned to five dietary treatment (n=40) according to initial body weight and sex, and fed with a basal diet supplemented with 0, 20, 40, 80, or 160 mg/kg iron in the form of FeSO<sub>4</sub>·H<sub>2</sub>O, respectively. After a 35-d trial, 8 rabbits per treatment were randomly selected to collect liver and duodenum samples. The results showed that the ADG of rabbits in 40 mg/kg iron group and the ADFI of rabbits in 20, 40 and 80 mg/kg iron groups were higher than those in 0 and 160 mg/kg iron groups. Furthermore, 40 mg/kg iron supplementation upregulated the mRNA expression levels of *DMT1* in duodenum as well as the mRNA expression levels of *Tf* and *HAMP* in liver. These results suggested that dietary appropriate level of iron supplementation could improve the growth performance of growth *Rex* rabbit and the optimal addition level of iron was 40 mg/kg in this study.

**Key words:** Rabbit, Iron, Growth performance, Iron metabolism.

## INTRODUCTION

Iron is one of the essential trace elements in almost all organisms that participates in various physiologic processes, including electron transfer, binding and transport of oxygen, as well as the production of adenosine triphosphate (Nakamura *et al.*, 2019). When iron intake is inadequate, symptoms such as iron deficiency in erythroblasts and even iron-deficiency anemia occur (Camaschella, 2019), and subsequently induce growth retardation. But Staniek *et al* (2018) reported that excess iron accumulation could also lead to cytotoxicity, inducing oxidative stress, extensive destruction of tissues and organs and then causing various diseases. Therefore, an adequate supply of iron is essential for the health of the body.

*Rex rabbits* are mainly farmed for their skin and meat. The meat of *Rex* rabbit contains the high-quality animal protein and has a high nutritional and health caring value (Dalle Zotte, 2002). But as far as we know, from now on, there is little literature available to evaluate dietary iron requirements of *Rex* rabbits. Thus, it is necessary to explore the appropriate addition level of iron in *Rex* rabbit diet.

The aim of this study was to investigate the effect of different levels of iron supplementation on growth performance and the underlying mechanisms responsible for the beneficial effect of iron supplementation in *Rex rabbits*. Our study will be helpful to clarify the nutritional iron requirements of *Rex* rabbits.

## MATERIALS AND METHODS

### Animals and experimental design

Two hundred healthy *Rex rabbits*, with an initial average body weight (BW) of 1947 ± 211 g, were selected and randomly assigned into five dietary treatment groups (n=40) according to initial body weight and sex. Rabbits were given a basal diet supplemented with 0, 20, 40, 80, or 160 mg/kg iron in

the form of FeSO<sub>4</sub>·H<sub>2</sub>O (provided by Mushen Feed Co., Ltd, Taian, China) and the measured values of iron in diets were 8.2, 25.4, 49.1, 85.6 and 179 mg/kg, respectively. After 7 d preliminary experiment, the rabbits were fed corresponding diets as described above for 35 d. The basal diet was formulated to meet the NRC (1977) recommendations for the nutrient requirements of growth rabbits. Ingredients and compositions of the basal diet were shown in Table 1. All rabbits were housed in individual cages and had free access to feed and water. The BW of rabbits were individually measured after overnight fasting at the morning of d 36 of the feeding trial, and the feed intake of each rabbit was collected weekly throughout the trial to calculate Average Daily Feed Intake (ADFI), Average Daily Gain (ADG) and Feed Conversion Ratio (FCR). At the end of the trial, eight rabbits with the average BW of each treatment were randomly selected and slaughtered with electric stun. Then, the abdomen was immediately opened, followed by the collection of liver and jejunal mucosa tissue samples.

**Table 1:** Ingredients composition and nutrient levels of basal diets (as-fed basis)

Ingredients	Content (%)	Nutrient concentrations	Content (%)
Corn	10.0	DE (MJ/kg)	10.8
Soybean meal	15.0	CP	17.7
Wheat bran	15.0	CE	3.53
Germ meal	15.0	CF	15.7
Sunflower meal	5.00	NDF	31.7
Peanut seedling	20.0	ADF	17.1
Alfalfa	10.0	Ash	3.60
Soybean straw powder	5.50	Xylogen	4.12
Soybean oil	0.50	Ca	1.20
Mineral and vitamin premix <sup>1</sup>	4.00	P	0.61

<sup>1</sup>Premix provided per kg diet: vitamin A, 8,250 IU; vitamin B1, 2.91 mg; vitamin B2, 7.2 mg; vitamin B12, 0.014 mg; vitamin D3, 4,050 IU; vitamin E, 11.85 IU; vitamin B6, 2.85 mg; vitamin K3, 1.29 mg; folic acid, 0.72 mg; nicotinamide, 29.4 mg; calcium pantothenate, 21.6 mg; biotin, 0.15 mg; antioxidant, 4.5 mg; choline chloride, 100 mg; Cu, 10 mg; Zn, 60 mg; Mn, 10 mg; I, 1 mg; CaHPO<sub>4</sub>, 4,500 mg; Lys, 7,000 mg; Met, 5,500 mg; enterococcus faecalis, 3 trillion CFU; bacillus subtilis, 153 trillion CFU; phytase, 1800 IU; bacillus licheniformis, 15 trillion CFU; xylanase, 3600 IU.

<sup>2</sup>The energy level was calculated and the remaining nutrient level was measured.

### Chemical Analyses

RNA extraction and quantitative real-time PCR of mucosa samples were performed as described previously (Chen *et al.*, 2018). The beta-actin gene was chosen as the reference gene to normalize mRNA expression of target genes. The relative expression ratio of target genes relative to the reference gene was calculated using the 2<sup>-ΔΔCT</sup> method (Livak *et al.*, 2001).

**Table 2:** Primers used for real-time quantitative PCR

Gene	Primer sequence (5'-3')		Accession No.
<i>β-actin</i>	F: GATGATGATATCGCCGCGCTC	R: GAATCCTTCTGACCCATGCCCA	AF 404278
<i>Tf</i>	F: GTTGAGTATGGAATAACAGTGGC	R: TCATATGTGTTTTGCCCCGAAG	HM 233548
<i>HAMP</i>	F: CTCCTTGTTCTCACTGGCCTGA	R: AGAAGATGCAGATGGGGAAGTG	XM 008249495
<i>DMT1</i>	F: ATGTTACAGTGAGACCCAGCCAG	R: ATGATGACAGCTCCCACGATG	NM 000617
<i>CYTb</i>	F: AATCCCCTCAATACTCCTCTCA	R: GGAGGAAGGGGATAATTGCTAGG	HM 233084

### Statistical Analysis

Data were analyzed using the one-way ANOVA procedure of SAS 8.0 (SAS Institute Inc., Cary, NC). Statistical differences among groups were determined by Duncan's multiple-range test. The results were expressed as means with RMSE. Statistical significance was considered as  $P < 0.05$ .

## RESULTS AND DISCUSSION

Exogenous iron supplementation is considered an effective way to prevent iron deficiency. Appropriate iron supplementation has a significant influence on the growth performance of animals (Table 3). Previous study showed that iron supplementation could improve the ADG of weaned pigs (Williams *et al.*, 2020). Similarly, in the present study, dietary iron supplementation at 40 mg/kg

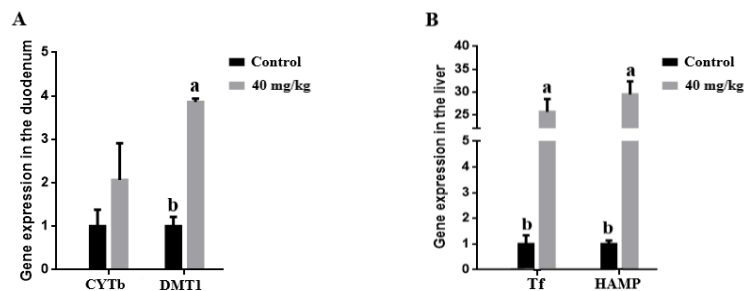
increased the ADG of rabbits compared with those in the 0 and 160 mg/kg iron groups. In addition, compared with the 0 mg/kg iron group, the ADFI was increased in rabbits fed 20, 40 and 80 mg/kg iron-supplemented diet. However, no significant difference in growth performance was observed between rabbits in the 0 and 160 mg/kg iron groups. These results indicated that dietary appropriate iron supplementation could promote the growth performance of rabbits. The optimal level of iron was 40 mg/kg in the present trial.

**Table 3:** Effects of dietary iron levels on growth performance in growing *Rex* rabbits<sup>1</sup>

Items	Iron level (mg/kg)					RMSE (n=40)	P-value
	0	20	40	80	160		
Initial BW (g)	1975	1965	1914	1948	1935	201	0.79
Final BW (g)	2515 <sup>ab</sup>	2587 <sup>a</sup>	2569 <sup>a</sup>	2548 <sup>a</sup>	2423 <sup>b</sup>	180	0.0058
ADG (g)	15.4 <sup>bc</sup>	17.8 <sup>ab</sup>	18.7 <sup>a</sup>	17.1 <sup>ab</sup>	13.9 <sup>c</sup>	5.10	0.0042
ADFI (g)	113 <sup>b</sup>	128 <sup>a</sup>	131 <sup>a</sup>	131 <sup>a</sup>	116 <sup>b</sup>	5.74	<0.001
FCR (g/g)	7.36	7.26	6.98	7.65	8.35	2.81	0.0721

<sup>1</sup>The different letters on the same row means differ significantly.

Exogenously added iron should be absorbed and transported before it works. The brush border of duodenal enterocytes is the main site of iron absorption while the liver is the place for iron storage (Hentze *et al.*, 2010). Therefore, we further evaluated the effect of 40 mg/kg iron on the expression levels of iron metabolism related genes (Figure 1). Duodenal cytochrome b (*Cytb*), a key reductase located at the brush border of the duodenum, could reduce the Fe<sup>3+</sup> to Fe<sup>2+</sup> (Mckie, 2008). Divalent metal transporter 1 (*DMT1*), an intestinal transporter, is responsible for the transportation of Fe<sup>2+</sup> to the epithelial cells (Shan *et al.*, 2009). Therefore, the expression levels of *Cytb* and *DMT1* are closely related to the iron absorption. In current study, the *DMT1* expression in duodenum was upregulated, indicating the iron absorption was increased. Moreover, parenteral pathway is another important way for iron absorption (Bogdan *et al.*, 2016). The free Fe<sup>3+</sup> can bound to transferrin (*Tf*) and then be delivered to cells by endocytosis of its complex with transferrin receptor (Cheng *et al.*, 2004). In the present study, we found that the *Tf* mRNA expression level in liver was increased, further indicating the iron absorption was enhanced. Another meaningful discovery in the present study was that the expression of liver hepcidin (*HAMP*) were upregulated when rabbits were fed the diet supplemented with 40 mg/kg iron. *HAMP* is a novel antimicrobial peptide mainly expressed in liver, acting as a negative regulatory hormone and playing a vital role in maintaining iron balance in animals (Nicolas *et al.*, 2001). It appears, therefore, that supplemented with 40 mg/kg iron in the diet of *Rex* rabbits was sufficient.



**Figure 1:** Effects of dietary iron on mRNA levels of iron metabolism related genes in duodenum (A) and liver (B) of growing *Rex* rabbits. Letters above the bars (a, b) indicate statistical significance ( $P < 0.05$ ) of genes expression between the two treatments. n=8. *CYTB*, cytochrome b; *DMT1*, divalent metal transporter 1; *Tf*, transferrin; *HAMP*, hepcidin.

## CONCLUSIONS

In conclusions, results of the present study indicated that dietary supplemented with appropriate iron can improve the growth performance of *Rex* rabbits partly by regulating the iron metabolism. The addition of 40 mg/kg iron is sufficient.

## ACKNOWLEDGEMENTS

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## REFERENCES

- Bogdan A.R., Miyazawa M., Hashimoto K., Tsuji Y. 2016. Regulators of iron homeostasis: new players in metabolism, Cell Death, and Disease. *Trends Biochem. Sci.*, 41, 274-286.
- Camaschella C. 2019. Iron deficiency. *Blood*, 133, 30-39.
- Chen J., Li Y., Yu B., Chen D., Mao X., Zheng P., et al. 2018. Dietary chlorogenic acid improves growth performance of weaned pigs through maintaining antioxidant capacity and intestinal digestion and absorption function. *J. Anim. Sci.*, 96, 1108-1118.
- Cheng Y., Zak O., Aisen P., Harrison S.C., Walz T. 2004. Structure of the human transferrin receptor-transferrin complex. *Cell*, 116, 565-576.
- Dalle Zotte A., 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livest. Prod. Sci.*, 75, 11-32.
- Hentze M.W., Muckenthaler M.U., Galy B., Camaschella, C. 2010. Two to tango: regulation of Mammalian iron metabolism. *Cell*, 142, 24-38.
- Livak K.J., Schmittgen T. D. J. M. 2001. Analysis of Relative Gene Expression data using real-time quantitative PCR. *Methods*, 25, 402-408.
- Mckie A.T. 2008. The role of Dcytb in iron metabolism: an update. *Biochem. Soc. T.*, 36, 1239-1241.
- Nakamura T., Naguro I., Ichijo, H. 2019. Iron homeostasis and iron-regulated ROS in cell death, senescence and human diseases. *BBA-Gen. Subjects*, 1863, 1398-1409.
- Nicolas G., Bennoun M., Devaux I., Beaumont C., Grandchamp B., Kahn A., et al. 2001. Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. *Clin. Infect. Dis.*, 98, 8780-8785.
- NRC. 1977. Nutrient requirements of rabbits. *National Academies Press*.
- Shan S.L., Apte S.S., Jr B.A., Andrews M.C., Schranzhofer M., Kahawita T., et al. 2009. Nramp1 promotes efficient macrophage recycling of iron following erythrophagocytosis in vivo. *Proc Natl Acad Sci USA.*, 106, 5960-5965.
- Staniek, Halina, Wojciak, Rafal W. 2018. The combined effects of iron excess in the diet and chromium (iii) supplementation on the iron and chromium status in female rats. *Biol. Trace Elem. Res.*, 184, 398-408.
- Williams H.E., Woodworth J.C., Derouchey J.M., Dritz S.S., Tokach M.D., Fry R.S., et al. 2020. Effects of feeding increasing levels of iron from iron sulfate or iron carbonate on nursery pig growth performance and hematological criteria. *J. Anim. Sci.*, 98,1-6.

# Effects of Dietary Iron Levels on Growth Performance and Iron Metabolism-related Genes Expression in Growing Rex Rabbits

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**2021-11-05**

# ***Introduction***



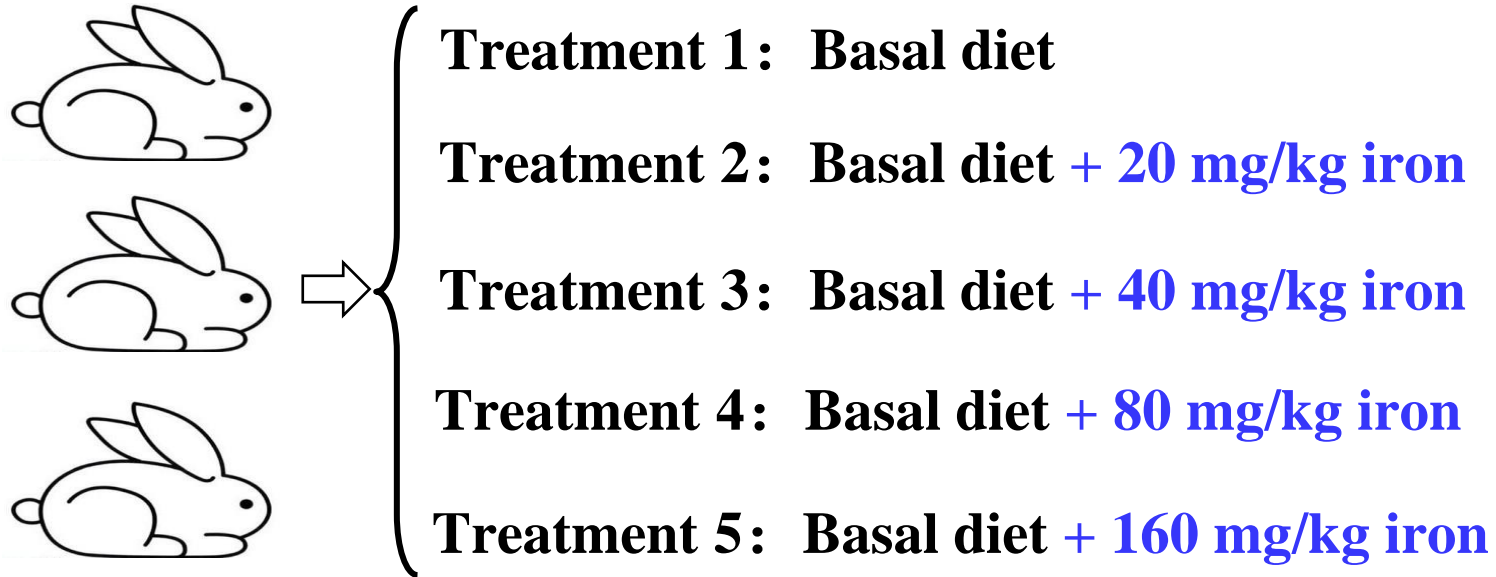
- **Iron is one of the essential trace elements in almost all organisms that participates in various physiologic processes.**
- **An adequate supply of iron is essential for the health of the body.**
- **Rex rabbits are mainly farmed for skin and meat, but there is little literature available to evaluate dietary iron requirements.**

**The aim of this study was to investigate the effect of different levels of iron supplementation on growth performance and the underlying mechanisms responsible for the beneficial effect of iron supplementation in rex rabbits.**

# Materials and methods



- **Two hundred** healthy rex rabbits were selected and randomly allotted into five dietary treatment groups (n=40) according to initial body weight and sex.



- The trial lasted for **35d**.
- Sample collection : After the trial, **liver and duodenal mucosa samples** were collected.



# Results and discussion

## ◆ Effects of dietary iron levels on growth performance in growing rex rabbits

Items	Iron level (mg/kg)					RMSE(n=40)	P-value
	0	20	40	80	160		
Initial BW (g)	1975	1965	1914	1948	1935	201	0.790
Final BW (g)	2515 <sup>ab</sup>	2587 <sup>a</sup>	2569 <sup>a</sup>	2548 <sup>a</sup>	2423 <sup>b</sup>	180	0.0058
ADG (g)	15.4 <sup>bc</sup>	17.8 <sup>ab</sup>	18.7 <sup>a</sup> ↑	17.1 <sup>ab</sup>	13.9 <sup>c</sup>	5.10	0.0042
ADFI (g)	113 <sup>b</sup>	128 <sup>a</sup> ↑	131 <sup>a</sup> ↑	131 <sup>a</sup> ↑	116 <sup>b</sup>	5.74	<0.001
FCR (g/g)	7.36	7.26	6.98	7.65	8.35	2.81	0.0721

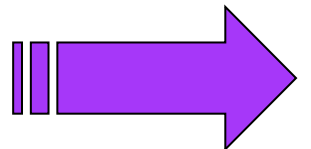
✓ Dietary appropriate iron supplementation could promote the growth performance of rabbits.

**The optimal level of iron was 40 mg/kg in the present trial.**

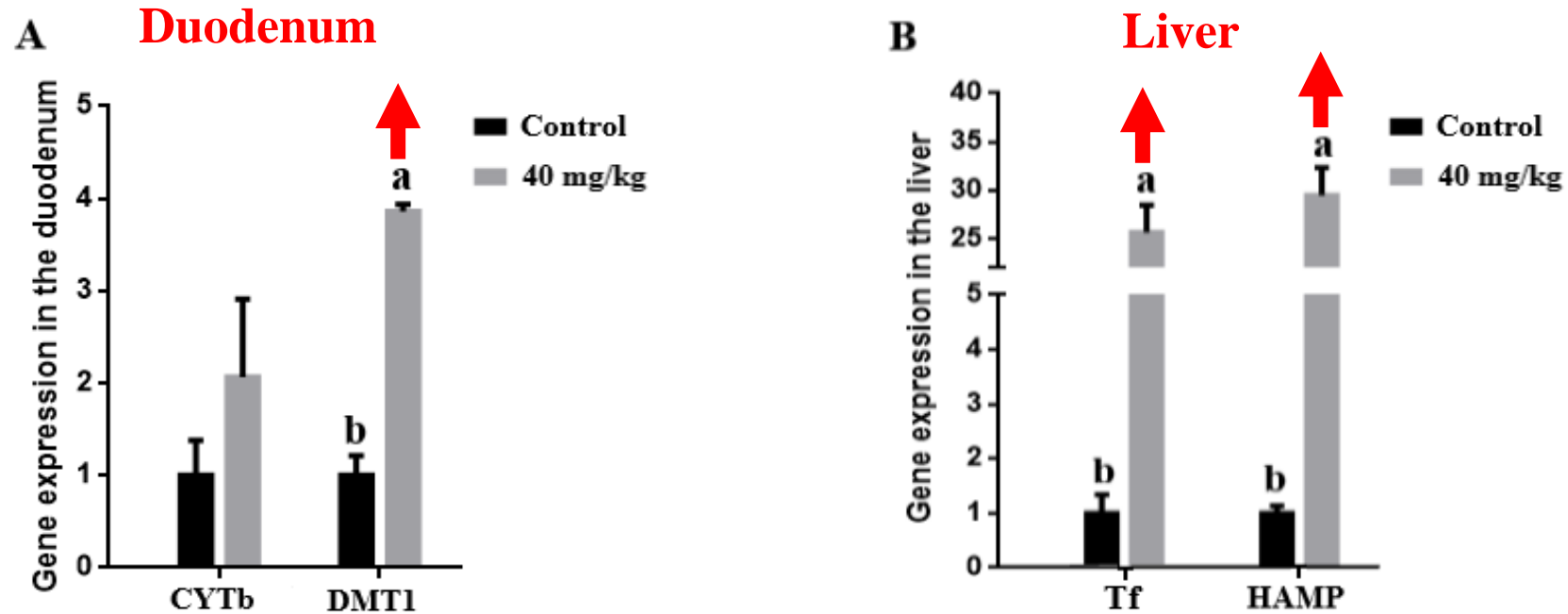


- **Exogenously added iron should be absorbed and transported before it works.**
- **The brush border of duodenal enterocytes is the main site of iron absorption.**
- **The liver is the place for iron storage.**

**We further evaluated the effect of 40 mg/kg iron on the expression levels of iron metabolism related genes.**



◆ Effects of dietary iron levels on mRNA levels of iron metabolism related genes in growing rex rabbits.



✓ The mRNA expression levels of DMT1 in duodenum and the mRNA expression levels of Tf and HAMP in liver were upregulated when rabbits were fed the diet supplemented with 40 mg/kg iron.



# *Conclusion*

- **Dietary supplemented with appropriate iron can improve the growth performance of rex rabbits partly by regulating the iron metabolism.**
- **The optimal dose of iron is 40 mg/kg in the present study.**

