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

Chen J.[1], Wang J.[1], Li F.[1] *

[1]Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, Department of Animal Science and Technology, Shandong Agricultural University, Tai'an, China
*Corresponding author: chlf@sdau.edu.cn

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 $FeSO_4 \cdot H_2O$, 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 (Nakmura *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 FeSO4·H2O (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[1]	4.00	P	0.61

[1]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; CaHPO4, 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.
[2]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 $2^{-\Delta\Delta CT}$ method (Livak *et al*., 2001).

Table 2: Primers used for real-time quantitative PCR

Gene	Primer sequence (5′-3′)		Accession No.
β-actin	F: GATGATGATATCGCCGCGCTC	R: GAATCCTTCTGACCCATGCCCA	AF 404278
Tf	F: GTTGAGTATGGAAATACAGTGGC	R: TCATATGTGTTTTTGCCCCGAAG	HM 233548
HAMP	F: CTCCTTGTTCTCACTGGCCTGA	R: AGAAGATGCAGATGGGGAAGTG	XM 008249495
DMT1	F: ATGTTACAGTGAGACCCAGCCAG	R: ATGATGACAGCTCCCACGATG	NM 000617
CYTb	F: AATCCCCTCAATACTCCTCCTCA	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[1]

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

[1]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 Fe3+ to Fe2+ (Mckie, 2008). Divalent metal transporter 1 (*DMT1*), an intestinal transporter, is responsible for the transportation of Fe2+ 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 Fe3+ 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.

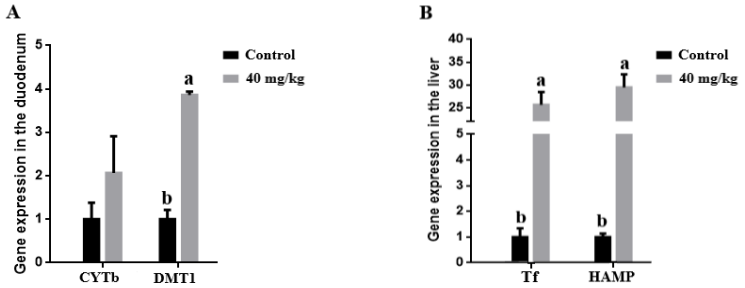

Figure 1: Effects of dietary iron on mRNA levels of iron metabolism related genes in duodenum (A) and liver (B) of growing *Rex* rabbits. Letters above the bars (a, b) indicate statistical significance ($P < 0.05$) of genes expression between the two treatments. n=8. *CYTb*, cytochrome b; *DMT1*, divalent 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 partily by regulating the iron metabolism. The addition of 40 mg/kg iron is sufficient.

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