Effect of Dietary Iron Level on Hepatic Lipid Metabolism in Broilers

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Abstract: This study investigated the effects of dietary iron (Fe) levels on the lipid metabolism in the liver of broilers. A total of 640 1-day-old AA broilers were fed with the basal diet added with 0, 60, 300, or 600 mg Fe/kg (Fe0, Fe60, Fe300, or Fe600) for 42 days, with 8 pens of 20 birds each in different treatment. The Fe level in Fe60, close to the requirement of the broilers, was used as the control. Both Fe0 and Fe600 treatments significantly increased the weight of abdominal adipose tissue. The Fe600 treatment significantly increased the concentrations of cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in serum and the concentrations of cis-aconitic acid and acetyl-CoA carboxylase and glyceraldehyde-3-phosphate dehydrogenase in liver. In parallel, it also significantly increased the expression of acyl-Co A synthetase long-chain family member 1 (*ACSL1*) mRNA in the liver, and decreased the expression of triglyceride lipase (*ATGL*) mRNA in abdominal adipose tissue. The Fe0 treatment significantly increased the mRNA expression of acetyl-CoA carboxylase (*ACC*), sterol regulatory element binding protein *1* (*SREBP1)* in liver and the fatty acid transposase (*CD36*) in abdominal adipose tissue. In conclusion, dietary high and low iron levels increased abdominal fat deposition in broilers by promoting TCA cycle and *ACSL1* expression or by promoting *ACC* and *SREBP1* expression, respectively.

Keywords: Iron; Lipid Metabolism; Broilers; Adipose Fat Tissue

1. Introduction

The liver plays a vital role in *de novo* fatty acid synthesis, and more fat deposits in abdominal adipose tissue of chickens. Hepatic synthetic fatty acids are the components of triglycerides which are secreted into blood as very low-density lipoprotein (VLDL), and then transported to the peripheral tissues [1]. In poultry, the 95% of the hepatic *de novo* fatty acids is deposited in the peripheral tissues, with 20% of total body fat in abdominal fat tissue [2]. Iron is an essential trace mineral and iron deficiency and iron overload can affect lipid metabolism in animals [3]. Several studies found that iron-overload decreased abdominal fat deposition and inhibited the proliferation and differentiation of precursor adipocytes [4]. Low iron increased lipid content in the body of mice and decreased blood triglyceride and cholesterol in broilers [5]. However, the underlying mechanisms of iron affecting the lipid metabolism remain unknown, although inconsistent results occur in previous studies. Therefore, this study aimed to investigate the effects of iron on hepatic lipid metabolism in broiler chickens, which could provide insight to regulate the lipid deposition in poultry.

2. Materials and Methods

All experimental protocols in this study were reviewed and approved by the Animal Experimental Committee of Sichuan Agricultural University (No.20181105).

2.1 Animals and Diets

A total of 640 one-day-old male broilers (Arbor Acres) were randomly allotted into 4 treatments with 8 replicates per group and 20 birds per replicate. The 4 treatments were the basal diet (Table 1) added with 0, 60, 300, or 600 mg Fe/kg (Fe0, Control, Fe300, or Fe600), respectively. The added iron source was $FeSO_4 \cdot 7H_2O$. The analyzed Fe concentrations were 72.19, 141.8, 335.5 and 727.7 mg Fe/kg for starter broiler and 64.12, 128.9, 319.6 and 653.5 mg/kg for grower broiler. The Fe levels in the Control group were close to the

requirement of broilers described by Ma et al. [6], and this group was used as the control. The nutrients levels except Fe in the basal diet met or exceeded the requirement of broilers recommended by Chinese Chicken Feeding Standard (NY/T 33-2004). The broilers were fed the different diet for 42 days. Water and feed were provided *ad libitum*.

Table 1. Composition and Nutrient Levels of Basal Diet (Air-Dry Basis) (%)

Items	Starter (d 1-21)	Grower (d22-35)
Ingredients		
Corn	36.70	43.75
Unhusked rice	20.10	14.61
Broken rice	12.00	12.90
Soy-protein	17.35	18.50
Casein	6.65	2.22
Soybean oil	2.00	1.80
DL-methionine	0.25	0.20
Calcium carbonate	1.78	1.65
Dicalcium phosphate	1.30	1.00
Sodium chloride	0.30	0.30
Corn starch	1.00	2.50
Choline chloride	0.34	0.34
Vitamin-minerals mix[1]	0.23	0.23
Nutrient levels		
Metabolizable energy (kcal/kg)	3202	3201
Crude protein	23.01	20.01
Calcium	1.01	0.91
Available phosphorus	0.49	0.39
Lysine	1.33	1.07
Methionine	0.67	0.52

[1]Provided the following (per kg of the diet): vitamin A 9000 IU, vitamin D3 1500IU, vitamin E 7.5 IU, vitamin B1 0.6 mg, vitamin B2 4.8 mg, vitamin B6 1.5 mg, vitamin B12 7.5 mg, folic acid 0.15 mg, nicotinamide 35 mg, Cu (CuSO4·5H2O) 8 mg, Mn (MnSO4·H2O) 120 mg, Zn (ZnSO4·H2O) 100 mg, I(KI) 0.7 mg, and Se (Na2SeO3) 0.3 mg.

2.2 Samples Collection

On day 42, 4 broilers per replicate were randomly selected. The blood samples were collected through jugular vein puncture and then separated serum. After sacrificed, the kidney, liver and abdominal fat tissue of one broiler each replicate were collected for iron analysis. Another batch of the liver and abdominal tissue subsamples were collected from another one bird each replicate for the m RNA expression of genes. The abdominal fat samples (the central part of the abdominal fat) were fixed in 4% paraformaldehyde for histology determination.

2.3 Growth Performance

On day 42, the broilers were weighed after 8 h fasting, and average daily gain (ADG) was calculated during day 1 to 42. Dietary consumption was determined to calculate average daily feed intake (ADFI) and the ratio of feed consumption: body weight gain (F: G).

2.4 Serum Biochemical Indices

The serum was separated by centrifugation at 3,000 g at 4℃ for 10 min. The concentrations or activities of creatinine, urea, total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), nonesterified fatty acid (NEFA), triglyceride (TG), alanine aminotransferase (ALT), and glutamine aminotransferase (AST) in serum samples were determined by a biochemical auto-analyzer (Hitachi automatic biochemical analyzer 7600, Tokyo, Japan). Serum iron, total iron binding capacity (TIBC), Ferritin and transferrin (Tf) were measured using specific commercial assay kits (purchased in Nanjing Bioengineering Institute, Nanjing, China).

2.5 Analysis of Fe Content in Tissues

About 0.5 g of liver, kidney, or adipose fat tissue were digested using 5 mL of acid mixture (HNO_3:$HClO_4$ = 4:1, v/v). The completely digested samples were re-dissolved using a certain volume of 5% nitric acid, and the concentration of Fe was determined by Atomic Absorption Spectroscopy. The Fe concentration was expressed by μg/g wet-tissue.

2.6 Enzymes Activities Determination

About 0.5 g of liver and abdominal fat tissue was homogenized using the saline at 4 ℃. And then, the homogenate was centrifuged at 3,500×g at 4 ℃ for 10 min, and the supernatant was used to determine enzyme activities. The activities of acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), aconitase (ACO), stearoyl-CoA desaturase (SCD1), citrate synthase (CS) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were determined using the commercial assay kits.

2.7 mRNA Expression of Target Genes

Total RNA was extracted from liver and abdominal fat tissue using the RNAiso Plus reagent (TaKaRa, Japan). Complementary DNA (cDNA) was synthesised from RNA using NovoScript Plus All-in-one 1st Strand cDNA Synthesis Mix (TaKaRa, Shiga, Japan) and stored at -20℃. Quantitative real-time PCR was done using a CFX96TM real-time system (Bio-Rad, USA). The PCR reaction system was from the TB Green Premix Ex Taq instructions. Primer sequences used for PCR are listed in Table 2. The reaction condition was as follows: 95℃ denaturation for 30 s, 40 cycles of denaturation at 95℃ for 5 s, annealing and extension at 60℃ for 10 s, and finally melting curve analysis (TaKaRa, Shiga, Japan). Using β-actin as a reference gene, relative quantification of gene expression was calculated as previous description [7]. The control group was used as a correction factor, defining mRNA expression of target gene in control as 1.

Table 2. Composition and Nutrient Levels of Basal Diet (Air-Dry Basis) (%)

Genes	Primer sequences (5'-3')	GeneBank No.
β-Actin	F:GAGAAATTGTGCGTGACATCA/R:CCTGAACCTCTCATTGCCA	NM_205518
FAS	F:CTGTCGGGTCACCTTCAAC/R:TCCTCTGCTGTCCCAGTCTT	NM_205155
ACC	F:TGTGGCTGATGTGAGCTTTC/R:ACTGTCGGGTCACCTTCAAC	NM_205505
LXRα	F:GACCTGAGCTATAATCGGGATG/R:CAGGTGATCATTTGGTCTGTTG	AF492498.1
PPARY	F:CACTGCAGGAACAGAACAAAGAA/R:TCCACAGAGCGAAACTGACATC	NM_001001460
SREBP1	F:CATCCATCAACGACAAGATCGT/R:CTCAGGATCGCCGACTTGTT	AJ310768.1
SREBP2	F:GCCTCTGATTCGGGATCACA/R:TCAGGTTCATCCTTAACCTTTGC	AJ310769.1
C/EBPβ	F:GCCGCCCGCCTTTAAA/R:CCAAACAGTCCGCCTCGTAA	NM_205253
HMGCS1	F:GGTACAGAAGTCGGTGGCAA/R:CAACCGTAGACTGAAGGCGT	NM_205411
ACSL5	F:GGTTCACAAGGAGAGTGCAGGAAG/R:TCTGAGGCTAGGAGCAGGAAGTTC	NM_001031237
CPT-1	F:GGGTTGCCCTTATCGTCACA/R:TACAACATGGGCTTCCGTCC	DQ314726
ACLY	F:CACCCAGAGGTGGATGTTCT/R:GTTGCAGGCCCAATGTTAGT	NM_001030540
FABP2	F:ACTTAGCAGCACATCAAC/R:CAGTCAC TAACCCATTCAT	NM_001007923
ACSL1	F:TACCCTGGTGGGTTTTGGTG/R:AGGAGAGAGGACCTTCGAGC	XM_046916038
ACSL6	F:AATGACACAGCTGAAGCGGA/R:ATTGAGCATGGTGGTACCCG	XM_040682779
ATGL	F:CACTGCCATGATGGTCCCCTA/R:CCACAAGGAGATGCTGAAGAA	NM_001113291
LPL	F:GACAGCTTGGCACAGTGCAA/R:CACCCATGGATCACCACAAA	NM_205282.1
FABP4	F:ATGAGACCACAGCAGATGACAGAA/R:TCCACCAGCAGGTTCCCATC	NM_204290
CD36	F:ACCAGACCAGTAAGACCGTGAAGG/R:ATGTCTAGGACTCCAGCCAGTGTG	NM_001030731

FAS = fatty acid synthase; ACC = acetyl CoA carboxylase; LXRα liver X receptor alpha =; PPARγ = peroxisome proliferator-activated receptors gamma; SREBP = sterol regulatory element binding protein; C/EBPβ = CCAAT/enhancer binding protein beta; HMGCS =; ACSL = long-chain lipoyl-CoA; CPT-1 = synthetase; ACLY = ATP-citrate lyase; FABP = fatty acid binding protein; ATGL = adipose triglyceride lipase; LPL = lipoprotein lipase; CD36 = fatty acid transporters.

2.8 Adipocyte Size Determination

Abdominal fat samples were stained with haematoxylin and eosin (H&E), fixed in 4% paraformaldehyde solution for 24 h, and then were dehydrated in graded ethanol concentrations and embedded in paraffin. Sections were deparaffined in xylene, hydrated in ethanol and stained with H&E. Adipocyte sizes were measured using a light microscope

(OLYMPUS, DP73, Nikon Corporation, Japan) and the supporting software.

2.9 Statistical Analysis

All data are showed as mean and standard deviation, and analyzed by one-way ANOVA analysis using GLM procedure of SAS Statistics version 9.4. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Growth Performance and Tissue Weights

Dietary Fe600 treatment increased F: G, the absolute weights and the percentage of liver and abdominal fat tissue relative to BW compared to control (Table 3). However, the Fe300 did not alter the BW, F: G, and the absolute weights and the percentage of liver and abdominal fat tissue relative to BW. And dietary iron treatments did not affect the ADG and ADFI during day 1-42 and BW at 42 days of age. The Fe0 treatment increased the absolute and relative weights of abdominal fat tissue ($P < 0.05$), but did not influence the the absolute weights and the percentage of liver relative to BW, F: G, and the aforementioned performance indices ($P > 0.10$).

3.2 Serum Biochemical Parameters

Dietary iron level did not alter the concentrations of creatinine and urea, and ALT and AST activities in serum (data not shown). The Fe600 treatment increased the concentrations of TC, HDLC and LDLC ($P < 0.05$) and the Fe300 increased serum HDLC level while decreased serum NEFA and TG levels ($P < 0.05$) in comparison with control (Table 4). The Fe0 treatment significantly reduced serum TC, HDLC and TG levels ($P < 0.05$).

3.3 Serum Iron Status

In comparison with control, the Fe600 significantly increased the concentrations of HGB, HCT, RBC, Fe, TIBC, and ferritin ($P < 0.05$), and the Fe300 also significantly increased the aforementioned indices except for TIBC (Table 5). The Fe0 treatment only decreased serum Fe level and Tf, while increased serum ferritin level ($P < 0.05$).

3.4 Iron Concentrations in Different Tissues

Compared with the control, the Fe600 and Fe 300 increased Fe concentration in abdominal fat tissue, kidney and liver, although the difference of liver Fe did not attain significant level between Fe300 and control groups (Table 6). However, the Fe0 treatment did not influence Fe concentration in abdominal adipose tissue, kidney, and liver compared with control.

3.5 Liver Lipid Deposition and Abdominal Adipocyte Size

The Fe600 treatment increased liver TC concentration, but did not change liver TG and abdominal adipocyte size (Table 7). The Fe300 did not alter the aforementioned parameters. The Fe0 treatment significantly decreased liver TG, but increased abdominal adipocyte size ($P < 0.05$). The Fe600 did not significantly affect liver TC and TG, and abdominal adipocyte size compared with Fe300 treatment.

Table 3. The Growth Performance and Different Tissue Weights of Broilers

Items[1]	Fe0	Control	Fe300	Fe600	SEM	*P*-Value
BW on day 1(g)	41.82	41.79	41.93	41.73	0.12	0.686
BW on day 42(g)	2566	2368	2337	2133	158.9	0.341
F: G (g/g)	1.56^{b}	1.55^{b}	1.57^{ab}	1.60^{a}	0.01	0.018
ADG(g)	60.10	55.39	54.63	49.79	3.78	0.341
ADFI(g)	93.28	86.64	85.79	79.31	5.63	0.426
Liver(g)	40.26^{b}	39.57^{b}	40.34^{b}	46.98^{a}	1.96	0.045
Liver weight/BW (%)	17.24^{b}	16.94^{b}	17.05^{b}	19.98^{a}	0.69	0.013
Abdominal adipose tissue (g)	35.62^{a}	28.97^{b}	34.39^{a}	37.51^{a}	1.72	0.019
Abdominal adipose tissue/BW (%)	15.24^{a}	12.40^{b}	14.40^{a}	15.94^{a}	0.68	0.011

[1]BW = body weight; F: G = feed consumption: body weight gain; ADG = average daily gain; ADFI = average daily feed intake.

[a, b]Values without same superscripts differ ($P < 0.05$).

Table 4. Serum Biochemical Indices of broilers

Items[1]	Fe0	Control	Fe300	Fe600	SEM	*P*-Value

TC (mmol/l)	3.27^{c}	3.66^{b}	3.96^{b}	4.34^{a}	0.14	<.001
HDLC (mmol/l)	2.34^{c}	2.66^{b}	2.91^{a}	2.98^{a}	0.10	<.001
LDLC (mmol/l)	0.44^{b}	0.51^{b}	0.52^{b}	0.73^{a}	0.07	0.026
NEFA (mmol/l)	0.62^{ab}	0.75^{a}	0.50^{b}	0.60^{ab}	0.05	0.049
TG (mmol/l)	0.43^{b}	0.56^{a}	0.43^{b}	0.53^{a}	0.04	0.002

[1]TC = total cholesterol; HDLC = high density lipoprotein cholesterol; LDLC = low density lipoprotein cholesterol; NEFA = non-esterificated fatty acids; TG = triglyceride.

[a, b]Values without same superscripts differ ($P < 0.05$).

Table 5. Serum Iron Status Relevant Indices

Items[1]	Fe0	Control	Fe300	Fe600	SEM	*P*-Value
HGB (g/L)	67.29^{bc}	65.88^{c}	69.25^{ab}	70.63^{a}	1.11	0.028
HCT (%)	28.71^{b}	27.96^{b}	30.76^{a}	31.13^{a}	0.55	0.001
RBC (10^{12}/L)	2.31^{ab}	2.23^{b}	2.42^{a}	2.44^{a}	0.05	0.025
Fe (μmol/l)	19.13^{c}	27.03^{b}	35.23^{a}	35.78^{a}	4.84	<.0001
TIBC (μmol/L)	55.83^{b}	57.18^{b}	58.42^{b}	75.93^{a}	4.78	0.019
Tf (%)	30.24^{c}	40.56^{b}	52.95^{a}	42.84^{b}	2.24	<.0001
Ferritin(ng/ml)	237.0^{a}	146.1^{c}	206.5^{b}	214.7^{ab}	8.99	<.0001

[1]HGB = hemoglobin; HCT = hematocrit; RBC = red blood cell; TIBC = total iron binding capacity; Tf = transferrin.

[a, b]Values without same superscripts differ ($P < 0.05$).

Table 6. Iron Concentrations in Different Tissues

Items	Fe0	Control	Fe300	Fe600	SEM	*P*-Value
Abdominal adipose (μg/g)	49.19^{b}	50.89^{b}	78.05^{a}	76.30^{a}	5.71	0.001
Kidney(μg/g)	43.05^{b}	42.66^{b}	53.05^{a}	53.28^{a}	3.33	0.041
liver(μg/g)	101.5^{c}	119.2^{bc}	146.9^{ab}	177.1^{a}	11.40	<0.001

[a-c]Values without same superscripts differ ($P < 0.05$).

Table 7. Liver Lipid Concentrations and Abdominal Adipocyte Size

Items	Fe0	Control	Fe300	Fe600	SEM	*P*-Value
Liver TG (mmol/gprot)	2.11^{b}	3.51^{a}	3.02^{ab}	3.65^{a}	0.37	0.038
Liver TC (mmol/gprot)	4.68^{b}	4.73^{b}	4.80^{b}	6.97^{a}	0.59	0.041
Abdominal adipocyte area (μm^2)	3633^{a}	2551^{b}	2346^{b}	2853^{b}	225.4	0.004

[a,b]Values without same superscripts differ ($P < 0.05$).

3.6 Concentrations or Activities of Enzymes Involving in Liver Lipid Metabolism

The Fe600 group significantly increased ACO activity, and the concentrations of G3PDH, and ACC, while decreased SCD1 activity ($P < 0.05$) (Table 8). The Fe300 group significantly increased ACO activity, while decreased SCD1 activity ($P < 0.05$). The Fe0 treatment tented to increase liver CS content ($P = 0.083$), but decreased SCD1 activity ($P < 0.05$).

3.7 mRNA Abundance of Target Genes in Liver

The Fe600 significantly increased the expression of long-chain fatty acyl CoA synthetase 1 (*ACSL1*) in liver, and the Fe300 significantly increased the mRNA expression of peroxisome proliferators-associated receptor γ (*PPARγ*) and sterol regulatory element-binding protein 1 (*SREBP1*) (Table 9). The Fe0 treatment increased the mRNA expression of *ACC*, *SREBP1*, and carnitine palmitoyltransferase 1 (*CPT-1*) in liver ($P < 0.05$). As dietary iron level increased, the mRNA expression of adipose acyl-CoA synthase (*ACSL6*) and citrate lyase (*ACLY*) tended to decrease ($P < 0.10$), and the 3-hydroy-3-methylglutaryl-CoA synthase 1 (*HMGCS1*) mRNA abundance tended to increase ($P = 0.074$).

Table 8. Concentrations or Activities of Enzymes Involving in Lipid Metabolism in Liver of Broilers

Items[1]	Fe0	Control	Fe300	Fe600	SEM	*P*-Value
ACO (U/L)	44.17^{b}	43.78^{b}	62.35^{a}	67.11^{a}	4.74	0.003
CS (ng/ml)	16.10	14.47	13.79	14.85	0.61	0.082

FAS (ng/ml)	2.68	2.59	2.70	2.47	0.19	0.822
ACC (ng/ml)	1.08^{c}	1.11^{bc}	1.12^{ab}	1.15^{a}	0.01	0.003
SCD1 (U/L)	56.20^{d}	107.7^{a}	86.80^{b}	75.79^{c}	2.65	<.0001
G3PDH (ng/ml)	4.20^{b}	3.70^{b}	4.51^{b}	6.35^{a}	0.57	0.033

1ACO = aconitase; CS = citrate synthase; FAS = fatty acid synthase; ACC = acetyl-CoA carboxylase; SCD1 = steroyl-CoA desaturase 1; G3PDH = glyceraldehyde-3-phophate dehydrogenase.
^{a,b}Values without same superscripts differ ($P < 0.05$).

Table 9. mRNA expression of Genes in Liver of Broilers

Items[1]	Fe0	Control	Fe300	Fe600	SEM	*P*-Value
LXRα	0.86	1.00	0.87	1.07	0.14	0.707
ACC	1.89^{a}	1.00^{b}	1.64^{ab}	1.20^{b}	0.18	0.047
FAS	1.58	1.00	1.86	2.88	0.66	0.304
PPARγ	1.03^{b}	1.00^{b}	2.85^{a}	1.57^{ab}	0.46	0.032
SREBP1	1.43^{a}	1.00^{b}	1.37^{a}	1.17^{ab}	0.11	0.048
SREBP2	0.88	1.00	1.00	1.27	0.20	0.569
C/EBPβ	1.35	1.00	1.15	1.61	0.34	0.661
HMGCS1	0.79	1.00	1.02	1.22	0.12	0.074
CPT-1	1.77^{a}	1.00^{b}	0.88^{b}	1.12^{b}	0.19	0.009
ACLY	1.19	1.00	0.80	0.74	0.13	0.087
FABP2	1.12	1.00	1.36	1.28	0.32	0.878
ACSL1	0.90^{b}	1.00^{b}	0.87^{b}	1.86^{a}	0.27	0.044
ACSL5	0.73^{b}	1.00^{ab}	0.71^{b}	1.84^{a}	0.29	0.034
ACSL6	1.75	1.00	1.05	0.87	0.26	0.059

[1]LXRα = liver X receptor α; ACC = acetyl-CoA carboxylase; FAS = fatty acid synthase; PPARγ = peroxisome proliferators-activated receptor γ; SREBP1 = sterol regulatory element-binding protein 1; SREBP2 = sterol regulatory element-binding protein 2; C/EBPβ = CCAAT/enhancer binding protein β; HMGCS1 = 3-hydroxy-3-methylglutaryl-CoA synthase 1; CPT-1 = carnitine palmitoyltransferase1; ACLY = ATP-citrate lyase; FABP2 = fatty acid binding protein 2; ACSL = adipose acyl-CoA synthetase.
^{a,b}Values without same superscripts differ ($P < 0.05$).

3.8 mRNA Abundance of Target Genes in Abdominal Adipose Tissue

The Fe600 and Fe300 treatment decreased the mRNA expression of adipose triglyceride lipase (*ATGL*) in abdominal adipose tissue, although the difference between Fe300 and control did not attain significant level (Table 10). They did not alter the mRNA expression of lipoprotein lipase (*LPL*), fatty acid binding protein 4 (*FABP4*) and fatty acid transporter (*CD36*). The Fe0 treatment significantly increased the mRNA expression of *CD36*, while had no significant effect on the mRNA abundance of *ATGL*, *LPL*, and *FABP4* in abdominal adipose tissue ($P < 0.05$).

Table 10. mRNA expression of Genes in Abdominal Adipose Tissue of Broilers

Items[1]	Fe0	Control	Fe300	Fe600	SEM	*P*-Value
ATGL	0.86^{a}	1.00^{a}	0.76^{ab}	0.52^{b}	0.11	0.043
LPL	1.38	1.00	1.36	1.34	0.21	0.569
CD36	1.43^{a}	1.00^{b}	1.08^{b}	0.78^{b}	0.12	0.014
FABP4	1.47	1.00	1.03	0.93	0.15	0.087

[1]ATGL = adipose triglyceride lipase; LPL = lipoprotein lipase; CD36 = platelet glycoprotein 4; FABP4 = fatty acid binding protein 4.
^{a,b}Values without same superscripts differ ($P < 0.05$).

4. Discussion

In this study, high dietary iron level decreased the feed conversion efficiency in broilers during day 1-42, which was in line with previous findings that high iron decreased the performance of broiler chickens [8, 9]. The decrease in broiler performance may be due to oxidative stress caused by excessive iron intake affecting intestinal absorptive capacity [8, 10]. Organ indices reflect the development and metabolic intensity of the organs. The decrease of liver index caused by Fe0 and Fe600 treatments present herein suggested that low iron and iron overload negative affect the development and metabolism of the liver [9]. No changes in serum creatinine, urea, and the

activities of serum AST, and ALT indicated that the high level in Fe600 treatment did not cause the toxic effect on the liver of broilers, although it might cause oxidative stress to the broilers. The previous study found that dietary addition of 40 mg Fe/kg could maintain the growth of major organs in animals [6]. In this study, the iron level in the basal diet was 64.12 mg/kg, which could meet the requirement of iron for broiler growth. Therefore, we need lower iron levels in the basal diet to cause the Fe-deficient broilers in future.

Blood HGB and HCT, and serum iron, TIBC, and ferritin concentrations can reflect the iron status of animals [7]. We found that the Fe600 and Fe300 treatment increased HCT, RBC and serum iron, which demonstrated that they all caused the Fe-overload broilers. The increase in serum Tf caused by the Fe300 treatment indicated the negative feedback of liver of broiler to mild iron-overload increased the synthesis of more Tf and ferritin binding to the iron ion. The increases in the blood HCT and RBC, and serum Fe was similar to the previous finding addition of 2% carbonyl iron significantly increased plasma Fe, ferritin, TIBC, unsaturated iron-binding capacity and Tf in mice [10]. In the present study, the serum iron and Tf caused by the Fe0 treatment indicated that the Fe0 treatment caused the Fe-deficient broilers although it did not alter the growth performance of broilers. In parallel, we also found that the Fe300 and Fe600 treatment increased iron level in liver, kidney, and abdominal adipose tissue of broilers, which also suggested the Fe-overload status.

In this study, the increase in TG content in the liver and serum and increase in abdominal adipose tissue weight indicated the iron-overload increased the TG synthesis and more TG synthesized in liver was transported to the epithelial tissue for deposition. The increases in serum HDL and LDL levels caused by the Fe600 treatment indicated an increase in more TC transporting back to the liver from the epithelial tissues. The present results was in agreement with the previous report in mice that high iron significantly increase liver TC and TG content in mice [10]. However, we found that low iron decreased liver TG, and serum HDL and TC, which indicated the iron-deficient broilers had low TG and TC synthesis capacity. In contrast, iron-deficient rats increase hepatic lipogenesis capacity, leading to cellular TG accumulation and steatosis. The discrepancy might be due to different *de novo* fatty acid capacity in liver among different animal species.

The ACC plays an important role in hepatic fatty acid synthesis [3]. In this study, the increases of liver ACC activity in Fe600 treatment suggested that iron-overload increased *de novo* fatty acid synthesis in broiler, which was in line with the previous finding in rats [5]. The ACO plays a vital role in the tricarboxylic acid (TCA) cycle and G3PDH is an enzyme of the glycolysis process [1, 4]. G3PDH is positively correlated with fat deposition, which can be used as an indirect marker of TG synthesis [1]. In the present study, the increases in liver ACO and G3PDH activity or concentration suggested the iron-overload promoted the TCA capacity, and simultaneously increased the liver TG synthesis. However, it was note that the Fe0 treatment did not alter the activity or concentration of ACC, ACO, and G3PDH, which seemed to indicate that the low Fe did not affect the lipogenesis in liver of broilers. The SCD1 functions the desaturation of fatty acids [1, 4]. In this study, the Fe600 and Fe0 treatments all decreased SCD1 enzyme levels in the liver. This suggests that iron-deficiency or iron-overload decreased the synthesis of long-chain fatty acid in liver of broilers.

The PPARs and SREBPs were the main signal pathways of lipid metabolism regulation in animals [4]. The SREBP-1c can activate the expression of *ACC* and *FAS* in the liver, promoting lipid synthesis, and SREBP2 regulates the cholesterol synthesis [1]. In this study, the Fe0 treatment increased *SREBP-1* and *ACC* mRNA, and the Fe600 increased *SREBP1* and *PPARγ*, which indicated that both iron-deficiency and iron-overload increased the lipogenesis in liver of broilers. These results were in agreement with the finding described by Davis [11] that rats fed a low-iron diet (3 mg/kg) showed a significant increase in the expression of genes related to adipogenesis. The CPT-1 is a rate-limiting enzyme in the β-oxidation process [1]. The increase of CPT-1 mRNA expression caused by the Fe0 treatment indicated that iron-deficiency enhanced the lipolysis, consequently reducing fat deposition in liver of broilers. In contrast, iron-overload did not affect hepatic CPT-1 mRNA expression, which suggested it did not promote the lipolysis

in liver of broilers. This is consistent with the previous results in mice that iron-overload increased CPT-1 mRNA expression in the liver [1,4]. The ACSL is involved in the whole-body energy metabolism process in animals, which can catalyze the generation of long-chain fatty acids into long-chain lipoyl-CoA, promoting the synthesis of triglycerides and intracellular fat deposition [1, 4]. In this study, the increases in ACSL1 and ACSL5 mRNA expression indicated that iron-overload promoted the triglycerides synthesis.

In adipose tissue, the FABP4 involveed in the lipid uptake in adipocytes from the epithelial blood [1,4]. In this study, the increase of FABP4 mRNA expression caused by the Fe0 treatment suggested that the iron-overload increased lipid uptake in the abdominal fat tissue of broilers. The CD36 involve in the uptake of long-chain fatty acids and the ATGL is responsible for the first step of TG hydrolysis and prevents excessive accumulation of fat in the body [1, 7]. The decrease in the mRNA expression of ATGL caused by the Fe600 group indicated that iron-overload reduced the degradation of TG in abdominal fat tissue. The increase in the mRNA expression of CD36 in the Fe0 group suggested that iron-deficiency increased the uptake of fatty acids in abdominal adipocytes, promoting abdominal fat deposition.

In conclusion, high dietary iron level enhanced hepatic lipid synthesis, evidenced by the increases in the mRNA expression of *PPARγ* and *SREBP1*, and their regulatory factors ACC, ACO and G3PDH, and promoted TCA cycle and glycolysis. The low dietary iron level also increased abdominal fat weight in broilers increased hepatic lipid synthesis, evidenced by increases in *SREBP1* and *ACC*, but did not affect the glycolysis in liver of broilers.

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