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Non Ruminant Nutrition

Dietary manganese supplementation decreases hepatic lipid deposition by regulating gene expression and enzyme activity involved in lipid metabolism in the liver of broilers

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Abstract

This study aimed to characterize the effects of different dietary forms of supplemental manganese (**Mn**) on hepatic lipid deposition, gene expression, and enzyme activity in liver fat metabolism in 42-d-old broiler chickens. In total 420 one-day-old Arbor Acres (AA) broilers (rooster:hen = 1:1) were assigned randomly based on body weight and sex to 1 of 6 treatments (10 replicate cages per treatment and 7 broilers per replicate cage) in a completely randomized design using a 2 (sex) × 3 (diet) factorial arrangement. The 3 diets were basal control diets without Mn supplementation and basal diets supplemented with either Mn sulfate or Mn proteinate. No sex × diet interactions were observed in any of the measured indexes; thus, the effect of diet alone was presented in this study. Dietary Mn supplementation increased Mn content in the plasma and liver, adipose triglyceride lipase (**ATGL**) activity, and *ATGL* mRNA and its protein expression in the liver by 5.3% to 24.0% ($P < 0.05$), but reduced plasma triglyceride (**TG**), total cholesterol, and low-density lipoprotein (**LDL-C**) levels, liver TG content, fatty acid synthase (**FAS**) and malic enzyme (**ME**) activities, mRNA expression of sterol-regulatory element-binding protein 1 (***SREBP1***), *FAS*, stearoyl-coA desaturase (***SCD***), and *ME*, as well as the protein expression of SREBP1 and SCD in the liver by 5.5% to 22.8% ($P < 0.05$). No differences were observed between the 2 Mn sources in all of the determined parameters. Therefore, it was concluded that dietary Mn supplementation, regardless of Mn source, decreased hepatic lipid accumulation in broilers by inhibiting SREBP1 and SCD expression, FAS and ME activities, and enhancing ATGL expression and activity.

Lay Summary

Dietary manganese supplementation regulates lipid deposition in broiler chickens, with the liver being a significant site of lipid metabolism. This study investigated the effects of different dietary forms of supplemental manganese on hepatic lipid deposition, gene expression, and enzyme activity in the liver fat metabolism of broiler chickens. The results showed that dietary manganese supplementation decreased the hepatic lipid accumulation of broilers by inhibiting the expression of sterol-regulatory element-binding protein 1 (**SREBP1**) and stearoyl-coA desaturase (**SCD**), as well as fatty acid synthase (**FAS**) and malic enzyme (**ME**) activities, and enhancing the expression and activity of adipose triglyceride lipase (**ATGL**). This reduction in excessive fat production will help improve poultry health and mitigate losses in the poultry industry.

Key words: broiler, enzyme activity, gene expression, liver lipid deposition, manganese source

Abbreviations: AA, Arbor Acres; ACC, acetyl-CoA carboxylase; ADFI, average daily feed intake; ADG, average daily gain; ATGL, adipose triglyceride lipase; DGAT2, diacylglycerol acyltransferase 2; ELISA, enzyme-linked immunosorbent assay; FAS, fatty acid synthase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HDL-C, high-density cholesterol; IDL, intermediate-density lipoproteins; LDL-C, low-density lipoprotein; ME, malic enzyme; Mn, manganese; PPARγ, peroxisome proliferator-activated receptor γ; SCD, stearoyl-coA desaturase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SREBP1, sterol-regulatory element-binding protein 1; TC, total cholesterol; TG, triglyceride

Introduction

In poultry, plasma lipids are primarily triglycerides (TGs), which are synthesized in liver cells and stored in fat cells (Wang et al., 2017; Nematbakhsh et al., 2021). Similar to humans, chickens primarily synthesize fatty acids in the liver (Lin et al., 2021; Cui et al., 2022). Understanding the intrinsic transcriptional and post-transcriptional regulatory mechanisms of fatty acid synthesis is important for the prevention of fatty liver in poultry.

Manganese (**Mn**) is an indispensable micromineral for poultry because it affects protein, fat, and carbohydrate metabolism (Li and Yang, 2018). The lipophilic factor Mn

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plays a key role in fat metabolism in poultry. Dietary Mn supplementation significantly reduced fat deposition (Lv et al., 2022). Numerous studies have demonstrated that adding 110 mg Mn/kg to diets in broilers could decrease abdominal fat accumulation (Lu et al., 2006, 2007). Reportedly, transcriptional factors, such as sterol-regulatory element-binding protein 1 (**SREBP 1**), regulate liver fat metabolism (Wang et al., 2009). SREBP1 is a critical putative transcriptional factor that initiates the initial lipid synthesis pathway. Meanwhile, it has been shown that modulation of adipocyte differentiation by SREBP1 is mediated through its effect on the expression or functionality of specific target genes in lipid metabolism (Wang et al., 2017; Wang, 2019). Acetyl-CoA carboxylase (**ACC**), fatty acid synthase (**FAS**), and stearoyl-CoA desaturase (**SCD**) are crucial enzymes related to lipid metabolism (Liu et al., 2018). SREBP1 regulates TG production and the expression of FAS, SCD, ACC, and other enzymes (Liu et al., 2010; Garcia Caraballo et al., 2017). Lu et al. (2017) found that Mn reduced chicken liver cell injury and regulated the expression of fat metabolism-related enzymes such as FAS and malic enzyme (**ME**) at the transcriptional level. In our previous experiment, the addition of dietary Mn decreased the abdominal lipid accumulation of broilers by downregulating the expression of peroxisome proliferator-activated receptor γ (**PPARγ**) and diacylglycerol acyltransferase 2 (**DGAT2**) and enhancing triglyceride lipase (**ATGL**) expression and activity in the abdominal fat of broilers. Furthermore, Mn proteinate with moderate chelation strength (Mn-Prot M, Q_f value = 51.8), but not Mn sulfate, effectively decreased the FAS activity compared with the Mn-unsupplemented control, although no difference was observed between Mn-Prot M and Mn sulfate (Cui et al., 2023). However, whether dietary Mn supplementation affects the liver fat accumulation of broilers and the possible mechanisms involved remain unclear. These studies suggest that Mn supplementation modulate fat metabolism by probably affecting SREBP1 expression and then regulating the transcription or translation of fat metabolism-related genes, including FAS, ACC, and SCD, in broilers. Therefore, we hypothesized that dietary Mn supplementation would decrease liver fat deposition by modulating fat metabolism-related enzyme activities and gene expression in the liver of broilers and that Mn-Prot M would be superior to Mn sulfate. Additionally, roosters and hens may respond differently to the modulating effects of dietary Mn addition.

This study aimed to investigate the effect of dietary added Mn source (Mn sulfate or Mn-Prot M) on plasma TG, total cholesterol (**TC**), low-density cholesterol (**LDL-C**), high-density cholesterol (**HDL-C**) levels, liver fat accumulation, and fat metabolism-related enzyme (e.g., ATGL, ACC, and FAS) activities, as well as the mRNA and protein expressions of fat metabolism-related genes (e.g., SREBP1, ACC, FAS, and ATGL) in the liver of roosters or hens to confirm previously stated scientific hypotheses.

Materials and Methods

Animal ethics

Animals were used and cared for according to the Committee on Animal Use of the Ministry of Agriculture of China (Beijing, China) guidelines. The Animal Ethics Committee of Yangzhou University approved this study (approval number: SYXK (Su) 2021-0027).

Experimental design and treatments

Six treatments in total were included in a completely randomized design with a 2 (sex) × 3 (diet) factorial arrangement. The 2 sexes were rooster and hen, and the 3 diets were used: basal control diets without Mn supplementation (control, 17.52 mg Mn/kg for days 1 to 21 and 15.62 mg Mn/kg for days 22 to 42 based on analysis, respectively) and basal diets supplemented with Mn (110 mg/kg for days 1 to 21 and 80 mg/kg for days 22 to 42) in the form of either feed grade Mn sulfate ($MnSO_4.H_2O$, 32.19% Mn by analysis) or feed grade Mn-Prot M (15.85% Mn and Q_f = 51.8 by analysis). The levels of dietary Mn added were based on the Mn requirements (total dietary Mn levels of approximately 130 mg/kg [1 to 21 d] and 100 mg/kg [22 to 42 d]) of broilers as estimated in our previous studies (Li et al., 2011; Lu et al., 2016). The 2 Mn sources and dietary supplemental Mn levels were previously reported by Cui et al. (2023).

Animals and diets

Based on the experimental design and treatments described above, 420 one-day-old Arbor Acres (**AA**) broilers (rooster:hen = 1:1) were randomly divided into 1 of 6 treatments based on body weight and sex, with 10 replicate cages of 7 broilers per cage for each treatment. Birds were fed the 3 diets for 42 d. The corn–soybean meal basal diets were formulated to meet or exceed the requirements of broilers for all other nutrients except for Mn, as recommended by the NRC (1994) and the Ministry of Agriculture of the People's Republic of China (2004). Table 1 lists the composition of the basal diets. Table 2 lists the analyzed Mn contents in the diets. Feeding management, body weight weighing, recording of feed consumption and dead birds, composition of the basal diets, formulation and mixing of all treatment diets, and analyzed Mn contents in all treatment diets throughout the experimental period were the same as in the study by Cui et al. (2023).

Samples collections and preparations

Samples of diets, tap water, Mn sulfate, and Mn-Prot M were collected and analyzed as described by Cui et al. (2023). At 42 d of age, 3 broilers were randomly selected from each replicate cage based on the cage's average body weight and fasted overnight without water restriction. Then, 5 mL of blood was collected into an anticoagulation tube (Anticoagulant: ethylene diamine tetraacetic acid) from the wing vein of each bird and centrifuged to harvest the plasma. After bleeding, the birds were slaughtered by the cervical dislocation. The liver was removed via dissection, and 1.5 g of the liver tip was taken into a 2-mL centrifuge tube, frozen in −196 °C liquid nitrogen, and then transferred to a −80 °C refrigerator for gene expression assays. The remaining liver sample was stored at −20 °C for enzyme activity and TG measurements. All samples of the 3 birds in each replicate cage were mixed in equal proportions to form a single sample before the assays.

Determinations of Mn and lipid contents in plasma and liver

Manganese contents in the plasma and liver were measured as described by Cui et al. (2023). Plasma levels of TG, TC, HDL-C, and LDL-C were measured using a fully automated biochemical analyzer (7180, Hitachi, Japan). Liver TG content was determined using a commercial kit (A110-1-1, Nanjing Jiancheng, China). The steps were as follows: the 50 mg

Table 1. Composition and nutrient levels of the basal diets for 1- to 42-d-old broilers

Item	Days 1 to 21	Days 22 to 42
Ingredients, %		
Corn	53.69	58.44
Soybean meal	37.40	33.00
Soybean oil	4.80	5.00
$CaHPO_4 \cdot 2H_2O$[1]	1.86	1.62
Limestone[2]	1.26	1.17
NaCl[2]	0.30	0.30
DL-Met[2]	0.32	0.16
Micronutrients[3]	0.22	0.16
Cornstarch[4]	0.15	0.15
Total	100.00	100.00
Nutrient levels		
ME, MJ/kg	12.65	12.92
CP[5], %	21.95	20.09
Lys, %	1.12	1.02
Met, %	0.61	0.44
Met + cys, %	0.91	0.72
Ca[5], %	1.03	0.89
Non-phytate P, %	0.45	0.40
Mn[5], mg/kg	17.52	15.62

[1]Reagent grade.
[2]Feed grade.
[3]Provided per kilogram of diet for days 1 to 21: VA 12,000 IU, VD_3 4,500 IU, VE 33 IU, VK_3 3 mg, VB_1 3 mg, VB_2 9.6 mg, VB_6 4.5 mg, VB_{12} 0.03 mg, Pantothenic acid calcium 15 mg, Niacin 54 mg, Folic acid 1.5 mg, Biotin 0.15 mg, Choline 700 mg, Cu ($CuSO_4 \cdot 5H_2O$) 8 mg, Fe ($FeSO_4 \cdot H_2O$) 40 mg, Zn ($ZnSO_4 \cdot H_2O$) 60 mg, Se (Na_2SeO_3) 0.15 mg, I ($Ca(IO_3)2 \cdot H_2O$) 0.35 mg; for days 22 to 42: VA 8,000 IU, VD_3 3,000 IU, VE 22 IU, VK_3 2 mg, VB_1 2 mg, VB_2 6.4 mg, VB_6 3 mg, VB_{12} 0.02 mg, Pantothenic acid calcium 10 mg, Niacin 36 mg, Folic acid 1.0 mg, Biotin 0.10 mg, Choline 500 mg, Cu ($CuSO_4 \cdot 5H_2O$) 8 mg, Fe ($FeSO_4 \cdot H_2O$) 30 mg, Zn ($ZnSO_4 \cdot H_2O$) 40 mg, Se (Na_2SeO_3) 0.15 mg, I ($Ca(IO_3)2 \cdot H_2O$) 0.35 mg.
[4]Mn supplements were added in place of equivalent weights of cornstarch.
[5]Analyzed values are based on triplicate determinations.

Table 2. Analyzed Mn contents in diets of broilers from 1 to 42 d of age[1]

Diet	Added Mn, mg/kg		Analyzed Mn contents, mg/kg	
	Days 1 to 21	Days 22 to 42	Days 1 to 21	Days 22 to 42
Control	0	0	17.52	15.62
Mn sulfate	110	80	124.63	94.59
Mn-Prot M[2]	110	80	122.58	94.98

[1]Values of analyzed Mn contents are based on triplicate determinations.
[2]Mn-Prot M = Mn proteinate with moderate chelation strength (Q_f = 51.8).

of the liver was homogenized using a homogenizer (N9548, HODER, Beijing, China). The mixture was centrifuged at 2,500 × *g* for 10 min at 4 °C, and the supernatant was carefully extracted. Then, 2.5 µL of the supernatant was added to 250 µL of the working solution and incubated at 37 °C for 10 min. After incubation, the absorbance value was measured at 500 nm. These assays were performed using a microplate reader (SPARK 30086376, Tecan Austria GmbH, Grodig, Austria).

Enzyme activity assays

Enzyme-linked immunosorbent assay (**ELISA**) commercial kits were used to determine the activities of FAS (catalog number: FC-9788), ACC (catalog number: FC-4780), ME (catalog number: FC-6637), and ATGL (catalog number: FC-6594) in the liver, following the manufacturer's instructions (Beijing Fangcheng Biotechnology Ltd, Beijing, China). Briefly, 50 mg of the liver sample was homogenized using a homogenizer (N9548, HODER), and the mixture was then centrifuged at 2,000 × *g* for 20 min at 4 °C. The supernatants were transferred to special 96-well plates coated with specific antibodies, incubated at 37 °C for 30 min, washed with washing solutions, and incubated with enzyme labeling reagents at 37 °C for another 30 min. Specific dyes were then added, and incubated at 37 °C for 15 min for color development, and the absorbance values were measured at 450 nm using a microplate reader (SPARK 30086376, Tecan Austria GmbH) to calculate the activities of the above-mentioned enzymes. The protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (catalog number: 23225, ThermoFisher Scientific, Rockford, IL, USA).

Quantitative real-time PCR

Total RNA was isolated from the liver using TRIzol reagent (Vazyme Biotechnology, Nanjing, China). cDNA was prepared for real-time fluorescence quantitative PCR (RT-qPCR) using a commercially available kit (Vazyme Biotechnology). AceQ SYBR Green Master Mix (Vazyme Biotechnology) was used for the RT-qPCR procedure. Real-time PCR was conducted under amplification conditions, and the primers were synthesized by Beijing Tsingke Biotech. The resulting data were normalized using the $2^{-\Delta\Delta Ct}$ method to calculate the relative mRNA expression. The geometric mean of 2 internal reference genes, namely β-actin and glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**), was employed for normalization. Table 3 presents the primer sequences of the relevant genes.

Total protein extraction and western blotting

The liver samples were homogenized using radioimmunoprecipitation assay buffer supplemented with 1% (v/v) phenylmethylsulfonyl fluoride using a homogenizer (N9548, HODER). Lysates were then centrifuged at 12,000 × *g* for 4 min at 4 °C. The liquid above the sediment was collected, and protein concentrations were determined. The liver lysates with a protein content of 40 µg were separated using either 8% or 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**SDS-PAGE**). Protein expression levels of the target genes in the liver were analyzed via Western blotting. Anti-SREBP1 (16643-1-AP14088-1-AP, dilution 1:1,000), anti-ATGL (55190-1-AP, dilution 1:1,000), and anti-SCD (23393-1-AP, dilution 1: 1,000) were purchased from Proteintech Group, Inc. (Chicago, IL, USA). The anti-β-actin (HX1829, dilution 1:5000) antibody was purchased from Huaxingbio (Beijing, China). As for secondary antibodies, anti-mouse (HX2032, dilution 1:5000) or goat anti-rabbit (HX2031, dilution 1:5,000) antibodies were purchased from Huaxingbio. Protein bands were observed using a chemiluminescent image scanner (Tanon Technology Co. Ltd, Shanghai, China).

Statistical analyses

All data were subjected to 2-way ANOVA using the GLM program of SAS 9.4 (SAS, Institute, 2013), with the model

Table 3. Primer sequences for real-time qPCR amplification

Genes	Primer sequence (5′ to 3′)	GenBank ID	Product length (bp)
SREBP1	F: CTACCGCTCATCCATCAACG R: CTGCTTCAGCTTCTGGTTGC	NM_204126.3	145
CD36	F: CTTTCTTGCAAAGCAGGAGGT R: TCTTCGTGAGAGAAGCTGTATG	NM_001030731.1	214
ApoB	F: TCTCAACTTCGTGCAAGCCT R: TGCTGCTTTACACCTTCTGCT	NM_001044633.2	250
ME	F: CATTACGGTTTAGCATTTCGG R: GGTAGGCACTCATAAGGTTTCAC	NM_204303.2	236
FAS	F: CAATGGACTTCATGCCTCGGT R: GCTGGGTACTGGAAGACAAACA	NM_205155.3	119
ACC	F: GCCTCCGAGAACCCAA R: CCAGCAGTCTGAGCCACTA	NM_205505.1	128
SCD	F: GGCTGACAAAGTGGTGATG R: GGATGGCTGGAATGAAGA	NM_204890.1	137
ATGL	F: AAGTCCTGCTGGTCCTCTCCTTG R: AGTGTTGTCCTCCATCTGGTCCTC	NM_001113291.2	94
β-Actin	F: CAGCCATCTTTCTTGGGTAT R: CTGTGATCTCCTTCTGCATCC	NM_205518.2	169
GAPDH	F: GCACGCCATCACTATCTT R: GGACTCCACAACATACTCAG	NM_204305.2	82

SREBP1, sterol-regulatory element-binding protein 1; *CD36*, thrombospondin receptor; *APOB* apolipoprotein B; *ME*, malic enzyme; *FAS*, fatty acid synthase; *ACC*, acetyl-CoA carboxylase; *SCD*, stearoyl-CoA desaturase; *ATGL*, adipose triglyceride lipase; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; F, forward; R, reverse.

including sex, diet, and their interactions. Different treatments were distinguished using the LSD method. Before analysis, the percentage of bird mortality data was transformed into arcsine values. Each replicate cage was treated as an experimental unit. $P < 0.05$ was considered statistically significant.

Results

Growth performance and mortality

The average daily feed intake, average daily gain, feed-to-gain ratio, and mortality of broilers were not affected by diet and sex × diet interaction. These results have been reported previously (Cui et al., 2023).

Manganese contents in the plasma and liver

As shown in Table 4, both sex and diet affected Mn contents in the plasma and liver of broilers on day 42 ($P < 0.0001$), except for the sex × diet interaction. In comparison with the control, Mn addition increased the Mn contents in the plasma and liver by 17.5% and 18.1% ($P < 0.0001$), respectively, and no differences were observed between the 2 Mn sources. Compared with hens, roosters had increased Mn contents in the plasma and liver of 14.1% and 5.65% ($P < 0.04$), respectively.

Plasma lipid indices

Diet affected plasma TC, TG, and LDL contents of broilers at 42 d of age ($P < 0.02$), whereas sex affected plasma TC, TG, LDL, and HDL contents ($P < 0.04$) (Table 5). However, diet had no effect on plasma HDL content, and sex × diet interaction had no effect on all of the above parameters. Compared with the control, Mn supplementation decreased plasma LDL, TC, and TG levels by 11.7%, 5.5%, and 11.3%, respectively ($P < 0.02$), and no differences were observed between the 2 Mn sources. Compared with roosters, the hens had decreased plasma TC, LDL, and HDL levels of 5.7%, 7.0%, and 7.4% ($P < 0.04$), respectively, but had increased plasma TG level of 9.7% ($P < 0.0001$).

Liver triglyceride content

Both sex and diet affected the liver TG content of broilers on day 42 ($P < 0.003$), but sex × diet interaction did not (Table 5). Compared with the control, Mn supplementation decreased liver TG content by 8.8% ($P < 0.001$), and no difference was observed between the 2 Mn sources. Compared with roosters, the hens had increased liver TG content of 6.6% ($P < 0.003$).

Lipid metabolism-related enzyme activities in the liver

Diet affected ATGL, FAS, and ME activities in the liver of broilers on day 42 ($P < 0.02$), except for liver ACC activity (Table 6). Sex and sex × diet interaction did not affect these parameters. Compared with the control, Mn supplementation increased liver ATGL activity by 5.3% ($P < 0.02$) but decreased liver FAS and ME activities by 14.7% and 22.8% ($P < 0.004$), respectively, but no differences were observed between the 2 Mn sources.

mRNA expression levels of lipid metabolism-related genes in the liver

Diet affected the mRNA expression of *FAS*, *SCD*, *ME*, *SREBP1*, and *ATGL* in the liver of broilers on day 42 ($P < 0.03$), whereas sex affected liver ACC and CD36 mRNA expression ($P < 0.0001$) (Table 7). However, diet did not affect the mRNA expression of *ACC*, *APOB*, and *CD36*, whereas sex did not affect the mRNA expression of *SREBP1*, *FAS*, *SCD*, *ME*, *APOB*, and *ATGL*. Sex × diet interaction did not affect these parameters. Compared with the control, Mn supplementation decreased the mRNA expression of *SREBP1*, *SCD*, *ME*, and *FAS* in the liver by 18.2%, 15.2%,

Table 4. Effect of dietary Mn supplementation on plasma and liver Mn contents of roosters or hens at 42 d of age

Sex	Diet	Plasma Mn content, μg/L	Liver Mn content, μg/g, fresh basis
Rooster[1]	Control	25.92	2.23
	Mn sulfate	31.48	2.52
	Mn-Prot M	32.09	2.70
Hen[1]	Control	23.75	2.07
	Mn sulfate	26.03	2.38
	Mn-Prot M	27.12	2.56
	SEM	1.035	0.083
Sex[2]			
	Rooster	29.83[a]	2.48[a]
	Hen	25.63[b]	2.34[b]
	SEM	0.609	0.048
Diet[3]			
	Control	24.84[b]	2.15[b]
	Mn sulfate	28.75[a]	2.45[a]
	Mn-Prot M	29.60[a]	2.63[a]
	SEM	0.732	0.058
P value			
	Sex	<0.0001	0.032
	Diet	<0.0001	<0.0001
	Sex × diet	0.244	0.985

[1]Values are the means of 9 to 10 replicate cages of 3 birds per replicate cage (n = 9 to 10).
[2]Values are the means of 29 to 30 replicate cages of 3 birds per replicate cage (n = 29 to 30).
[3]Values are the means of 19 to 20 replicate cages of 3 birds per replicate cage (n = 19 to 20).
[a,b]Means with different superscripts within the same column differ ($P < 0.05$).
Mn-Prot M, Mn proteinate with moderate chelation strength (Q_f = 51.8).

Table 5. Effect of dietary Mn supplementation on plasma lipid indices and liver triglyceride content of roosters or hens at 42 d of age

Sex	Diet	Plasma TG, mmol/L	Plasma TC, mmol/L	Plasma HDL, mmol/L	Plasma LDL, mmol/L	Liver TG, mmol/g prot
Rooster[1]	Control	0.59	3.29	2.04	0.93	0.222
	Mn sulfate	0.52	3.16	2.03	0.88	0.205
	Mn-Prot M	0.48	3.07	2.00	0.78	0.210
Hen[1]	Control	0.65	3.10	1.92	0.87	0.243
	Mn sulfate	0.59	2.96	1.89	0.78	0.218
	Mn-Prot M	0.60	2.92	1.83	0.75	0.217
	SEM	0.022	0.066	0.066	0.034	0.022
Sex[2]						
	Rooster	0.53[b]	3.17[a]	2.03[a]	0.86[a]	0.212[b]
	Hen	0.61[a]	2.99[b]	1.88[b]	0.80[b]	0.226[a]
	SEM	0.013	0.038	0.038	0.019	0.004
Diet[3]						
	Control	0.62[a]	3.20[a]	1.98	0.90[a]	0.233[a]
	Mn sulfate	0.56[b]	3.06[b]	1.96	0.83[b]	0.211[b]
	Mn-Prot M	0.54[b]	2.99[b]	1.92	0.76[b]	0.214[b]
	SEM	0.015	0.047	0.047	0.024	0.015
P value						
	Sex	<0.0001	0.002	0.010	0.035	0.003
	Diet	0.003	0.014	0.640	0.0007	0.0003
	Sex × diet	0.324	0.939	0.935	0.589	0.425

[1]Values are the means of 9 to 10 replicate cages of 3 birds per replicate cage (n = 9 to 10).
[2]Values are the means of 29 to 30 replicate cages of 3 birds per replicate cage (n = 29 to 30).
[3]Values are the means of 19 to 20 replicate cages of 3 birds per replicate cage (n = 19 to 20).
[a,b]Means with different superscripts within the same column differ ($P < 0.05$).
Mn-Prot M, Mn proteinate with moderate chelation strength (Q_f = 51.8); TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 6. Effect of dietary Mn supplementation on liver enzyme activities of roosters or hens at 42 d of age

Sex	Diet	ATGL activity, pmol/g prot	FAS activity, nmol/g prot	ACC activity, pmol/g prot	ME activity, ng/g prot
Rooster[1]	Control	91.98	3.004	184.14	29.67
	Mn sulfate	95.47	2.672	179.68	23.52
	Mn-Prot M	96.95	2.454	177.37	23.15
Hen[1]	Control	92.54	3.055	196.20	30.03
	Mn sulfate	98.12	2.772	181.15	21.77
	Mn-Prot M	98.03	2.531	179.91	23.85
	SEM	1.754	0.129	7.679	2.176
Sex[2]					
	Rooster	94.80	2.710	180.40	25.44
	Hen	96.23	2.770	185.75	25.22
	SEM	1.013	0.078	4.433	1.279
Diet[3]					
	Control	92.26[b]	3.030[a]	190.17	29.85[a]
	Mn sulfate	96.80[a]	2.679[b]	180.41	22.64[b]
	Mn-Prot M	97.49[a]	2.492[b]	178.64	23.50[b]
	SEM	1.240	0.094	5.430	1.539
P value					
	Sex	0.328	0.591	0.401	0.902
	Diet	0.011	0.001	0.281	0.004
	Sex × diet	0.828	0.994	0.753	0.840

[1]Values are the means of 8 to 10 replicate cages of 3 birds per replicate cage (n = 8 to 10).
[2]Values are the means of 26 to 30 replicate cages of 3 birds per replicate cage (n = 26 to 30).
[3]Values are the means of 17 to 20 replicate cages of 3 birds per replicate cage (n = 17 to 20).
[a,b]Means with different superscripts within the same column differ ($P < 0.05$).
ATGL, adipose triglyceride lipase; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; ME, malic enzyme; Mn-Prot M, Mn proteinate with moderate chelation strength (Q_f = 51.8).

13.7%, and 18.7% ($P < 0.03$), respectively, but increased the ATGL expression by 24.0% ($P < 0.001$). No differences were observed between the 2 Mn sources. Compared with the roosters, the hens exhibited upregulated mRNA expression of liver *ACC* and *CD36* of 27.5% and 31.0% ($P < 0.0001$), respectively.

Protein expression levels in the liver

Diet affected the protein expression of SREBP1, SCD, and ATGL in the liver of broilers on day 42 ($P < 0.0007$), but the sex and the sex × diet interaction did not affect these parameters (Table 8, Figure 1). Compared with the control, Mn addition decreased liver SREBP1 and SCD protein expression by 7.7% and 8.9% ($P < 0.001$), respectively, but increased liver ATGL protein expression by 7.1% ($P < 0.001$). No differences were observed between the 2 Mn sources.

Discussion

This study showed that Mn sulfate or Mn-Prot M added to diets effectively increased Mn contents in the plasma and liver, ATGL activity and its mRNA and protein expressions in the liver, but significantly decreased plasma TG, TC, LDL-C, and liver TG content, FAS and ME activities, mRNA expression of *SREBP1*, *FAS*, *SCD*, and *ME*, and protein expression of SREBP1 and SCD in the liver. These results suggest that dietary Mn supplementation decrease hepatic lipid accumulation in broilers by inhibiting SREBP1 and SCD expression, as well as FAS and ME activities and enhancing ATGL activity and expression, which partly supports our hypotheses. However, there were no differences between the 2 added Mn sources as well as no sex × diet interactions in all of the measured indices, indicating that both Mn sulfate and Mn proteinate were equally effective. The roosters or hens responded similarly to the above modulating aspects of dietary Mn addition, which did not support our hypotheses, possibly because the Mn concentrations in the basal diets were not low enough and/or the above lipid metabolism-related enzymes are not Mn-containing enzymes. Therefore, the assays of the above enzymes were not sensitive enough to detect these changes. These findings, which have not been reported before, offer a new insight into the rational addition of Mn to diets in broiler production.

Previous studies have shown that adding Mn to the diet of broilers effectively reduced hepatic fat deposition (Luo et al., 1998), which agrees with this study's results. Hepatic Mn content has been identified as an indicator of the Mn nutritional status of organisms. Some studies have demonstrated that with increasing dietary Mn levels, plasma and liver Mn contents increase significantly (Mwangi S et al., 2019; Groff Urayama et al., 2023), which also agrees with this study's results. Plasma-free fatty acids and TGs are crucial for fat deposition in broilers (Nematbakhsh et al., 2021). The liver produces key lipoprotein particles, including HDL, LDL, and intermediate-density lipoproteins. These lipoproteins are carriers for components that are then transported to adipocytes (Alvarenga et al., 2011; Na et al., 2018). Significant reductions in liver LDL and TC contents due to Mn addition were observed in this study, suggesting that Mn would be associated with hepatic cholesterol metabolism. LDL transports

Table 7. Effect of dietary Mn supplementation on mRNA expression levels of lipid metabolism-related genes in the liver of roosters or hens at 42 d of age

Sex	Diet	*SREBP1*	*FAS*	*ACC*	*SCD*	*ME*	*CD36*	*APOB*	*ATGL*
		(RQ)[1]							
Rooster[2]	Control	1.006	1.010	1.007	1.008	1.017	1.010	1.009	1.008
	Mn sulfate	0.823	0.900	1.019	0.873	0.857	0.948	0.961	1.148
	Mn-Prot M	0.812	0.907	1.014	0.878	0.869	0.951	0.981	1.260
Hen[2]	Control	1.013	1.097	1.423	1.085	1.065	1.381	1.115	0.877
	Mn sulfate	0.826	0.916	1.341	0.910	0.841	1.203	0.975	1.146
	Mn-Prot M	0.842	0.912	1.428	0.889	0.820	1.231	0.971	1.121
	SEM	0.038	0.054	0.046	0.063	0.057	0.053	0.046	0.057
Sex[3]									
	Rooster	0.880	0.939	1.013^{b}	0.919	0.914	0.970^{b}	0.984	1.139
	Hen	0.893	0.975	1.398^{a}	0.962	0.908	1.271^{a}	1.021	1.048
	SEM	0.023	0.032	0.027	0.038	0.033	0.031	0.027	0.033
Diet[4]									
	Control	1.009^{a}	1.053^{a}	1.215	1.046^{a}	1.041^{a}	1.196	1.062	0.942^{b}
	Mn sulfate	0.824^{b}	0.908^{b}	1.180	0.892^{b}	0.849^{b}	1.075	0.968	1.147^{a}
	Mn-Prot M	0.827^{b}	0.910^{b}	1.221	0.883^{b}	0.844^{b}	1.090	0.976	1.190^{a}
	SEM	0.028	0.038	0.032	0.045	0.041	0.039	0.033	0.041
P value									
	Sex	0.683	0.432	<0.0001	0.436	0.905	<0.0001	0.337	0.062
	Diet	<0.0001	0.016	0.633	0.023	0.002	0.066	0.097	0.0002
	Sex × diet	0.935	0.729	0.514	0.876	0.695	0.542	0.438	0.427

[1]The mRNA expression level was calculated as the relative quantity of the target gene mRNA to the geometric mean of *β-actin* and *GAPDH* mRNA using the $2^{-\Delta\Delta CT}$ method.
[2]Values are the means of 8 to 10 replicate cages of 3 birds per replicate cage (n = 8 to 10).
[3]Values are the means of 26 to 30 replicate cages of 3 birds per replicate cage (n = 26 to 30).
[4]Values are the means of 17 to 20 replicate cages of 3 birds per replicate cage (n = 17 to 20).
[a,b]Means with different superscripts within the same column differ ($P < 0.05$).
SREBP1, sterol-regulatory element-binding protein 1; *FAS*, fatty acid synthase; *ACC*, acetyl-CoA carboxylase; *SCD*, stearoyl-CoA desaturase; *ME*, malic enzyme; *CD36*, thrombospondin receptor; *APOB*, apolipoprotein B; *ATGL*, adipose triglyceride lipase, Mn-Prot M, Mn proteinate with moderate chelation strength (Q_f = 51.8).

cholesterol synthesized in the liver to the bloodstream (Qiao et al., 2007). Consequently, the significant decreases in plasma LDL and cholesterol levels, coupled with the reduction in TG contents in the plasma and liver, indicated that Mn was actively involved in hepatic lipid metabolism in broilers. However, insignificant differences were observed between the 2 Mn sources in these indices, indicating that the 2 Mn sources were equally effective in this study.

Previous investigations conducted in cattle have substantiated the pivotal role of SREBP1 as a master regulator of cholesterogenesis (Matsuhashi et al., 2011). Furthermore, several downstream genes were affected by fluctuations in the expression levels of the SREBP gene, resulting in significant changes in adipose tissue composition (Gamarra et al., 2021). Our study showed that dietary supplementation with Mn in broilers reduced the SREBP1 mRNA and protein expressions in the liver. This finding underscores the critical role of down-regulated SREBP1 expression as a mechanism through which Mn exerts its inhibitory effect on hepatic lipid deposition. Therefore, SREBP1, acting as a transcriptional factor, might play a crucial role in modulating the hepatic lipid metabolism of broilers by participating in the synthesis of cholesterol and fatty acids.

Both FAS and ME are essential rate-limiting enzymes that facilitate the scratch production of long-chain fatty acids in animals (Goodridge et al., 1989), and their activities are closely related to hepatic fat synthesis. The addition of Mn reduces the activities and mRNA expression of FAS and ME enzymes (Katsurada et al., 1987). In this study, dietary Mn supplementation reduced fatty acid synthesis in the liver by decreasing FAS and ME expression, and then reduced liver lipid deposition, which is consistent with the results of Lu et al. (2017). Lu et al. (2017) reported that Mn chloride and Mn-amino acid complexes decreased hepatic lipid synthesis by inhibiting FAS activity in broiler hepatocytes. However, Mn-Prot M and Mn-sulfate were equally effective in affecting these indices in the liver of broilers, which is consistent with the other results obtained in this study.

SCD catalyzes the conversion of paleic and stearic acids to saturated palmitoleic (**C16:1**) and oleic (**C18:1**) acids, respectively (Wang et al., 2006). This enzyme plays various roles, including participation in lipid synthesis, insulin responsiveness, and oxidation of genetically determined fatty acids in the liver, muscle, and fat tissues. Moreover, SCD serves as a regulatory gene in the metabolic pathways of unsaturated fatty acids (Payne et al., 2009; Huang et al., 2015). In this study, Mn addition decreased both the mRNA and protein expressions of liver SCD, possibly reducing the scratch production of unsaturated fatty acids and fat deposition, which is consistent with the report of Lin et al. (2022) in mice.

Table 8. Effect of dietary Mn supplementation on protein expression levels of the genes involved in lipid metabolism in the liver of rooster or hen broilers at 42 d of age

Sex	Diet	SREBP1 (RQ)[1]	SCD (RQ)[1]	ATGL (RQ)[1]
Rooster[2]	Control	0.636	0.712	0.684
	Mn sulfate	0.595	0.647	0.718
	Mn-Prot M	0.597	0.667	0.733
Hen[2]	Control	0.644	0.710	0.677
	Mn sulfate	0.582	0.642	0.734
	Mn-Prot M	0.599	0.638	0.726
	SEM	0.014	0.012	0.015
Sex[3]				
	Rooster	0.609	0.675	0.712
	Hen	0.605	0.663	0.712
	SEM	0.008	0.007	0.008
Diet[4]				
	Control	0.640[a]	0.711[a]	0.680[b]
	Mn sulfate	0.589[b]	0.644[b]	0.726[a]
	Mn-Prot M	0.592[b]	0.652[b]	0.730[a]
	SEM	0.010	0.008	0.014
P value				
	Sex	0.670	0.216	0.972
	Diet	0.0004	<0.0001	0.0006
	Sex × diet	0.694	0.452	0.611

[1]The protein expression level was calculated as the relative quantity of the target gene protein band intensity to the β-actin protein band intensity.
[2]Values are the means of 9 to 10 replicate cages of 3 birds per replicate cage (n = 9 to 10).
[3]Values are the means of 29 to 30 replicate cages of 3 birds per replicate cage (n = 29 to 30).
[4]Values are the means of 19 to 20 replicate cages of 3 birds per replicate cage (n = 19 to 20).
[a,b]Means with different superscripts within the same column differ (P < 0.05).
SREBP1, sterol-regulatory element-binding protein 1; SCD, stearoyl-CoA desaturase; ATGL, adipose triglyceride lipase; Mn-Prot M, Mn proteinate with moderate chelation strength (Q_f = 51.8).

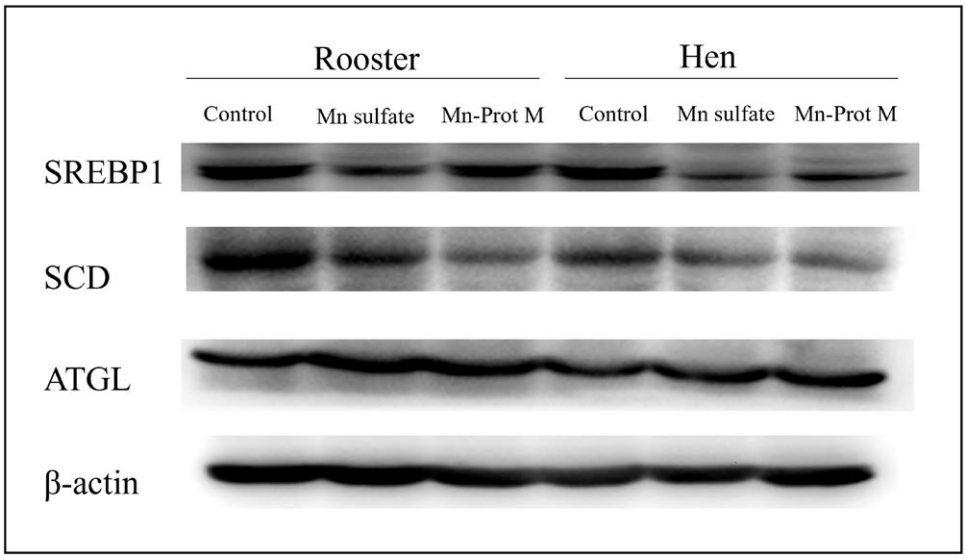

Figure 1. Representative immunoblots of SREBP1, SCD, and ATGL in the liver of broilers at 42 d of age. SREBP1, sterol-regulatory element-binding protein 1; SCD, stearoyl-CoA desaturase; ATGL, adipose triglyceride lipase; Mn-Prot M, Mn proteinate with moderate chelation strength (Q_f = 51.8).

Adipose triglyceride lipase is a vital and rate-limiting enzyme for triglyceride catabolism in the liver, and it controls most intracellular lipid turnover (Ghosh et al., 2016). Lipid deposition in primary chicken hepatocytes was correlated negatively with the ATGL gene expression (Liu et al., 2018). In this study, dietary Mn supplementation increased liver ATGL expression and activity and decreased liver fat deposition in broilers. However, Mn-Prot M and Mn-sulfate were equally effective in affecting these indices in the liver of broilers, which is in agreement with other outcomes reported here for plasma

and liver Mn contents, plasma TG, TC, and LDL-C contents, liver TG content, liver FAS and ME activities, and expression of SREBP1, FAS, SCD, and ME in this study.

In conclusion, dietary Mn supplementation decreased the hepatic lipid accumulation of broilers by inhibiting the expression of SREBP1 and SCD as well as FAS and ME activities and enhancing ATGL expression and activity. Furthermore, both Mn sulfate and Mn proteinate were equally effective in affecting the above aspects of broilers.

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Conflict of interest statement.

None of the authors have any conflicts of interest to declare.

Literatuers Cited

Alvarenga, R. R., M. G. Zangeronimo, L. J. Pereira, P. B. Rodrigues, and E. M. Gomide. 2011. Lipoprotein metabolism in poultry. World's Poult. Sci. J. 67:431–440. doi:10.1017/s0043933911000481

Cui, X. Y., K. Abouelezz, Z. Y. Jiang, Z. Y. Gou, Y. B. Wang, and S. Q. Jiang. 2022. Effects of metabolic energy intervention on lipid content and liver transcriptome in finisher yellow-feathered chickens. Ital. J. Anim. Sci. 21:1362–1370. doi:10.1080/1828051X.2022.2116607

Cui, X. Y., K. Yang, W. Y. Zhang, L. Y. Zhang, D. Li, W. Wu, Y. Hu, T. T. Li, and X. G. Luo. 2023. Effect of dietary manganese supplementation on abdominal fat deposition of broilers. J. Integr. Agric. 2095. doi:10.1016/j.jia.2023.08.004

Gamarra, D., N. Aldai, A. Arakawa, M. M. de Pancorbo, and M. Taniguchi. 2021. Effect of a genetic polymorphism in SREBP1 on fatty acid composition and related gene expression in subcutaneous fat tissue of beef cattle breeds. Anim. Sci. J. 92:E13521. doi:10.1111/asj.13521

Garcia Caraballo, S. C., T. M. Comhair, C. H. C. Dejong, W. H. Lamers, and S. E. Koehler. 2017. Dietary treatment of fatty liver: high dietary protein content has an antisteatotic and antiobesogenic effect in mice. Biochim. Biophys. Acta Mol. Basis Dis. 1863:1789–1804. doi:10.1016/j.bbadis.2017.04.022

Ghosh, M., S. Niyogi, M. Bhattacharyya, M. Adak, D. K. Nayak, S. Chakrabarti, and P. Chakrabarti. 2016. Ubiquitin ligase COP1 controls hepatic fat metabolism by targeting ATGL for degradation. Diabetes. 65:3561–3572. doi:10.2337/db16-0506

Goodridge, A. G., J. F. Crish, F. B. Hillgartner, and S. B. Wilson. 1989. Nutritional and hormonal regulation of the gene for avian malic enzyme. J. Nutr. 119:299–308. doi:10.1093/jn/119.2.299

Groff Urayama, P. M., J. M. Cruvinel, C. Y. Oura, T. S. dos Santos, F. K. de Lima-Krenchinski, J. S. Batistioli, P. A. D. Rodrigues, K. V. Z. Augusto, Y. Han, and J. R. Sartori. 2023. Sources and levels of copper and manganese supplementation influence performance, carcass traits, meat quality, tissue mineral content, and ileal absorption of broiler chickens. Poult. Sci. 102:102330. doi:10.1016/j.psj.2022.102330

Huang, H. Y., R. R. Liu, G. P. Zhao, Q. H. Li, M. Q. Zheng, J. J. Zhang, S. F. Li, Z. Liang, and J. Wen. 2015. Integrated analysis of microRNA and mRNA expression profiles in abdominal adipose tissues in chickens. Sci. Rep. 5:16132. doi:10.1038/srep16132

Katsurada, A., N. Iritani, H. Fukuda, T. Noguchi, and T. Tanaka. 1987. Influence of diet on the transcriptional and post-transcriptional regulation of malic enzyme induction in the rat liver. Eur. J. Biochem. 168:487–491. doi:10.1111/j.1432-1033.1987.tb13443.x

Li, L., and X. Yang. 2018. The essential element manganese, oxidative stress, and metabolic diseases: links and interactions. Oxid. Med. Cell. Longevity 2018:7580707. doi:10.1155/2018/7580707

Li, S. F., Y. X. Lin, L. Lu, L. Xi, Z. Y. Wang, S. F. Hao, L. Y. Zhang, K. Li, and X. G. Luo. 2011. An estimation of the manganese requirement for broilers from 1 to 21 days of age. Biol. Trace Elem. Res. 143:939–948. doi:10.1007/s12011-010-8931-7

Lin, C. W., T. W. Huang, Y. J. Peng, Y. Y. Lin, H. J. Mersmann, and S. Ding. 2021. A novel chicken model of fatty liver disease induced by high cholesterol and low choline diets. Poult. Sci. 100:100869. doi:10.1016/j.psj.2020.11.046

Lin, X., Y. J. Lu, N. Wang, and Y. B. Feng. 2022. The role and mechanisms of selenium supplementation on fatty liver-associated disorder. Antioxidants. 11:922. doi:10.3390/antiox11050922

Liu, S., Y. Wang, L. Wang, N. Wang, Y. Li, and H. Li. 2010. Transdifferentiation of fibroblasts into adipocyte-like cells by chicken adipogenic transcription factors. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 156:502–508. doi:10.1016/j.cbpa.2010.04.003

Liu, Y., J. Shen, X. Yang, Q. Sun, and X. Yang. 2018. Folic acid reduced triglycerides deposition in primary chicken hepatocytes. J. Agric. Food Chem. 66:13162–13172. doi:10.1021/acs.jafc.8b05193

Lu, L., C. Ji, X. G. Luo, B. Liu, and S. X. Yu. 2006. The effect of supplemental manganese in broiler diets on abdominal fat deposition and meat quality. Anim. Feed Sci. Technol. 129:49–59. doi:10.1016/j.anifeedsci.2005.12.005

Lu, L., X. G. Luo, C. Ji, B. Liu, and S. X. Yu. 2007. Effect of manganese supplementation and source on carcass traits, meat quality, and lipid oxidation in broilers1. J. Anim. Sci. 85:812–822. doi:10.2527/jas.2006-229

Lu, L., B. Chang, X. D. Liao, R. L. Wang, L. Y. Zhang, and X. G. Luo. 2016. Use of molecular biomarkers to estimate manganese requirements for broiler chickens from 22 to 42 d of age. Br. J. Nutr. 116:1512–1518. doi:10.1017/S0007114516003640

Lu, L., M. Wang, X. Liao, L. Zhang, and X. Luo. 2017. Manganese influences the expression of fatty acid synthase and malic enzyme in cultured primary chicken hepatocytes. Br. J. Nutr. 118:881–888. doi:10.1017/S0007114517002987

Luo, X. G., Q. Su, Y. Q. Duan, and J. C. Han. 1998. Effect of the trace element manganese on liver fat deposition in broiler pullets. China Academic Journal Electronic Publishing House; 1–2.

Lv, W. H., T. Zhao, K. Pantopoulos, G. H. Chen, X. L. Wei, D. G. Zhang, and Z. Luo. 2022. Manganese-induced oxidative stress contributes to intestinal lipid deposition via the deacetylation of PPARγ at K339 by SIRT1. Antioxid. Redox. Signal. 37:417–436. doi:10.1089/ars.2021.0190

Matsuhashi, T., S. Maruyama, Y. Uemoto, N. Kobayashi, H. Mannen, T. Abe, S. Sakaguchi, and E. Kobayashi. 2011. Effects of bovine fatty acid synthase, stearoyl-coenzyme A desaturase, sterol regulatory element-binding protein 1, and growth hormone gene polymorphisms on fatty acid composition and carcass traits in Japanese Black cattle. J. Anim. Sci. 89:12–22. doi:10.2527/jas.2010-3121

Ministry of Agriculture of the People's Republic of China. 2004. Feeding standard of chicken (NY/T 33–2004). Beijing: Agricultural Press of China.

Mwangi, S., J. Timmons, T. Ao, M. Paul, L. Macalintal, A. Pescatore, A. Cantor, and K. A. D. 2019. Effect of manganese preconditioning and replacing inorganic manganese with organic manganese on performance of male broiler chicks. Poult. Sci. 98:2105–2113. doi:10.3382/ps/pey564

Na, W., Y. Wu, P. Gong, C. Wu, B. Cheng, Y. Wang, N. Wang, Z. Du, and H. Li. 2018. Embryonic transcriptome and proteome analyses on hepatic lipid metabolism in chickens divergently selected for abdominal fat content. BMC Genomics. 19:384. doi:10.1186/s12864-018-4776-9

Nematbakhsh, S., C. Pei Pei, J. Selamat, N. Nordin, L. H. Idris, and A. F. Abdull Razis. 2021. Molecular regulation of lipogenesis, adipogenesis and fat deposition in chicken. Genes. 12:414. doi:10.3390/genes12030414

NRC. 1994. Nutrient requirements of poultry. 9th ed. Washington, DC, USA: The National Academies Press.

Payne, V. A., W. S. Au, C. E. Lowe, S. M. Rahman, J. E. Friedman, S. O'Rahilly, and J. J. Rochford. 2009. C/EBP transcription factors regulate SREBP1c gene expression during adipogenesis. Biochem. J. 425:215–223. doi:10.1042/BJ20091112

Qiao, F., H. Yao, H. Q. Li, and J. D. Wang. 2007. Effect of supplemental organic manganese or chromium on growth performance, carcass traits and dynamic lipid metabolism indexes in broilers. J. Beijing Univ. Agric. 22:32–38. doi:10.13473/j.cnki.issn

SAS. Institute, 2013. SAS proprietary software release 9.4. Cary, NC: SAS Inst. Inc.

Wang, W. 2019. Research advances of adipocyte differentiation in poultry. World's Poult. Sci. J. 75:535–546. doi:10.1017/S004393391900059X

Wang, Q., H. Li, N. Li, L. Leng, and Y. Wang. 2006. Tissue expression and association with fatness traits of liver fatty acid-binding protein gene in chicken. Poult. Sci. 85:1890–1895. doi:10.1093/ps/85.11.1890

Wang, P. H., Y. H. Ko, H. J. Chin, C. Hsu, S. T. Ding, and C. Y. Chen. 2009. The effect of feed restriction on expression of hepatic lipogenic genes in broiler chickens and the function of SREBP1. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 153:327–331. doi:10.1016/j.cbpb.2009.04.003

Wang, G., W. K. Kim, M. A. Cline, and E. R. Gilbert. 2017. Factors affecting adipose tissue development in chickens: A review. Poult. Sci. 96:3687–3699. doi:10.3382/ps/pex184