Folic acid perfusion administration reduced abdominal fat deposition in starter Arbor Acres broilers

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ABSTRACT With intensive selection for meat production in broilers, excessive fat accumulation is also accompanied and causes economic concerns. Folic acid was reported to be involved in lipid metabolism. The present study was conducted to investigate the role of folic acid in reducing abdominal fat deposition. A total of 105 one-day-old healthy Arbor Acres broilers were randomly distributed into 3 treatments, including the control (Con), saline-perfusion group (NS), and folic acid perfusion group (FA). The growth performance, biochemical characteristics in serum, and lipid metabolism in the liver and abdominal fat tissues were evaluated. Results have shown that folic acid significantly reduced abdominal fat percentage ($P < 0.05$) and had no effects on BW, ADFI, ADG, and FCR ($P > 0.05$). Serum triglycerides (TG), total cholesterol (TC), and alanine aminotransferase (ALT) levels were lower in FA group but albumin concentration was higher ($P < 0.05$). Hepatic ACC, SCD, ELOVL6, PI3K, LDLR, HMGCR, and ABCA1 mRNA abundance were all down-regulated in FA group ($P < 0.05$) when compared with the Con and NS groups, while CPT1 and PPARα were not affected. In addition, MTTP mRNA abundance was higher in the liver of birds subjected to folic acid ($P < 0.05$). There was no difference about TG deposition in the liver among all groups based on hematoxylin−eosin (HE) and Oil Red O staining. On the other hand, ELOVL6, PPARγ, IGF1, and TGFβ2 expression were notably decreased in the abdominal fat in FA group ($P < 0.05$). In conclusion, our data demonstrated that folic acid has reduced abdominal fat percentage by decreasing hepatic lipogenesis and suppressing adipocytes proliferation and differentiation. And the inhibiting effect of adipocytes might be mediated by IGF1 and TGFβ2 down-regulation.

Key words: broilers, folic acid, fat deposition, adipocytes proliferation

INTRODUCTION

Over the last few decades, broiler chickens have been simultaneously selected for genetic breeding based on rapid growth, meat production, and carcass yield, while excessive abdominal fat was also accompanied due to its positive inheritance and genetic relationships with BW (Le et al., 1998). About 150 to 200 g fat per kg BW is contained in modern broilers, over 85% of which is physiologically inessential (Mingan Choct et al., 2000). Abdominal fat deposition is a negative phenomenon for economic benefit in poultry industry because it reduces feed efficiency, ketone body production, and increases the cost for processing waste adipose tissue (Wang et al., 1991; Moreira et al., 2018). Abdominal fat deposition is regulated by many complex factors such as endocrine hormones, diets, and multiple behavioral factors (Resnyk et al., 2015).

The previous study has summarized possible mechanisms of nutritional factors that beneficially regulate abdominal fat deposition such as enhancing fatty acid oxidation, inhibiting fatty acid synthesis, adipose cell numbers or size, and so on (Fouad and El-Senousey, 2014). On the other hand, unlike in mammals, the main site of de novo lipogenesis in chickens is the liver (Noble and Cocchi, 1990; Laliotis et al., 2010), thus, hepatic lipid metabolism is closely related to triglyceride accumulation in abdominal fat. Our previous study has revealed that 15 mg/L folic acid addition could reduce lipid deposition in primary chicken hepatocytes (Liu et al., 2018). In addition, it was reported that both hyperplastic and hypertrophic growth of adipocytes contributed to the expansion of adipose tissue (Arner et al., 2010). Yu et al. (2014) found that more than 8 mg/L folic acid increased proliferation of chicken adipocytes after 96 or 144 h induction, but this was accompanied with a decrease in lipid accumulation, suggesting that folic acid could reduce lipid contents on per-cell basis. In vivo, maternal high folate intake contributed to the risk of obese phenotype in male offspring in humans (Xie et al., 2018), whereas other study reported that maternal diets deficient in folic acid

and related methyl donors modified mechanisms associated with lipid metabolism in the fetal liver of the rat (Mcneil et al., 2009). Serum cholesterol was decreased by dietary 12.5 mg/kg folic acid addition in sows during lactation and increased at 50 and 100 mg/kg addition levels (Wang et al., 2011). But reports of folic acid on lipid metabolism in chickens are rare in vivo.

Considering the difference of lipid metabolism between chickens and mammals, correlational research in mammals might not be applicative in poultry. It is reported that total cell numbers in abdominal adipose tissue of chickens increase steadily from 3 D to 7 wk of ages, and the hypertrophic growth occurs before 5 wk of age (Guo, 2011). Based on our previous study (Liu et al., 2018) and Yu's (Yu et al., 2014) in vitro study, we speculate whether folic acid could reduce the accumulation of abdominal fat in broilers in vivo. Therefore, the current study was carried out to reveal the effects of folic acid on lipid metabolism and further explore its regulation mechanism, aiming to provide a theoretical basis for folic acid application in poultry.

MATERIALS AND METHODS

Animals and Experimental Design

A total of 105 one-day-old Arbor Acres broiler were randomly allotted into 3 treatments with 7 replicates, and each replication contained 5 chickens. The treatment groups were: (1) non-perfusion control group (**Con**), (2) diluent-perfusion control group (**NS**, 0.9% saline solution, wt/vol), and (3) folic acid solution-perfusion group (**FA**, 800 mg/L). Saline solution was purchased from Cisen Pharmaceutical Co., Ltd (Jining, China). With the aid of 10 mL 10% ammonium hydroxide (Yu et al., 2014) 0.4 g folic acid standard ($\geq$97%) from Sigma-Aldrich LLC was firstly dissolved and then diluted to 800 mg/L in 0.9% NaCl solution; finally the solution is adjusted to a neutral pH. The basal diets were bought from HuaQin Farming Co., Ltd (Yangling, China), and the formulation and approximate composition are shown in Table 1. Based on intake standard provided by Aviagen company (Huntsville, AL), birds in folic acid perfusion (**FA**) group were given extra folic acid perfusion solution containing 10 times as much as folic acid intake every afternoon at 1:00 to 2:00 pm, while chickens in saline-perfusion (**NS**) group were given the same volume of saline. Perfusion solutions firstly are inhaled using the Eppendorf and then put into the mouth of chicks, which drink the solutions just like drinking water without any stress. The perfusion details were provided in Supplemental Table S1. All birds had ad libitum access to feed and water throughout the experiment. All the birds and experimental protocol in this study were approved by the Institution Animal Care and Use Committee

Table 1. Formulation and proximate composition of experimental diets.

Items	Values
Ingredients (%)	
Corn	62.00
Soybean meal	24.50
Corn powder	4.45
Corn bran	4.00
Limestone	1.35
Dicalcium phosphate	1.23
Premix[1]	1.00
L-lysine sulphate (70%)	0.58
Soybean oil	0.30
Sodium chloride	0.30
Choline chloride (50%)	0.10
Preservatives	0.10
L-threonine	0.06
DL-methionine	0.03
Total	100.00
Nutrient levels	
Metabolism energy (kcal/kg)	2810
Crude protein (%)	18.51
Ca (%)	1.00
Total P (%)	0.59
Digestible P (%)	0.35
Lysine (%)	1.20
Methionine (%)	0.43
Methione + cysteine (%)	0.76

[1]The premix provided the following per kilogram of diets: vitamin A, 8.4 KIU; vitamin D, 3.0 KIU; vitamin E, 54.90 mg; vitamin K, 2.70 mg; vitamin B_1, 1.93 mg; vitamin B_2, 7.92 mg; vitamin B_6, 4.70 mg; vitamin B_{12}, 0.04 mg; niacin, 50.30 mg; folic acid, 1.30 mg; pantothenic acid, 15.73; biotin, 0.20 mg; manganese, 83.20 mg; zinc, 93.60 mg; iron, 122.4 mg; iodine, 0.40 mg; copper, 10.00 mg; selenium, 0.39 mg; cobalt, 0.15 mg.

of the Northwest A&F University (protocol number NWAFAC1008).

Growth Performance

At 11 D of age, birds and feed were weighted to determine ADG, ADFI, and FCR. Abdominal fat weight was measured and also calculated as a percentage of BW (AFP). Broilers mortality was recorded daily.

Sample Collection

At 11 D of age, 1 bird per replicate (7 birds per treatment) close to the average BW of the replicate was selected. The blood samples were taken from the wing vein and collected in vacuum tubes. The tubes were centrifuged at 2,500 rpm for 10 min to obtain serum samples. The serum was stored at $-20°C$ for further analysis. After the blood sampling, broilers were sacrificed. The liver and abdominal adipose tissue were quickly collected in RNAase-free tubes. About 10 cm mid-sections of duodenum and jejunum were obtained and rinsed using saline to remove their contents. Then intestinal segments were opened longitudinally with small sharp scissors. Mucosa were scraped and collected in RNAase-free tubes. All tubes were snap-frozen in liquid nitrogen immediately. Frozen samples were stored at $-80°C$ until analysis. In addition, liver samples were also fixed for 48 h with 4% formaldehyde at room

temperature. Fixed liver samples were processed for sectioning and then stained with hematoxylin−eosin (**HE**) or oil red O by Wuhan goodbio technology Co., Ltd. (Wuhan, China).

Serum Biochemical Characteristics Analysis

Serum biochemical parameters such as alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), alkaline phosphatase (**ALP**), total protein (**TP**), albumin (**ALB**), globulin (**GLO**), total bilirubin (**TBIL**), serum urea nitrogen (**BUN**), creatinine (**CRE**), glucose (**GLU**), and uric acid (**UA**) were determined by the automated biochemical analyser (Hitachi, Tokyo, Japan). Serum high density lipoprotein (**HDL**), low density lipoprotein (**LDL**), low density lipoprotein (**VLDL**), triglyceride (**TG**), total cholesterol (**TC**), high density lipoprotein cholesterol (**HDL-c**), low density lipoprotein cholesterol (**LDL-c**), apolipoprotein B (**Apo-B**) and lipase were detected using commercial kits (Enzyme-linked Biotechnology Co., Ltd, Shanghai, China) based on kits instructions.

Gene Expression

Total RNA extraction and cDNA synthesis in liver, abdominal adipose tissue, and mucosa were performed according to reagent protocols using TRIzol and Primer Script RT Reagent Kits (TaKaRa, Dalian, China). The genes relative expression was quantified by real-time quantitative PCR. The assay was carried out via SYBR Premix Ex Taq kit (TaKaRa, Dalian, China) on the IQ5 (Bio-Rad, Hercules, CA). Detailed reaction system was referred to our previous introduction (Liu et al., 2017b). Primer sequences used were all obtained from GenBank (shown in Supplemental Table S2). All samples were run in triplicate, and the average cycle threshold (Ct) values were calculated for quantification using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Measurement of Hepatic Lipid Metabolism

Before analysis, liver tissues were defrosted, washed, and homogenized together with PBS. Then homogenate solutions were centrifuged at 3,000 rpm for 15 min at 4°C. The supernatant was collected to examine contents of hepatic HDL, LDL, VLDL, TG, TC, HDL-c, LDL-c, Apo-B, and lipase using commercial kits (Enzyme-linked Biotechnology Co., Ltd, Shanghai, China) based on kits instructions. Protein level was also determined using BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA).

Statistical Analysis

All data were shown as means and analyzed by one-way ANOVA using the GLM procedure of SPSS

Table 2. Effects of folic acid perfusion on growth performance of starter broilers.

Items	Con	NS	FA	SEM	P-value
BW$_1$ (g)	45.34	45.73	45.27	0.201	0.635
BW$_{11}$ (g)	310.9	311.2	310.4	1.83	0.988
ADFI (g)	35.25	34.73	34.09	0.412	0.536
ADG (g)	26.43	26.50	26.50	0.193	0.986
FCR	1.33	1.31	1.29	0.018	0.628
AFP (%)	0.87[a]	0.83[a]	0.67[b]	0.030	0.002

BW = body weight; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio. [a, b]Mean values within a row with different superscript letters were significantly different ($P < 0·05$).

20.0 (SPSS Inc., Chicago, IL). P values less than 0.05 were considered to be statistically significant and the notable differences between groups were identified by Duncan's multiple comparisons test. Instances in which $0.05 < P < 0.10$ were considered trends.

RESULTS

Performance Growth

The effects of folic acid perfusion on growth performance, including BW, ADFI, ADG, FCR, and AFP were shown in Table 2. No significant difference was found about these parameters except AFP. When compared with the control and NS groups, folic acid perfusion significantly reduced abdominal fat percentage of broilers ($P < 0.05$). What's more, as shown in Figure 1, the abdominal fat in the FA group seemed to be thinner than those in other 2 groups.

Serum Biochemical Characteristics

The effects of folic acid perfusion on serum biochemical parameters were exhibited in Table 3. Folic acid perfusion significantly decreased ALT level and increased ALB concentration in serum when compared with the control and NS group. In addition, levels of TG, TC, HDL-c, LDL-c, and HDL were all lower in the FA group than those in control and NS groups. However, there was no difference about LDL, VLDL, Apo-B, and lipase of serum among 3 groups as well as AST, ALP, TP, GLO, TBIL, BUN, CRE, GLU, and UA.

Hepatic Biochemical Parameters About Lipid Metabolism

The effects of folic acid perfusion on hepatic biochemical parameters about lipid metabolism were shown in Table 4. Hepatic LDL content in FA group was higher than that in the control or NS group, while no difference was observed about HDL, Apo-B, VLDL, TC, TG, HDL-c, lipase, and LDL-c among 3 groups. On the other hand, as shown in Supplemental Figure S1, hepatic HE and oil red O staining also indicated that folic acid perfusion did not affect lipid droplets in the liver of broilers.
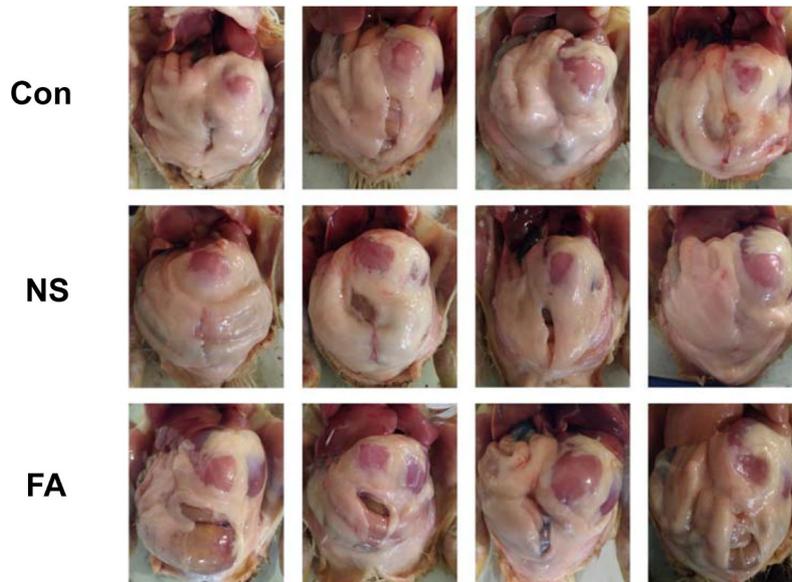
Figure 1. Pictures about abdominal fat in broilers among control, NS and FA groups.

Table 3. Effects of folic acid perfusion on serum biochemical parameters of starter broilers.

Items	Con	NS	FA	SEM	P-value
ALT (U/L)	8.85[a]	8.12[a,b]	6.48[b]	0.401	0.029
AST (U/L)	199.3	191.4	193.8	3.801	0.707
ALP (U/L)	9273	8539	9173	174.2	0.203
TP (g/L)	26.84	27.76	28.40	0.635	0.627
ALB (g/L)	14.54[b]	14.79[b]	16.82[a]	0.352	0.015
GLO (g/L)	12.30	12.97	12.82	0.296	0.644
TBIL (μmol/L)	36.10	35.00	36.61	1.339	0.892
BUN (mmol/L)	0.33	0.24	0.20	0.035	0.299
CRE (μmol/L)	3.12	2.76	3.70	0.252	0.432
GLU (mmol/L)	12.89	12.24	12.52	0.184	0.364
UA (mmol/L)	0.33	0.37	0.38	0.015	0.337
HDL (mg/dL)	51.4[a]	49.9[a]	32.9[b]	3.22	0.024
LDL (mmol/L)	2.22	2.17	1.98	0.145	0.793
VLDL (mmol/L)	2.14	2.22	1.80	0.138	0.416
TG (mmol/L)	1.70[a]	1.74[a]	1.08[b]	0.119	0.023
TC (mmol/L)	2.49[a]	2.55[a]	1.31[b]	0.222	0.027
HDL-c (μmol/L)	579.3[a]	490.6[a]	331.4[b]	37.3	0.017
LDL-c (mmol/L)	4.71[a]	3.89[a]	2.24[b]	0.404	0.033
Apo-B (μg/mL)	222.1	222.4	178.3	11.5	0.203
Lipase (U/L)	79.6	77.5	77.3	2.72	0.934

[a,b]Mean values within a row with different superscript letters were significantly different ($P < 0 \cdot 05$).

Table 4. Effects of folic acid perfusion on hepatic lipid metabolism of starter broilers.

Items	C	NS	FA	SEM	P-value
HDL (mg/gprot)	15.6	14.3	14.7	0.886	0.839
LDL (μmol/gprot)	134.5[b]	119.7[b]	193.4[a]	12.09	0.022
VLDL (μmol/gprot)	87.1	77.5	67.1	4.96	0.272
TG (μmol/gprot)	49.1	41.3	56.0	3.39	0.217
TC (μmol/gprot)	31.5	37.9	33.5	2.64	0.623
HDL-c (μmol/gprot)	17.2	13.2	15.4	1.24	0.433
LDL-c (μmol/gprot)	78.8	65.9	81.7	7.21	0.198
Apo-B (mg/gprot)	8.44	8.04	9.12	0.443	0.624
Lipase (U/gprot)	3.80	3.34	3.72	0.202	0.634

[a,b]Mean values within a row with different superscript letters were significantly different ($P < 0 \cdot 05$).

Genes Expression About Folic Acid Transport and Absorption in the Intestines

As demonstrated in Figures 2A and 2B, duodenal and jejunal genes expression about folic acid transport and absorption were analyzed respectively, including glutamate carboxypeptidase (**GCP**), proton coupled folate transporter (**PCFT**), reduced folate carrier (**RFC**), and folate receptor (**FR**). In duodenum, GCP and PCFT were not affected by folic acid perfusion, while jejunal GCP mRNA abundance was significantly reduced. On the other hand, RFC and FR expression were respectively down-regulated and up-regulated in both duodenum and jejunum.

Genes Expression About Lipid Metabolism in the Liver and Abdominal Fat

As shown in Figure 3, folic acid perfusion reduced ACC, SCD, ELOVL6, and PI3K mRNA expression in the liver which were all related to lipogenesis. Hepatic LPL and MTTP mRNA abundance were higher in the FA group while CPT1 and PPARα related to β-oxidation of fatty acid were not affected. On the other hand, when compared with the control, LDLR, HMGCR, and ABCA1 mRNA levels were down-regulated by folic acid perfusion, which were all associated with cholesterol metabolism.

In the abdominal adipose tissue, only ELOVL6 was detected to be down-regulated by folic acid among genes about lipogenesis examined in the study (Figure 4A). On the other hand, all lipolysis related genes in the abdominal fat detected in the current study were not affected (Figure 4B). Further, we also analyzed genes expression in the abdominal fat which could promote lipid deposition or affect proliferation
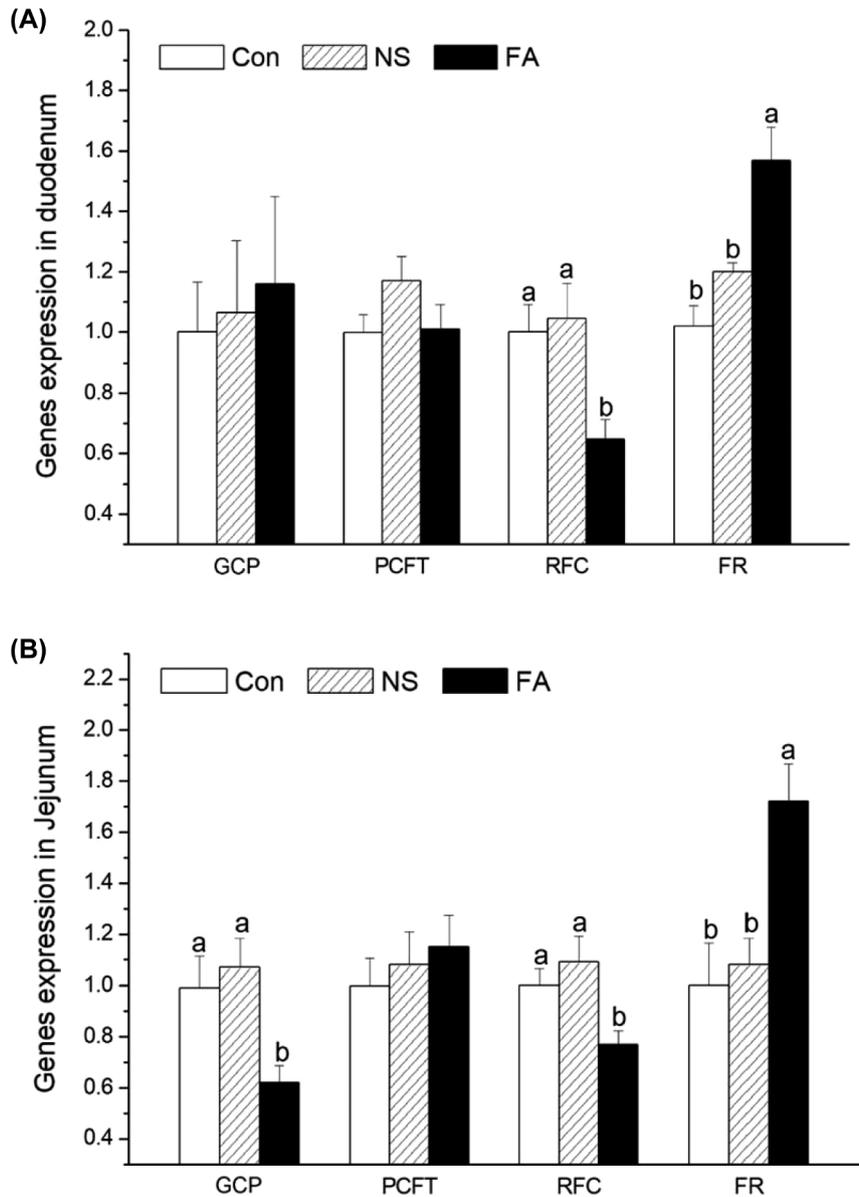
Figure 2. The effects of folic acid perfusion on genes expression about folic acid transport and absorption in the intestines. Data are expressed as mean $\pm$ SEM (n = 7), bars with different letters differ indicated statistically significant differences (one-way ANOVA, $P < 0.05$). GCP: glutamate carboxypeptidase; PCFT: proton coupled folate transporter; RFC: reduced folate carrier; FR: folate receptor.

and differentiation of preadipocytes. As shown in Figure 5, folic acid perfusion significantly reduced PPARγ, IGF1, and TGFβ2 mRNA expression in the abdominal fat, but improved IGF1R mRNA abundance.

DISCUSSION

The liver in new-born chicken is full of lipids which are absorbed from the yolk during the last 7 D of embryonic incubation (Noble and Cocchi, 1990; Liu et al., 2018). After hatching, chickens begin to receive feed consumption and the accumulated lipid in the liver declines rapidly during the first 6 D of post-hatch; the utilization and accumulation of lipids are changed mainly from either diets or de novo lipogenesis in the liver (Richards et al., 2010). Because the liver is the predominant site for lipogenesis, a higher abdominal fat deposition was closely related with the rate of TG synthesis and secretion from the liver (Zhao et al., 2007). These events reflected that the switch period in hepatic lipid metabolism (within the first few days after hatching; Richards et al., 2010) had a significant impact on adipose tissue of starter chickens.

The level of 10 times as much folic acid addition (10 mg/L) could decrease genes expression about lipogenesis of primary chicken hepatocytes in my preliminary experiment (Liu et al., 2017a). The 8, 12, and 16 mg/L folic acid were found to reduce mRNA expression associated lipid synthesis and lipid accumulation in chicken adipocytes (Yu et al., 2014). We supposed that folic acid could reduce abdominal fat deposition in broilers and then birds were administrated for folic
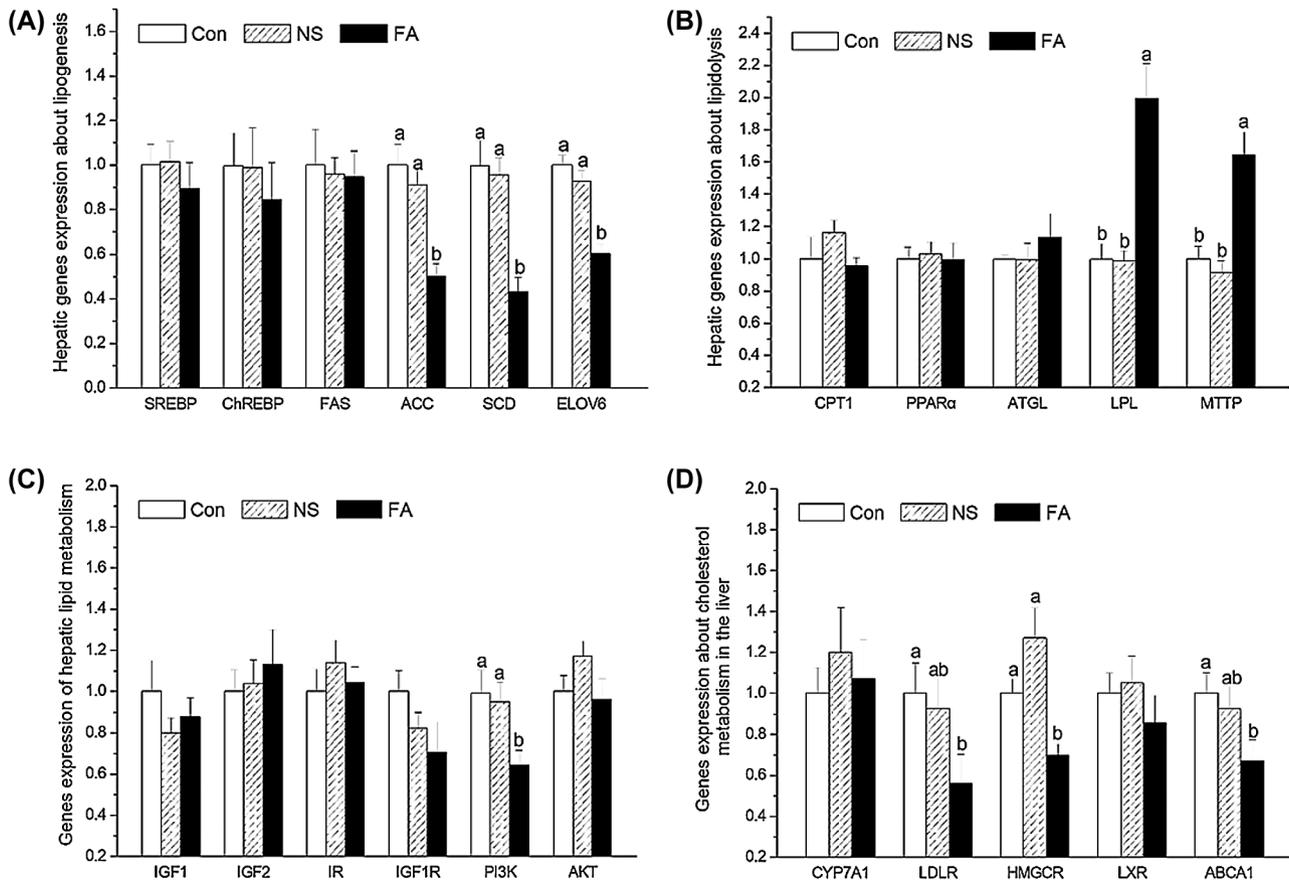
Figure 3. The effects of folic acid perfusion on genes expression related to lipid metabolism in the liver. Data are expressed as mean ± SEM (n = 7), bars with different letters differ indicated statistically significant differences (one-way ANOVA, $P < 0.05$). SRRBP: sterol regulatory element binding proteins; PPAR: peroxisome proliferator-activated receptor; ELOVL: elongase of very long chain fatty acids; MTTP: microsomal triglyceride transfer protein; CYP7A: cholesterol 7-alpha-hydroxylase; HMGCR: HMG-CoA reductase; ABCA1: ATP-binding cassette transporterA1; LXR: liver X receptor.

acid perfusion containing 10 times as much as folic acid intake during the starter period. Exactly as we assumed, results revealed that folic acid perfusion significantly reduced abdominal fat percentage when compared with the control and NS group. No difference was found about other performance parameters such BW, ADFI, ADG, and FCR.

In order to explore the regulation mechanism of folic acid, we firstly detected genes expression about folic acid transport and absorption in the intestines. Dietary folate in the polygutamate form must be converted to the monoglutamate form by GCP prior to intestinal absorption (Bailey and Caudill, 2012). Our results showed that jejunal GCP expression was down-regulated in FA group, suggesting folic acid perfusion in the monoglutamate form is enough for prior utilization. On the other hand, RFC, a bi-directional transmembrane protein mediated folic acid transport, is decreased by folic acid perfusion, though it is optimal at near-neutral pH. PCFT expression is not affected among 3 groups which is a unidirectional transport protein and has high affinity for folic acid at low pH (Jing et al., 2013). In addition, FR is found to be highly expressed in FA group when compared with the control and NS group. These

all implied that folic acid perfusion played an important role via FR-mediated transport and absorption.

Next, we analyzed lipid metabolism in the serum, liver, and abdominal fat tissues, respectively. Lower serum ALT and higher ALB levels were detected in the FA group; serum TG, TC, HDL-c, and LDL-c levels decreased by folic acid perfusion, which are all considered as risk factors for cardiovascular diseases. No change was found in hepatic TG, TC, HDL-c, and LDL-c contents. Lipoproteins are responsible for lipid storage and transportation. Folic acid improved hepatic LDL levels, implying that hepatic cholesterol could be transferred out for utilization of other organs. On the other hand, HMGCR is a limited enzyme for cholesterol synthesis, and hepatic lower HMGCR expression also suggested that folic acid might inhibit endogenous TC synthesis. Moreover, LDLR down-regulation in the liver by FA perfusion also reflected that folic acid reduced hepatic TC intake, which was in line with serum lower TC level in the study. These data indicated to some extent that folic acid improved liver function of birds, which was consistent with previous reports that folic acid had protective effects against hepatotoxicity (Allah and Badary, 2017). Hepatic fatty acid synthesis
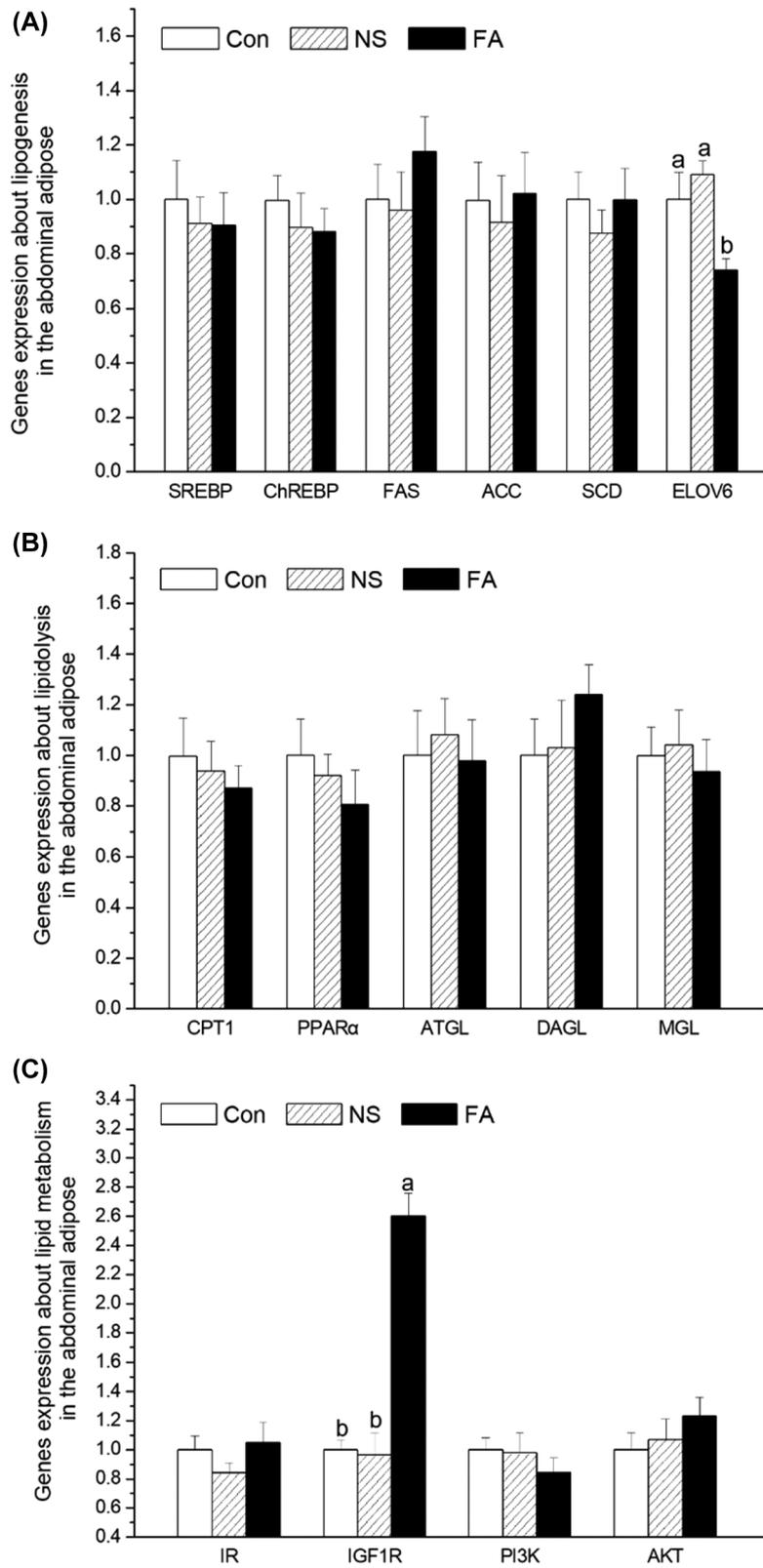
Figure 4. The effects of folic acid perfusion on genes expression related to lipid metabolism in the abdominal fat. Data are expressed as mean ± SEM (n = 7), bars with different letters differ indicated statistically significant differences (one-way ANOVA, $P < 0.05$).
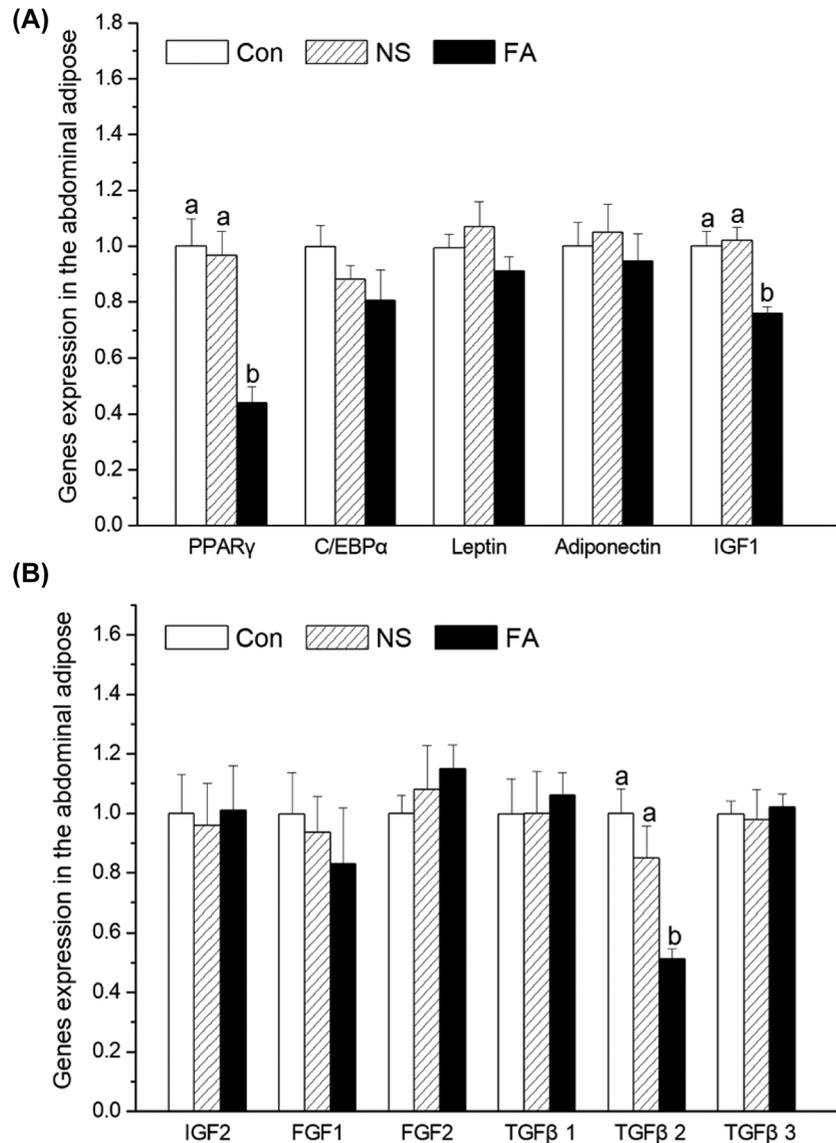
Figure 5. The effects of folic acid perfusion on genes expression related to fat deposition or proliferation and differentiation of preadipocytes in the abdominal fat. Data are expressed as mean $\pm$ SEM (n = 7), bars with different letters differ indicated statistically significant differences (one-way ANOVA, $P < 0.05$). IGF: insulin-like growth factor; FGF: fibroblast growth factor; TGF: transforming growth factor.

was inhibited by reducing ACC, SCD, and ELOVL6 mRNA expression in the FA group, while fatty acid β-oxidation might not be affected because no difference was found about CPT1 and PPARα expression among 3 groups. It was reported that MTTP was responsible for the assembly and subsequent secretion of VLDL from hepatocytes and controlled the incorporation of TGs into apolipoprotein B (Liu et al., 2016). In the current study, MTTP was up-regulated significantly in FA group, suggesting folic acid enhanced TG export from liver for energy requirement of other tissue growth in the starter period.

Meanwhile, only ELOVL6 expression was found to be down-regulated in abdominal fat by folic acid perfusion among all detected genes associated lipogenesis, and lipolysis was also not affected. PPARγ was reported to be probably a key regulator of chicken preadipocyte proliferation and differentiation, and may be a major

gene affecting fat deposition (Wang et al., 2008). Lower PPARγ level in FA group also indicated that fat deposition was restrained to some degree. On the other hand, de novo lipogenesis in adipose tissue occupy less in chicken (O'Hea and Leveille, 1968; Leveille et al., 1975), thus we considered whether folic acid could have an impact on proliferation of adipocytes in order to comprehensively demonstrate the possible reasons for abdominal fat reduction.

In mammal, fat cell cluster size increases steadily with fetal age, but the number of clusters does not change significantly before birth; after birth, the growth of adipose tissue is also mainly due to an increase in size of fat cell clusters rather than numbers (Ailhaud et al., 1992). However, unlike mammals, both the size and numbers of adipocyte all contribute to the growth of abdominal fat in chickens (Guo, 2011). The previous review has given an introduction to the processes

involved in chicken adipocytes development and described the potential regulatory role of growth factors (Butterwith, 1997) and other dietary factors (Wang et al., 2017). We mainly focused on IGF, TGF, FGF, and adiponectin expression in abdominal fat which were all associated with proliferation and differentiation of adipocytes. Both IGF1 and IGF2 promote chick adipocyte proliferation with equal potency via their autocrine−paracrine or endocrine actions (Butterwith and Goddard, 1991). In the current study, IGF1 expression was reduced in FA group while IGF2 did not. FGFs were expressed in proliferating and differentiated chicken adipocytes (Burt et al., 1992), and have been shown to stimulate adipocytes proliferation (Butterwith et al., 1993). However, no difference was found about FGF1 and FGF2 expression among all groups as well as adiponectin which was reported to stimulate mitochondrial biogenesis and reduce lipid content in adipocytes (Lu et al., 2015). In addition, TGFβ 1 to 3 had been detected in both proliferating and differentiated chicken adipocyte precursor cells (Burt et al., 1992). TGFβ also was related with adipocytes proliferation (Butterwith and Gilroy, 1991). Among TGFβ 1 to 3, only TGFβ2 was notably down-regulated in abdominal fat by folic acid perfusion. These results declared that folic acid might suppress adipocytes proliferation and differentiation by decreasing IGF1 and TGFβ2 expression in abdominal fat.

In conclusion, our results indicated that folic acid perfusion has improved liver functions, and reduced abdominal fat percentage by decreasing hepatic lipogenesis and suppressing adipocytes proliferation and differentiation. The inhibiting effect of adipocytes might be mediated by IGF1 and TGFβ2 down-regulation. These findings will provide a theoretical basis for folic acid application for abdominal fat deposition in poultry.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

Table S1. Table S1 detailed folic acid perfusion procedure.
Table S2. Forward and reverse primer sequences for PCR analysis.
Figure S1. Hepatic HE and Oil Red O staining (magnification: 10 × 20).

ACKNOWLEDGMENTS

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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