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Effects of folic acid supplementation to basal diets of broilers on growth performance, slaughter performance, *IGF2* gene expression and methylation

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Abstract: Folic acid (FA) is an important water-soluble vitamin, and plays an important role as a cofactor and coenzyme in animal growth and development, and regulation of gene expression and methylation. A total of 270 female broiler chickens (1-day-old) were randomly allotted to three dietary treatments supplemented with 0 mg/kg (control group), 5 mg/kg, and 10 mg/kg FA in basal diets for 42 days, respectively. Each treatment had six replicate cages with 15 birds per cage. Dietary supplementation of 5 mg/kg FA significantly enhanced average body weight and average daily gain of 21-day-old broilers ($P < 0.05$), but significantly reduced subcutaneous fat thickness and widths of intermuscular fat band of 42-day-old broilers by dietary FA treatments ($P < 0.05$). Also, a diet with 10 mg/kg FA supplementation significantly increased the relative heart weight of 42-day-old chickens ($P < 0.05$). Furthermore, dietary FA supplementation significantly improved the serum insulin-like growth factor 2 (IGF2) concentrations ($P < 0.01$) and *IGF2* mRNA expression in the abdominal fat ($P < 0.05$), but no statistical differences were found in the methylation of *IGF2* promoter ($P > 0.05$). The present study demonstrated that dietary FA supplementation may have positive effects on chicken growth through increased *IGF2* gene expression.

Keywords: chicken; fat; folate; growth traits; gene regulation

With the advantages of high efficiency and low cost, the chicken industry has developed rapidly into one of the industries with the highest degree of industrialization in the field of animal husbandry in China. However, with the rapid development of intensive and large-scale chicken industry, broiler breeding is facing prominent problems, such as excessive fat deposition, as well as rough, soft, tasteless meat. Improving the growth performance and meat quality of broilers by means of nutritional regulation has become one of the hot research topics.

Folate/folic acid (FA), also known as pteroylglutamic acid, is one type of water-soluble vitamin and one of the essential micronutrients for the body. FA is taken up from the small intestine and then it enters the liver through the portal vein.

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Folate metabolism involves at least 30 different enzymes in the liver, such as dihydrofolate reductase (DHFR), 5,10-methylenetetrahydrofolate reductase (5,10-MTHFR), tetrahydrofolic acid (THF) and so on (Lightfoot et al. 2005). The enzyme DHFR catalyzes the reduction of dietary FA or dihydrofolate to THF in the liver, and 5,10-MTHFR carries out a central reaction in folate metabolism (Nazki et al. 2014). Therefore, FA plays an important role in many physiological processes, such as cell division, cell repair, and tissue growth (Bailey et al. 2015). As a methyl donor, FA can be directly involved in synthesis and maintenance of RNA, DNA, and protein as well as DNA methylation and epigenetic modification (Friso et al. 2017), thus regulating animal reproduction, growth, and development (Crott 2017). Inadequate uptake or oversupply of FA, and disorders in absorption, metabolism, and utilization of FA can lead to the intracellular dysfunction of FA, resulting in the nuclear acid synthesis disorder and/or methylation disorder (Kim et al. 1997). Thus, a diet rich in FA is important in general and in the prevention of some diseases, for example, cleft lip and palate, neural tube malformations, autism spectrum disorders, megaloblastic anaemia, cancer (Czeizel et al. 2013). At present, studies on the FA application in livestock and poultry diets have been increasing. In pigs, it was reported that dietary FA supplementation prevented the harmful effect of intrauterine growth retardation on hepatic antioxidant function and mtDNA biogenesis in weanlings, and it also altered the expression of several hepatic proteins (Liu et al. 2013). Studies in chickens indicated that folate increases biochemical constituents, enhances the generation of total IgG in serum, as well as improves production performance and decreases the glucose level in young laying hens (Jing et al. 2014). Moreover, insulin-like growth factor 2 (IGF2) is a growth factor secreted mainly by the liver, and plays a key role in animal development, cell proliferation, and metabolism (Perkins et al. 2012; Pereira et al. 2019). It was also found that FA affected hepatic *IGF2* expression and methylation level of *IGF2* promoter of embryo growth of broilers (Liu et al. 2016).

In this study, it can be hypothesized that FA supplementation of basal diets may improve broiler performance. Hence, the aim of this study was to evaluate the effects of FA supplementation of broiler diets on growth performance, slaughter performance, *IGF2* expression, and methylation patterns.

MATERIAL AND METHODS

Experimental procedures and diets

A total of 270 female broiler chickens of Arbor Acres breed with an average body weight of 46 ± 0.9 g at one day of age that were purchased from a local hatchery (Liuhe Breeding Farm Co.) were used in this study. The birds were randomly allotted to three dietary treatments supplemented with 0 mg/kg (control group), 5 mg/kg, and 10 mg/kg FA in basal diets for 42 days, respectively. Each treatment had six replicate cages with 15 birds per cage. A standard basal diet containing maize and soybean meal was fed as mash, and the composition of the basal diet (Table 1) was formulated to meet or exceed the nutrition requirements of broiler chickens according to the Feeding Standard of Chickens (NY/T 33-2004, China). During the experimental period, water and mash feed were provided *ad libitum*, the lighting time was 24 h a day for the first week and then reduced to 16 h during 8–42 days. The room temperature was 33 °C during the first week and then decreased by 2 °C every week until 24 °C.

Measurement of growth performance

On days 21 and 42, the broilers of each group were weighed after feed deprivation for 12 h with free drinking water, respectively. The feed intake of each group was recorded. Average body weight (ABW), average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (F/G) were calculated for each group.

Slaughtering and sampling

At 21 and 42 days of age, two birds (similar body weights) were randomly selected from each replicate for live body weight and then euthanized after feed deprivation for 12 h, respectively. Immediately, blood samples were collected from the jugular vein and sera were obtained by gentle centrifugation at 3 000 × *g* for 20 min, and then they were stored at –80 °C until analysis. After slaughtering, carcass attributes were measured according to the published reference (Kolbadinejad and Rezaeipour 2020). That is, dressed weight, eviscerated weight, breast muscle weight, leg muscle weight, and

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Table 1. Content and nutrition level of diet for broilers (air-dry basis)

Items	Day 1–21	Day 22–42
Material Ingredient (%)		
Corn	54.50	57.60
Soybean	32.71	30.42
Fish meal	3.00	2.00
Shell powder	1.22	1.05
$CaHPO_4{\cdot}2H_2O$	1.70	1.60
Soybean oil	3.00	4.00
Wheat bran	2.50	2.00
Salt	0.20	0.19
DL-Methionine (98%)	0.17	0.14
Premix[a]	1.00	1.00
Total	100.00	100.00
Calculated nutrient level		
Apparent metabolic energy (MJ/kg)	12.52	12.74
Crude protein (%)	21.52	20.05
Calcium (%)	0.97	0.89
Available phosphorus (%)	0.45	0.40
Lysine (%)	1.21	1.18
Methionine (%)	0.53	0.47
Methionine + cysteine (%)	0.91	0.82
Folic acid (mg/kg)	0.36	0.33

[a]Premix provided per kilogram of diets: manganese sulphate, 55 mg; zinc sulphate, 55 mg; ferrous sulphate, 44 mg; copper sulphate, 5.5 mg; potassium iodine, 0.44 mg; selenium selenite, 0.099 mg; vitamin A, 770 IU; vitamin B_1, 1.2 mg; vitamin B_2, 4.8 mg; vitamin B_6, 1.6 mg; vitamin B_{12}, 0.011 mg; vitamin D_3, 255 IU; vitamin E, 15 mg; vitamin K, 2.2 mg; pantothenic acid, 11 mg; niacin, 35.5 mg; folic acid, 0.66 mg; biotin, 0.11 mg; choline, 500 mg; xanthine, 33.1 mg; antioxidant, 120 mg

abdominal fat weight were recorded to calculate dressing percentage, eviscerated percentage, breast muscle percentage, leg muscle percentage, and abdominal fat percentage. Weights of internal organs (heart, spleen, liver, proventriculus, gizzard) were also measured, and the organ index was calculated as the percentage of live body weight. Meanwhile, abdominal fat samples (one chick from each replicate) of 42-day-old broilers were isolated immediately, washed briefly with PBS, then snap-frozen and stored in liquid nitrogen at –80 °C for total RNA and DNA extraction. Subcutaneous fat thickness and width of intermuscular fat band were also measured with a vernier calliper.

Measurement of IGF2 in serum samples

IGF2 in serum samples was analyzed using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's guidelines (Shanghai Yuping Biotech Co., Ltd., Shanghai, China).

mRNA expression levels of *IGF2*

Total RNA was extracted from abdominal fat of 42-day-old broilers using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols, and then treated with DNase I to remove DNA contamination. The amount and quality of RNA were measured by loading total RNA onto a 0.8% agarose gel that was stained with ethidium bromide, and the absorption values of RNA at 260/280 nm and 260/230 nm were detected by a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA, USA). First-strand cDNA was synthesized with 2 μg of total RNA using a reverse transcription kit (Promega, Madison, USA) according to the manufacturer's instructions. Primers used for real-time quantitative PCR (qPCR) were designed by Xing et al. (2018), and the primer sequences are shown in Table 2. The *ACTB* was used as an internal control for the relative quantification of *IGF2* expression. The qPCR was carried out with SYBR Premix Ex Taq in a LightCycler 480 Real-Time PCR System (Roche, Basel, Switzerland). All reactions were performed in triplicate with a negative control. Relative expression levels of the *IGF2* gene were analyzed by the 2^{-DDCt} method.

Bisulphite sequencing and methylation analysis of *IGF2* gene

Genomic DNA was also isolated from the abdominal fat of 42-day-old broilers using the Genomic DNA Purification Kit (Thermo Fisher Scientific, Shanghai, China) according to the manufacturer's protocols. For each group, an equal quantity of DNA from the two samples which were used for qPCR analysis of *IGF2* gene was mixed to form three DNA pools. Sodium bisulphite treatment of each DNA pool was performed using the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA, USA). The *IGF2* gene promoter region ranging from –648 to –479 was amplified by PCR. The methyla-

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Table 2. Primer sequences, product sizes and annealing temperature applied for qPCR and methylation analysis

Primers	Primer sequences (5' to 3')	Product size (bp)	Annealing temperature (°C)	Usage	GenBank accession
IGF2-E-F	CTATGCGTTGGATTCAGC	133	58	Gene expression	NM_001030342
IGF2-E-R	CCTGTTATTTCGTCCCAC				
ACTB-F	GTGACATCAAGGAGAAGC	105	55	Gene expression	L08165
ACTB-R	CATCAGGGAGTTCATAGC				
IGF2-M-F	TGGTTGTGTTGTAGATTTTTTTTGT	170	62	Methylation	NC_052536
IGF2-M-R	ACACTAAATTTCACCTCCCATTTT				

tion PCR primers for *IGF2* were taken from Liu et al. (2016). Bisulphite sequencing was performed according to our previous study (Xing et al. 2009). For each pool, five positive clones were randomly selected for sequencing.

Statistical analysis

Group data were analyzed using SPSS v20.0 (IBM Corp., Armonk, NY, USA). Data for multiple comparisons were analyzed by one-way ANOVA. Multiple comparisons between the groups were performed using the S-N-K method. Values are given as the mean ± SD. The *P*-value lower than 0.05 was interpreted as statistically significant.

RESULTS

Growth performance

As shown in Table 3, ABW and ADG of 21-day-old chickens increased in the group that received 5 mg/kg FA as compared with the groups that received 0 mg/kg FA and 10 mg/kg FA ($P < 0.05$), while ABW and ADG of 42-day-old broilers were not affected by the FA ($P > 0.05$). No significant differences were observed in the ADFI and F/G of 21- and 42-day-old broilers from three groups ($P > 0.05$).

Slaughter performance

Table 4 shows the slaughter performance of broilers. In 42-day-old broilers, compared with the control group, the supplementation of 5 mg/kg FA to broiler diets led to a reduction in subcutaneous fat thickness ($P < 0.05$), while no significant difference was observed in 10 mg/kg group ($P > 0.05$). Similarly, widths of intermuscular fat band of 42-day-old broilers were also decreased significantly by supplementing 5 mg/kg FA and 10 mg/kg FA to basal diets ($P < 0.05$). However, subcutaneous fat thickness and widths of intermuscular fat band of 21-day-old broilers were not affected ($P > 0.05$) by dietary treatments. Other slaughter traits, i.e., dressing percentage, eviscerated percentage, breast muscle percentage, leg muscle percentage, abdominal fat percentage, were not altered by dietary FA supplementation either ($P > 0.05$).

Table 3. Effects of FA supplementation in basal diets on growth performance of broilers ($n = 30$)

Items	Day	0 mg/kg	5 mg/kg	10 mg/kg	*P*-value
ABW (g)	21	951.2 ± 27.4^{a}	1 077.2 ± 13.4^{b}	962.1 ± 33.7^{a}	0.034
	42	2 857.4 ± 43.3	3 098.3 ± 132.2	2 904.4 ± 119.8	0.395
ADG (g)	21	43.1 ± 1.3^{a}	49.1 ± 2.5^{b}	43.6 ± 2.0^{a}	0.043
	42	66.9 ± 2.3	72.7 ± 2.6	68.0 ± 3.0	0.326
ADFI (g)	21	58.8 ± 4.6	65.3 ± 5.2	59.5 ± 4.1	0.462
	42	125.4 ± 12.3	132.3 ± 11.3	126.3 ± 9.7	0.563
F/G	21	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	0.324
	42	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	0.425

ABW = average body weight; ADFI = average daily feed intake; ADG = average daily gain; F/G = feed to gain ratio
^{a,b}Different superscripts in the same row indicate significant differences ($P < 0.05$)

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Table 4. Effects of folic acid supplementation to basal diets on carcass characteristics of broilers (n = 12)

Items	Day	0 mg/kg	5 mg/kg	10 mg/kg	P-value
Dressing percentage	21	82.62 ± 1.32	84.78 ± 1.56	84.12 ± 2.11	0.406
	42	87.65 ± 2.21	89.67 ± 1.21	88.47 ± 2.68	0.426
Eviscerated percentage	21	72.60 ± 2.76	73.38 ± 2.91	72.46 ± 1.45	0.325
	42	72.15 ± 3.11	74.04 ± 1.38	74.24 ± 2.77	0.428
Breast muscle percentage	21	26.35 ± 1.02	27.05 ± 1.44	27.68 ± 1.12	0.423
	42	28.65 ± 2.61	29.30 ± 2.10	29.90 ± 3.11	0.654
Leg muscle percentage	21	20.95 ± 1.54	21.69 ± 1.66	21.86 ± 1.23	0.321
	42	21.59 ± 1.34	21.78 ± 1.03	21.65 ± 2.30	0.752
Abdominal fat percentage	21	1.44 ± 0.34	1.19 ± 0.16	1.41 ± 0.15	0.174
	42	2.79 ± 0.38	2.09 ± 0.20	2.21 ± 0.24	0.206
Subcutaneous fat thickness (mm)	21	5.21 ± 0.42	5.12 ± 0.20	5.47 ± 0.09	0.692
	42	6.66 ± 0.36^{a}	5.32 ± 0.43^{b}	6.38 ± 0.40^{ab}	0.047
Width of intermuscular fat band (mm)	21	9.27 ± 0.46	9.34 ± 0.65	10.54 ± 0.23	0.191
	42	14.33 ± 0.79^{a}	11.51 ± 0.45^{b}	12.19 ± 0.42^{b}	0.048

^{a,b}Different superscripts in the same row indicate significant differences (P < 0.05)

Organ index

In 42-day-old broilers, the group with supplementation of 10 mg/kg FA had higher heart weights than the groups with 0 mg/kg FA or 5 mg/kg FA supplementation (P < 0.05), but heart weights of 21-day-old broilers were not affected by the supplementation of FA to basal diets (P > 0.05) (Table 5). There were no significant differences in relative weights of liver, spleen, proventriculus, and gizzard between all groups at 21 and 42 days of age (P > 0.05) (Table 5).

Serum IGF2 concentrations

Serum IGF2 concentrations for 21- and 42-day-old broilers are given in Table 6. In general, serum IGF2 concentrations were increased with the FA supplemented diets in 21- and 42-day-old broilers (P < 0.01).

IGF2 mRNA expression in abdominal fat

The *IGF2* mRNA expression in the abdominal fat of 42-day-old broilers is shown in Figure 1. The *IGF2*

Table 5. Effects of folic acid supplementation to basal diets on the internal organ weight of broilers (g/kg live weight) (n = 12)

Items	Day	0 mg/kg	5 mg/kg	10 mg/kg	P-value
Liver	21	24.02 ± 2.24	23.65 ± 2.11	23.84 ± 2.5	0.491
	42	17.99 ± 3.82	18.59 ± 3.50	19.66 ± 2.87	0.700
Spleen	21	0.81 ± 0.17	0.90 ± 0.22	0.77 ± 0.15	0.488
	42	1.05 ± 0.33	1.31 ± 0.25	1.15 ± 0.29	0.342
Heart	21	5.42 ± 0.72	5.51 ± 0.62	5.79 ± 0.94	0.692
	42	3.67 ± 0.48^{a}	3.86 ± 0.34^{ab}	4.44 ± 0.72^{b}	0.046
Proventriculus	21	5.97 ± 0.69	6.04 ± 0.34	6.49 ± 0.67	0.782
	42	6.55 ± 0.78	6.89 ± 0.58	7.02 ± 0.63	0.462
Gizzard	21	18.20 ± 0.54	19.22 ± 0.49	19.68 ± 0.48	0.563
	42	19.24 ± 0.65	20.78 ± 1.16	21.09 ± 2.24	0.651

^{a,b}Different superscripts in the same row indicate significant differences (P < 0.05)

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Table 6. Effects of folic acid supplementation to basal diets on IGF2 concentration in the serum of broilers (n = 12)

Items	Day	0 mg/kg	5 mg/kg	10 mg/kg	P-value
IGF2 (ng/l)	21	907.98 ± 28.21^{A}	1 146.57 ± 20.32^{B}	1 189.40 ± 22.86^{B}	< 0.001
	42	885.98 ± 21.91^{A}	1 095.33 ± 26.06^{B}	1 140.97 ± 21.10^{B}	< 0.001

^{A,B}Different superscripts in the same row indicate significant differences (P < 0.01)

mRNA expression was significantly increased in the abdominal fat of 42-day-old broilers by supplementing 10 mg/kg FA to basal diets (P < 0.05), but the *IGF2* mRNA expression did not reach significance in 42-day-old broilers consuming the 5 mg/kg FA supplementation in the diet (P > 0.05).

IGF2 DNA methylation in abdominal fat

The results of bisulphite sequencing indicated that, for all the CpG sites analysed, the overall methylation percentage of *IGF2* promoter was 10.00%, 18.33%, and 15.00% for three groups at 42-day-old broilers, respectively (Figure 2); however, there was no difference in the methylation percentage of *IGF2* promoter between groups with different dietary treatments (P > 0.05).

DISCUSSION

Rich in FA are leafy vegetables, fruit, cereals, yeast, liver and dairy products, and it is known to be an essential dietary requirement for animals. Here, we demonstrated the influence of the supplementation of FA to broiler diets at 5 mg/kg and 10 mg/kg concentration on growth performance, slaughter performance, organ index, serum IGF2 parameters, *IGF2* mRNA expression and methylation patterns.

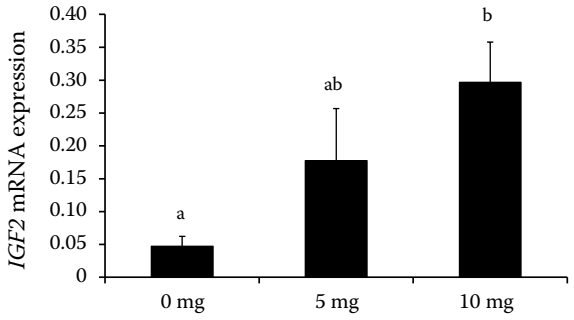

Figure 1. Effect of dietary folic acid supplementation on *IGF2* mRNA expression in abdominal fat of the 42-day-old broilers

^{a,b}Superscripts denote significant differences (P < 0.05)

The data presented here showed that the addition of 5 mg/kg FA to the diet positively influenced ABW and ADG of 21-day-old broilers compared to the control group and 10 mg/kg group. A similar finding was that the birth weight of new-hatched chickens with *in ovo* 150 μg FA injection was markedly higher than in the other groups (Liu et al. 2016). In addition, Liu et al. (2020) reported that FA supplementation increased ADG of Holstein dairy calves. Conversely, breeder cocks with extra folate exhibited a reduced body weight (Wu et al. 2019). Similarly, in pigs, dietary FA supplementation significantly reduced ADFI and ADG (Yao et al. 2013). It was reported that production performance of laying hens was not affected by FA supplementation (Tactacan

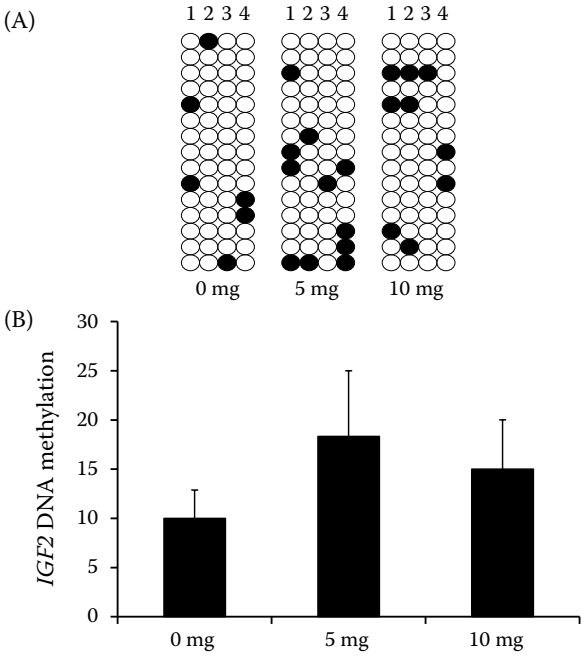

Figure 2. Effect of dietary folic acid supplementation on DNA methylation patterns of *IGF2*

(A) Bisulfite sequencing in DNA from abdominal fat of 42-day-old broilers, each horizontal row represents independent clone and each circle represents single CpG dinucleotide, and black circles and white circles indicate methylated and unmentylated CpG sites, respectively. (B) Bar graph represents of total methylation percentages of *IGF2* promoter of 42-day-old broilers

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et al. 2012). Additionally, the young and older laying hens responded differently to dietary FA (Jing et al. 2014). Recently, Zhang et al. (2020) have indicated that maternal FA deficiency had no influence on production performance and slaughter performance. It can be seen from the above studies that the role of FA in the animal body is very complex, and different results may be associated with differences between animal species used in the studies.

Chicken is frequently regarded as healthy food with fine muscle fibres, low fat content and high content of unsaturated fatty acids. However, modern commercial chickens exhibit excessive fat deposition in the abdominal area (Fouad and El-Senousey 2014). Thus, some researchers try to find solutions from dietary composition and feeding strategies to reduce body fat accumulation. The present study indicated that dietary FA supplementation could reduce the subcutaneous fat thickness and widths of intermuscular fat band of 42-day-old broilers. Similar to our study, another studies demonstrated that FA could reduce lipid accumulation in chicken adipocytes (Yu et al. 2014). Recently, it has been shown that the lipid and glucose metabolism of breeder cocks and broiler offspring was affected by paternal FA supplementation (Wu et al. 2019). On the contrary, it has been reported that the subcutaneous fat thickness at the age of 21 days of broiler chickens was increased significantly by maternal FA deficiency (Zhang et al. 2020). These results suggest that FA possibly contributes to lipogenesis and lipolysis (Yu et al. 2014).

In the present study, no significant effects on relative organ weight were observed by dietary supplementation of FA, except for the heart of 42-day-old broilers, the weight of which increased with 10 mg/kg FA supplementation. One recent study showed that extra folate exhibited increased organ indexes of the liver and bursa of Fabricius in breeder cocks, whereas no significant alterations were found in organ indexes of the spleen, testes and heart by FA (Wu et al. 2019). The same results were found by Liu et al. (2016) for chicken embryos injected with FA. Therefore, the effect of FA on the organ index is different, which is related to different chicken breeds, gender, age, and organ types.

The *IGF2* is a paternally expressed imprinted gene that plays an important role in DNA and protein synthesis, glucose, energy and even lipid metabolism (Perkins et al. 2012). The *IGF2* controls somatic growth and body composition, especially in early life, through a balance of cell differentiation, proliferation, embryo growth and development, and tumour cell proliferation (Huang et al. 2012). Previous studies indicated that aberrant DNA methylation of *IGF2* was associated with elevated plasma *IGF2* protein concentrations and increased *IGF2* gene expression, and risk of overweight, obesity and overgrowth disorders (Hoyo et al. 2012; Perkins et al. 2012). A similar result demonstrated that greater *IGF2*/*H19* methylation was associated with greater subcutaneous fat measures, but not overall, visceral or central adiposity in young adults (Huang et al. 2012). Conversely, lower circulating *IGF2* levels and decreased expression of *IGF2* were associated with fat mass and increased risk of weight gain and obesity in humans (Sandhu et al. 2003). The *IGF2* was also the major gene affecting the fatness traits of chickens (Li et al. 2004). Implantation with recombinant human *IGF2* in chickens could significantly increase the relative weight of the abdominal fat pads, while it significantly reduced the breast muscle (Li et al. 2004). In the present study, the breast muscle percentage was not modified by treatments or differences in *IGF2* gene expression and serum content. Indeed, there is a trend in the breast muscle percentage which was increased with FA supplementation.

Some studies demonstrated that maternal periconceptional FA use was associated with *IGF2* methylation in maternal blood and cord blood in women (Steegers-Theunissen et al. 2009; Hoyo et al. 2012). Steegers-Theunissen et al. (2009) found that children of mothers who used folic acid had a 4.5% higher methylation of *IGF2* than children who were not exposed to folic acid. In mice, the post-weaning diet lacking FA affected the methylation status of the *IGF2* gene and permanently affected the expression of *IGF2*, leading to *IGF2* loss of imprinting in adults (Waterland 2006). However, FA use in women after 12 weeks of gestation was associated with a higher level of methylation in *IGF2* of offspring (Haggarty et al. 2013). In contrast, a recent study reported that there were no significant associations between FA supplement before pregnancy and imprinting control of either *H19* in placental tissue or *IGF2* in cord blood (Tserga et al. 2017). Thus, FA supplementation during pregnancy plays different roles in the methylation of *IGF2*.

Liu's research group systematically investigated the relationship between folic acid and *IGF2* gene expression and methylation in chickens. They found that 150 μg FA injected on the 11th em-

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bryonic day of incubation significantly increased *IGF2* expression and decreased the methylation level of *IGF2* promoter in the liver of born chickens and spleen of 42-day-old chickens, respectively, and chromatin looseness of the *IGF2* promoter region was enhanced by 150 μg FA (Liu et al. 2016). In agreement with these studies, our results revealed that serum IGF2 concentrations and *IGF2* gene expression in abdominal fat were increased in 42-day-old broilers with the FA supplemented diets. Thus, whether FA affected *IGF2* expression in tissues by modulating DNA hypomethylation needs further study.

CONCLUSION

The supplementation of FA enhanced ABW and ADG of 21-day-old broilers, increased the relative heart weight of 42-day-old chickens, but decreased subcutaneous fat thickness and widths of intermuscular fat band of 42-day-old broilers. Meanwhile, dietary FA supplementation improved the serum IGF2 concentrations and *IGF2* mRNA expression in the abdominal fat, but no statistical differences were found in the methylation of *IGF2* promoter. Data from the present study suggest that FA is an important factor influencing chicken performance and gene expression. This study may provide a molecular nutrition basis for the studies of FA function in poultry.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory JF, Mills JL, Pfeiffer CM, Fazili Z, Zhang M, Ueland PM. Biomarkers of nutrition for development – Folate review. J Nutr. 2015 Jul 1;145(7):1636S-80S.

Crott JW. Effects of altered parental folate and one-carbon nutrient status on offspring growth and metabolism. Mol Aspects Med. 2017 Feb 1;53:28-35.

Czeizel AE, Dudas I, Vereczkey A, Banhidy F. Folate deficiency and folic acid supplementation: The prevention of neural-tube defects and congenital heart defects. Nutrients. 2013 Nov;5(11):4760-75.

Fouad AM, El-Senousey HK. Nutritional factors affecting abdominal fat deposition in poultry: A review. Asian-Australas J Anim Sci. 2014 Jul;27(7):1057-68.

Friso S, Udali S, De Santis D, Choi SW. One-carbon metabolism and epigenetics. Mol Aspects Med. 2017 Apr;54:28-36.

Haggarty P, Hoad G, Campbell DM, Horgan GW, Piyathilake C, McNeill G. Folate in pregnancy and imprinted gene and repeat element methylation in the offspring. Am J Clin Nutr. 2013 Jan 1;97(1):94-9.

Hoyo C, Fortner K, Murtha AP, Schildkraut JM, Soubry A, Demark-Wahnefried W, Jirtle RL, Kurtzberg J, Forman MR, Overcash F, Huang ZQ. Association of cord blood methylation fractions at imprinted insulin-like growth factor 2 (IGF2), plasma IGF2, and birth weight. Cancer Causes Control. 2012 Apr;23(4):635-45.

Huang RC, Galati JC, Burrows S, Beilin LJ, Li X, Pennell CE, van Eekelen J, Mori TA, Adams LA, Craig JM. DNA methylation of the IGF2/H19 imprinting control region and adiposity distribution in young adults. Clin Epigenetics. 2012 Nov 13;4(1): 11 p.

Jing M, Munyaka PM, Tactacan GB, Rodriguez-Lecompte JC, House JD. Performance, serum biochemical responses, and gene expression of intestinal folate transporters of young and older laying hens in response to dietary folic acid supplementation and challenge with Escherichia coli lipopolysaccharide. Poult Sci. 2014 Jan 1;93(1):122-31.

Kim YI, Pogribny IP, Basnakian AG, Miller JW, Mason JB. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Am J Clin Nutr. 1997 Jan 1;65(1):46-52.

Kolbadinejad A, Rezaeipour V. Efficacy of ajwain (Trachyspermum ammi L.) seed at graded levels of dietary threonine on growth performance, serum metabolites, intestinal morphology and microbial population in broiler chickens. J Anim Physiol Nutr. 2020 Sep;104(5):1333-42.

Li ZH, Li H, Wang QG, Zhan JG, Wang YX. The study on correlation analysis of single nucleotide polymorphism of IGF2 gene and body fatness traits in chicken. Agr Sci China. 2004 Jan 1;3(10):789-94.

Lightfoot TJ, Skibola CF, Willett EV, Skibola DR, Allan JM, Coppede F, Adamson PJ, Morgan GJ, Roman E, Smith MT. Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. Cancer Epidemiol Biomarkers Prev. 2005 Dec 1;14(12):2999-3003.

Liu JB, Yao J, Yu B, Mao XB, Huang ZQ, Chen DW. Effect of maternal folic acid supplementation on hepatic proteome in newborn piglets. Nutrition. 2013 Jan 1;29(1):230-4.

Liu YL, Zhi LH, Shen J, Li SZ, Yao JH, Yang XJ. Effect ofin ovo folic acid injection on hepatic IGF2 expression and embryo growth of broilers. J Anim Sci Biotechnol. 2016 Jul 22;7(1): 9 p.

https://doi.org/10.17221/76/2021-CJAS

Liu YR, Du HS, Wu ZZ, Wang C, Liu Q, Guo G, Huo WJ, Zhang YL, Pei CX, Zhang SL. Branched-chain volatile fatty acids and folic acid accelerated the growth of Holstein dairy calves by stimulating nutrient digestion and rumen metabolism. Animal. 2020 Jun;14(6):1176-83.

Nazki FH, Sameer AS, Ganaie BA. Folate: Metabolism, genes, polymorphisms and the associated diseases. Gene. 2014 Jan 1;533(1):11-20.

Pereira SS, Monteiro MP, Costa MM, Moreira A, Alves MG, Oliveira PF, Jarak I, Pignatelli D. IGF2 role in adrenocortical carcinoma biology. Endocrine. 2019 Aug 4;66(2):326-37.

Perkins E, Murphy SK, Murtha AP, Schildkraut J, Jirtle RL, Demark-Wahnefried W, Forman MR, Kurtzberg J, Overcash F, Huang ZQ, Hoyo C. Insulin-like growth factor 2/H19 methylation at birth and risk of overweight and obesity in children. J Pediatrics. 2012 Jul;161(1):31-9.

Sandhu MS, Gibson JM, Heald AH, Dunger DB, Wareham NJ. Low circulating IGF-II concentrations predict weight gain and obesity in humans. Diabetes. 2003 Jun 1;52(6):1403-8.

Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, Slagboom PE, Heijmans BT. Periconceptional maternal folic acid use of 400 μg per day is related to increased methylation of the IGF2 gene in the very young child. PLoS One. 2009 Nov 16;4(11): 5 p.

Tactacan GB, Rodriguez-Lecompte JC, House JD. The adaptive transport of folic acid in the intestine of laying hens with increased supplementation of dietary folic acid. Poult Sci. 2012 Jan 1;91(1):121-8.

Tserga A, Binder AM, Michels KB. Impact of folic acid intake during pregnancy on genomic imprinting of IGF2/H19 and 1-carbon metabolism. FASEB J. 2017 Dec;31(12):5149-58.

Waterland RA. Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (Igf2) locus. Hum Mol Genet. 2006 Mar 1;15(5):705-16.

Wu S, Guo W, Li X, Liu Y, Li Y, Lei X, Yao J, Yang X. Paternal chronic folate supplementation induced the transgenerational inheritance of acquired developmental and metabolic changes in chickens. Proc R Soc B. 2019 Sep 11;286(1910): 9 p.

Xing JY, Kang L, Hu Y, Xu QY, Zhang NB, Jiang YL. Effect of dietary betaine supplementation on mRNA expression and promoter CpG methylation of lipoprotein lipase gene in laying hens. J Poult Sci. 2009 Jul 25;46(3):224-8.

Xing JY, Jing WQ, Zhang YJ, Liu L, Xu JJ, Chen XW. Identification of differentially expressed genes in broiler offspring under maternal folate deficiency. Physiol Genomics. 2018 Dec 1;50(12):1015-25.

Yao Y, Yu B, Chen DW, Tian G, Mao XB, Zheng P, Liu JB. Effect of dietary folic acid supplementation on growth performance and hepatic protein metabolism in early-weaned intrauterine growth retardation piglets. J Integr Agr. 2013 May;12(5):862-8.

Yu X, Liu R, Zhao G, Zheng M, Chen J, Wen J. Folate supplementation modifies CCAAT/enhancer-binding protein α methylation to mediate differentiation of preadipocytes in chickens. Poult Sci. 2014 Oct 1;93(10):2596-603.

Zhang Y, Jing W, Zhang N, Hao J, Xing J. Effect of maternal folate deficiency on growth performance, slaughter performance, and serum parameters of broiler offspring. J Poult Sci. 2020 Oct 25;57(4):270-6.

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