

The impact of folic acid on genome-wide DNA methylation profiles in the liver of broilers

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Electronic Supplementary Material (ESM)

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Table S1. Content and nutrition level of diet for broiler (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 ·2H ₂ O	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
Prem ¹ xa	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 + cystine (%)	0.91	0.82
Folic acid (mg/kg)	0.36	0.33

^a Premix provided per kilogram of diets: Mn, 55 mg; Zn, 55 mg; Fe, 44 mg; Cu, 5.5 mg; I, 0.44 mg; Se, 0.099 mg; VA, 770 IU; VB₁, 1.2 mg; VB₂, 4.8 mg; VB₆, 1.6 mg; VB₁₂, 0.011 mg; VD₃, 255 IU; VE, 15 mg; VK, 2.2 mg; pantothenic acid, 11 mg; niacin, 35.5 mg; folic acid, 0.66 mg; biotin, 0.11 mg; choline, 500 mg; xanthin, 33.1 mg; antioxidant, 120 mg.

Table S2. Primers of qRT-PCR and methylation

Primers	Primer sequence (5'-3')	Annealing temperature (°C)	Product size (bp)	Usage
<i>ECE1</i> -E-F	TTGGAGTTTGAGACAGCG	60	189	gene expression
<i>ECE1</i> -E-R	ATACACCACCACAGGCTCT			
<i>METTL21A</i> -E-F	GGAGCGTTTGACTTCATC	50	142	
<i>METTL21A</i> -E-R	TGTTATCCCGTTCGTAGC			
<i>β-actin</i> -F	GTGACATCAAGGAGAAGC	55	105	
<i>β-actin</i> -R	CATCAGGGAGTTCATAGC			
<i>ECE1</i> -M-F	AGGTTTGTATGAATGAGAGTAAGAT	59	191	methylation
<i>ECE1</i> -M-R	AATATTCCATCCTCCCAACTAAAA			
<i>METTL21A</i> -M-F	GTGGGAAAGTAGGGTTGTTTTTT	60	257	
<i>METTL21A</i> -M-R	CAATTCCTTAAACCTACTCCAAAAC			

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Table S3. Top 10 hypermethylated and hypomethylated regions between each comparison group

Groups	Hypermethylation/ hypomethylation	Chromosome	Start	End	P-value	Methylation difference (%)
C vs M	hypermethylation	Chr1	193348026	193348026	1.29E-05	100.00
		Chr15	10862357	10862357	1.29E-08	100.00
		Chr4	28807249	28807249	3.41E-10	97.06
		Chr15	1599359	1599359	7.32E-06	95.83
		Chr11	1703266	1703266	8.56E-07	95.83
		Chr3	104132403	104132403	5.72E-07	95.00
		Chr2	400690	400690	6.03E-20	94.87
		Chr13	12561957	12561957	5.66E-08	93.75
		Chr4	12893349	12893349	1.10E-05	92.31
		Chr26	1629488	1629488	2.83E-09	90.00
	hypomethylation	Chr14	12944854	12944854	3.42E-06	-100.00
		Chr14	14046757	14046757	1.24E-05	-100.00
		Chr19	6757304	6757304	1.90E-17	-100.00
		Chr21	6549953	6549953	8.21E-18	-100.00
		Chrw	876419	876419	8.18E-07	-100.00
		Chr6	22836220	22836220	1.05E-11	-95.45
		Chr13	7886848	7886848	6.20E-59	-94.95
		Chr20	10968578	10968578	8.03E-09	-94.87
		Chr7	22044314	22044314	2.20E-07	-94.74
		Chr18	10814128	10814128	8.38E-07	-94.44
C vs H	hypermethylation	Chr19	5592738	5592738	5.65E-06	100.00
		Chr8	20088050	20088050	1.27E-07	100.00
		Chr12	18478939	18478939	8.48E-10	96.77
		Chr13	9346617	9346617	4.93E-09	93.75
		Chr26	1629488	1629488	2.69E-06	91.67
		Chr8	20088051	20088051	1.73E-08	90.91
		Chrz	637450	637450	4.60E-24	90.14
		Chr1	59864981	59864981	3.29E-09	90.00
		Chr13	12561957	12561957	3.20E-06	90.00
		Chr25	139605	139605	3.11E-11	89.24
	hypomethylation	Chr8	19161650	19161650	3.93E-09	-100.00
		Chr1	1614423	1614423	6.16E-06	-96.83
		Chr21	6549952	6549952	2.49E-10	-94.12
		Chr2	1.43E+08	1.43E+08	2.87E-09	-93.33
		Chr5	16222445	16222445	6.85E-06	-92.86
		Chr19	4222374	4222374	6.44E-27	-91.30
		Chr21	6549953	6549953	1.13E-08	-90.00
		Chr24	5917903	5917903	1.38E-40	-87.31
		Chr2	66279124	66279124	3.28E-21	-86.30
		Chr8	19161649	19161649	9.45E-14	-86.30

Table S3 to be continued

Groups	Hypermethylation/ hypomethylation	Chromosome	Start	End	P-value	Methylation difference (%)
M v. H	hypermethylation	Chr10	925610	925610	1.04E-07	100.00
		Chr17	464083	464083	3.10E-08	100.00
		Chr19	5592738	5592738	2.65E-06	100.00
		Chr7	22095189	22095189	3.06E-07	100.00
		Chr8	20088050	20088050	1.05E-08	100.00
		Chr7	34956974	34956974	7.03E-59	95.83
		Chr20	10968578	10968578	3.44E-06	94.87
		Chr13	12192293	12192293	1.66E-05	94.44
		Chr12	18478939	18478939	4.31E-09	93.75
		Chr12	2877839	2877839	1.00E-12	92.31
	hypomethylation	Chr1	20133506	20133506	1.24E-05	-100.00
		Chr11	1143451	1143451	4.08E-06	-100.00
		Chr14	4159190	4159190	3.06E-07	-100.00
		Chr15	10862357	10862357	8.60E-06	-100.00
		Chr3	66326995	66326995	1.74E-09	-100.00
		Chr2	142872258	142872258	1.72E-09	-96.15
		Chr19	4123176	4123176	1.01E-05	-93.75
		Chr6	7270907	7270907	1.05E-05	-93.33
		Chr23	4987617	4987617	6.53E-09	-91.67
		Chr2	6488245	6488245	4.72E-10	-89.29

C = control group (0 mg/kg FA); Chr = chromosome; H = high folic acid group (10 mg/kg FA); M = medium folic acid group (5 mg/kg FA)

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Table S4. The top 10 significant DMGs in the promoter region

Groups	Position	ID	P-value	Up/down	Q value	Symbol
C vs M	chr10:1941038:1943038:+	NM_205147	0.000 000	up	0.000 006	<i>CYP1A4</i>
	chr10:1941050:1943050:-	NM_205146	0.000 000	up	0.000 013	<i>CYP1A1</i>
	chr10:2011307:2013307:-	NM_001256157	0.000 000	up	0.000 019	<i>ARID3B</i>
	chr10:2011869:2013869:+	NM_001007824	0.000 000	up	0.000 026	<i>ACTG1</i>
	chr10:2135427:2137427:+	NM_001080876	0.000 000	up	0.000 032	<i>NEIL1</i>
	chr13:9740537:9742537:+	NM_001030610	0.000 000	up	0.000 045	<i>KIAA1191</i>
	chr13:9741782:9743782:-	NM_001277714	0.000 000	up	0.000 058	<i>ARL10</i>
	chr18:10114363:10116363:+	NM_204600	0.000 000	up	0.000 070	<i>SOCS3</i>
	chr18:10115046:10117046:-	NM_001008463	0.000 000	up	0.000 077	<i>PGS1</i>
	chr23:1808792:1810792:+	NM_001006311	0.000 000	up	0.000 083	<i>NUDC</i>
C vs H	chr10:2011307:2013307:-	NM_001256157	0.000 000	up	0.000 007	<i>ARID3B</i>
	chr10:2011869:2013869:+	NM_001007824	0.000 000	up	0.000 013	<i>ACTG1</i>
	chr10:2135427:2137427:+	NM_001080876	0.000 000	up	0.000 020	<i>NEIL1</i>
	chr13:9740537:9742537:+	NM_001030610	0.000 000	up	0.000 033	<i>KIAA1191</i>
	chr13:9741782:9743782:-	NM_001277714	0.000 000	up	0.000 046	<i>ARL10</i>
	chr14:12694121:12696121:-	NM_001004377	0.000 000	down	0.000 059	<i>PAM16</i>
	chr23:1808792:1810792:+	NM_001006311	0.000 000	up	0.000 066	<i>NUDC</i>
	chr23:1809396:1811396:-	NM_001030893	0.000 000	up	0.000 079	<i>NR0B2</i>
	chr23:4833741:4835741:+	NM_001030902	0.000 000	up	0.000 092	<i>SMAP2</i>
	chr23:4834663:4836663:-	NM_001271988	0.000 000	up	0.000 099	<i>COL9A2</i>
M vs H	chr10:2011307:2013307:-	NM_001256157	0.000 000	up	0.000 007	<i>ARID3B</i>
	chr10:2011869:2013869:+	NM_001007824	0.000 000	up	0.000 020	<i>ACTG1</i>
	chr10:2135427:2137427:+	NM_001080876	0.000 000	up	0.000 033	<i>NEIL1</i>
	chr13:9740537:9742537:+	NM_001030610	0.000 000	up	0.000 046	<i>KIAA1191</i>
	chr13:9741782:9743782:-	NM_001277714	0.000 000	up	0.000 059	<i>ARL10</i>
	chr18:10114363:10116363:+	NM_204600	0.000 000	down	0.000 073	<i>SOCS3</i>
	chr18:10115046:10117046:-	NM_001008463	0.000 000	down	0.000 079	<i>PGS1</i>
	chr23:1808792:1810792:+	NM_001006311	0.000 000	up	0.000 086	<i>NUDC</i>
	chr23:1809396:1811396:-	NM_001030893	0.000 000	up	0.000 099	<i>NR0B2</i>
	chr23:4833741:4835741:+	NM_001030902	0.000 000	up	0.000 112	<i>SMAP2</i>

C = control group (0 mg/kg FA); Chr = chromosome; H = high folic acid group (10 mg/kg FA); M = medium folic acid group (5 mg/kg FA); Q value = false discovery rate (FDR)-adjusted *P*-value; Symbol = official gene symbol

Table S5. Enrichment results of GO terms in the top 10 of DMGs

Groups	Category	GO: ID	Description	P-value	Genes
C vs M	BP	GO:0014707	branchiomic skeletal muscle development	0.001 361	3
		GO:0002478	antigen processing and presentation of exogenous peptide antigen	0.001 361	3
		GO:0044381	glucose import in response to insulin stimulus	0.001 361	3
		GO:2001273	regulation of glucose import in response to insulin stimulus	0.001 361	3
		GO:0007517	muscle organ development	0.001 526	18
		GO:0060537	muscle tissue development	0.002 303	20
		GO:0009410	response to xenobiotic stimulus	0.002 397	4
		GO:0061061	muscle structure development	0.002 716	24
		GO:0048625	myoblast fate commitment	0.003 224	3
		GO:0019884	antigen processing and presentation of exogenous antigen	0.003 224	3
	CC	GO:0005737	cytoplasm	0.000 007	201
		GO:0005739	mitochondrion	0.001 620	48
		GO:0002080	acrosomal membrane	0.005 708	3
		GO:0044424	intracellular part	0.020 889	238
		GO:0005622	intracellular	0.026 007	243
		GO:0042613	MHC class II protein complex	0.026 397	2
		GO:0043073	germ cell nucleus	0.026 397	2
		GO:0000781	chromosome, telomeric region	0.032 381	4
		GO:0098687	chromosomal region	0.032 381	4
		GO:0030135	coated vesicle	0.040 383	6
	MF	GO:0003955	NAD(P)H dehydrogenase (quinone) activity	0.000 349	3
		GO:0030332	cyclin binding	0.003 137	3
		GO:0032453	histone demethylase activity (H3-K4 specific)	0.004 975	2
		GO:0004419	hydroxymethylglutaryl-CoA lyase activity	0.004 975	2
		GO:0004157	dihydropyrimidinase activity	0.004 975	2
		GO:0016833	oxo-acid-lyase activity	0.004 975	2
		GO:0016829	lyase activity	0.007 54	10
		GO:0005212	structural constituent of eye lens	0.007 678	4
		GO:0030544	Hsp70 protein binding	0.009 867	3
		GO:0051117	ATPase binding	0.010 483	4

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Table S5 to be continued

Groups	Category	GO: ID	Description	P-value	Genes
C vs H	BP	GO:0007422	peripheral nervous system development	0.000 012	9
		GO:0019228	neuronal action potential	0.000 164	9
		GO:0042552	myelination	0.000 273	9
		GO:0007272	ensheathment of neurons	0.000 346	9
		GO:0008366	axon ensheathment	0.000 346	9
		GO:0019226	transmission of nerve impulse	0.000 820	9
		GO:0050808	synapse organization	0.001 185	10
		GO:0035637	multicellular organismal signaling	0.001 396	10
		GO:0009410	response to xenobiotic stimulus	0.001 865	4
		GO:0001508	action potential	0.002 822	9
	CC	GO:0034518	RNA cap binding complex	0.000 606	4
		GO:0005845	mRNA cap binding complex	0.001 159	3
		GO:0044815	DNA packaging complex	0.004 290	6
		GO:0000786	nucleosome	0.009 054	5
		GO:1990104	DNA bending complex	0.009 054	5
		GO:0043073	germ cell nucleus	0.024 892	2
		GO:0005737	cytoplasm	0.028 904	176
		GO:0031201	SNARE complex	0.029 312	4
		GO:0016328	lateral plasma membrane	0.035 214	4
		GO:0030667	secretory granule membrane	0.041754	4
	MF	GO:0003955	NAD(P)H dehydrogenase (quinone) activity	0.000 293	3
		GO:0047710	bis(5'-adenosyl)-triphosphatase activity	0.001 114	3
		GO:0004551	nucleotide diphosphatase activity	0.001 114	3
		GO:0004157	dihydropyrimidinase activity	0.004 426	2
		GO:0016655	oxidoreductase activity, acting on NAD(P)H, quinone or similar compound as acceptor	0.011 342	4
		GO:0004708	MAP kinase kinase activity	0.012 691	2
		GO:0051920	peroxiredoxin activity	0.012 691	2
		GO:0070402	NADPH binding	0.012 691	2
		GO:0070330	aromatase activity	0.012 691	2
		GO:0016780	phosphotransferase activity, for other substituted phosphate groups	0.012755	3

Table S5 to be continued

Groups	Category	GO: ID	Description	P-value	Genes
M vs H	BP	GO:0046329	negative regulation of JNK cascade	0.000 546	4
		GO:0048268	clathrin coat assembly	0.001 070	3
		GO:0032873	negative regulation of stress-activated MAPK cascade	0.001 769	4
		GO:0070303	negative regulation of stress-activated protein kinase signalling cascade	0.001 769	4
		GO:0042990	regulation of transcription factor import into nucleus	0.002 188	7
		GO:0042991	transcription factor import into nucleus	0.002188	7
		GO:1900180	regulation of protein localization to nucleus	0.002 317	11
		GO:0006901	vesicle coating	0.002 545	3
		GO:0046822	regulation of nucleocytoplasmic transport	0.004 301	11
		GO:0002904	positive regulation of B cell apoptotic process	0.004 307	2
	CC	GO:0005737	cytoplasm	0.015 509	174
		GO:0030118	clathrin coat	0.017 849	4
		GO:0070062	extracellular vesicular exosome	0.020 802	66
		GO:0043230	extracellular organelle	0.021350	66
		GO:0065010	extracellular membrane-bounded organelle	0.021 350	66
		GO:0005925	focal adhesion	0.022 430	18
		GO:0005924	cell-substrate adherens junction	0.022 430	18
		GO:0030055	cell-substrate junction	0.022 430	18
		GO:0005736	DNA-directed RNA polymerase I complex	0.023 749	2
		GO:0005605	basal lamina	0.023 749	2
	MF	GO:0001786	phosphatidylserine binding	0.001073	3
		GO:0004419	hydroxymethylglutaryl-CoA lyase activity	0.004 314	2
		GO:0016833	oxo-acid-lyase activity	0.004 314	2
		GO:0030276	clathrin binding	0.008 088	3
		GO:0005543	phospholipid binding	0.010 174	11
		GO:0032266	phosphatidylinositol-3-phosphate binding	0.012 316	3
		GO:0030976	thiamine pyrophosphate binding	0.012 378	2
		GO:0051920	peroxiredoxin activity	0.012 378	2
		GO:0008429	phosphatidylethanolamine binding	0.012 378	2
		GO:0070330	aromatase activity	0.012 378	2

ATPase = adenosine triphosphatase; BP = biological process; C = control group (0 mg/kg FA); CC = cellular component; DMG = differentially methylated gene; GO = Gene Ontology; H = high folic acid group (10 mg/kg FA); Hsp70 = heat shock protein 70; JNK = c-Jun N-terminal kinase; M = medium folic acid group (5 mg/kg FA); MAPK = mitogen-activated protein kinase; MF = molecular function; MHC = major histocompatibility complex; mRNA = messenger RNA; NAD(P)H = nicotinamide adenine dinucleotide phosphate; P = probability; SNARE = soluble NSF attachment protein receptor