Animal Nutrition 4 (2018) 11–16

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Review Article

Role of methionine on epigenetic modification of DNA methylation and gene expression in animals

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ARTICLE INFO

Article history:
Received 25 May 2017
Received in revised form
25 August 2017
Accepted 30 August 2017
Available online 19 September 2017

Keywords:
Epigenetics
Methionine
DNA methylation
Gene expression
Epigenetic modification

ABSTRACT

DNA methylation is one of the main epigenetic phenomena affecting gene expression. It is an important mechanism for the development of embryo, growth and health of animals. As a key nutritional factor limiting the synthesis of protein, methionine serves as the precursor of S-adenosylmethionine (SAM) in the hepatic one-carbon metabolism. The dietary fluctuation of methionine content can alter the levels of metabolic substrates in one-carbon metabolism, e.g., the SAM, S-adenosylhomocysteine (SAH), and change the expression of genes related to the growth and health of animals by DNA methylation reactions. The ratio of SAM to SAH is called 'methylation index' but it should be carefully explained because the complexity of methylation reaction. Alterations of methylation in a specific cytosine-guanine (CpG) site, rather than the whole promoter region, might be enough to change gene expression. Aberrant methionine cycle may provoke molecular changes of one-carbon metabolism that results in deregulation of cellular hemostasis and health problems. The importance of DNA methylation has been underscored but the mechanisms of methionine affecting DNA methylation are poorly understood. Nutritional epigenomics provides a promising insight into the targeting epigenetic changes in animals from a nutritional standpoint, which will deepen and expand our understanding of genes, molecules, tissues, and animals in which methionine alteration influences DNA methylation and gene expression.

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1. Introduction

Epigenetics is receiving more and more attention in molecular biology fields. Diet nutrient changes can alter gene expression without changing the DNA sequence and affect the development of embryo, the growth and health of animals by epigenetics mechanisms (Feil and Fraga, 2012; Gluckman, 2011). By exploiting the relationship between nutrition and gene expression, the epigenetic phenomena related to growth and health processes can be regulated (Anderson et al., 2012; Burdge and Lillycrop, 2010; Ji et al., 2016; Zhang, 2015). This dynamic relationship between nutrition and gene expression throughout the lifetime of an organism has now been recognized as a subfield called 'nutritional epigenomics' and provides a promising insight into the targeting growth and health of animals from a nutritional standpoint (Anderson et al., 2012; Reik, 2007).

Epigenetic processes include the modification of DNA by cytosine-guanine (CpG) sites methylation (Jaenisch and Bird, 2003; Jones and Takai, 2001; Triantaphyllopoulos et al., 2016), which is one form of epigenetic modification widely studied (Anckaert and Fair, 2015; Day et al., 2015; Farkas et al., 2015; Parrish et al., 2015). Methionine, an essential amino acid, serves as the precursor of S-adenosylmethionine (SAM) in the one-carbon metabolism (Mentch and Locasale, 2016). Recent studies demonstrate its essential role in diverse cellular processes (Castellano et al., 2015; Rolland et al., 2015; Zubiete-Franco et al., 2016) and the liver is the site where nearly half of the methionine metabolism and 85% of all methylation reactions takes place (Lu and Mato, 2012). The fluctuation of methionine can influence DNA methylation and consequently contribute to the dysregulation of gene expression, finally cause the pathogenesis and progression of liver disease

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2017.08.009

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(Mato and Lu, 2007). However, reviews focused on the relationship between methionine and epigenetics are fairly poor in the past decades, except that by Waterland (2006) who reviewed the effects of high methionine intake on DNA methylation. In this paper, we will focus on the effects of dietary methionine fluctuation on one-carbon metabolic substrates and the critical epigenetic role of DNA methylation in alteration of gene expression in animals.

2. Nutritional epigenomics

Nutrients can affect gene expression via epigenetic mechanisms (Zhang, 2015). Epigenetics is the phenomenon related to mitotically heritable yet potentially reversible DNA modifications but with no change of the DNA sequence (Triantaphyllopoulos et al., 2016), providing a connection between environment (e.g., nutritional factors) and health of animals and humans. Epigenetics builds a firm link between phenotypic plasticity and fixed genotype, and plays an important role for creating and keeping the diversity of gene expression patterns in animals. Epigenetic modifications consist of DNA methylation, histone modification, microRNA and non-coding RNA regulation (Turner, 2007). DNA methylation is widespread in animals and suppresses gene transcription by directly or indirectly methylating the CpG islands in gene promoters, playing critical roles in epigenetic processes (Jaenisch and Bird, 2003). DNA methyltransferases (DNMT) are key enzymes for activating DNA methylation in animals (Miranda and Jones, 2007). Three active DNMT (DNMT1, DNMT3a, and DNMT3b) are responsible for *de novo* methylation (Arand et al., 2012). DNA methyltransferase 1 prefers for hemimethylated DNA and keeps the methylation pattern intact after DNA replication (Jeltsch, 2006; Jurkowska et al., 2011) while DNMT3a and DNMT3b do not distinguish between methylated substrates (Jurkowska et al., 2011). Furthermore, DNA methylation occurs dramatically in the early development stage of mammals (Santangelo et al., 2009). More interestingly, the methylation in the body of genes can actually activate gene transcription compared with methylation in promoters (Langevin and Kelsey, 2013). The genome is constant, but the gene regulation effects of DNA methylation may vary across positions of methylation in genes, tissues, and development stages (Ibeaghaawemu and Zhao, 2015). Nutrients may have a critical role in affecting epigenetic modifications, providing possible ways through which nutrients may regulate gene expression and final phenotypes in animals (Ross, 2003).

Methionine is vital in epigenetic reactions by donating methyl groups to methylating cytosine in CpG islands (Tehlivets et al., 2013). Actually, it is the DNMT that converts SAM to S-adenosylhomocysteine (SAH) (Tehlivets et al., 2013). Importantly, the SAM is generated from methionine through the adenylation of methionine catalyzed by methionine adenosyltransferase (MAT) (Finkelstein, 1990). Generally, SAH is hydrolyzed by adenosylhomocysteinase (Ahcy) into homocysteine (Hcy) and adenosine as long as the latter is removed through other metabolic pathways. If not, the reverse reaction will occur to increase the concentration of SAH (Richards et al., 1978). The thermodynamics of Ahcy favors SAH biosynthesis rather than hydrolysis (Finkelstein, 1990) except Hcy is rapidly removed, which is essential to preventing the accumulation of SAH (Lu and Mato, 2012). There exit 2 outlets for Hcy: one is regeneration of methionine by betaine–homocysteine methyltransferase (BHMT) or 5-methyltetrahydrofolate–homocysteine methyltransferase (MS), another way is entering the trans-sulfuration pathway and to generate cystathionine by cystathionine β-synthase (Lu and Mato, 2012).

Nutritional factors, e.g., methionine, influencing the metabolism of SAM and SAH, may have impact on DNA methylation. The establishment of epigenome is vulnerable to nutritional factors especially during the prenatal and early postnatal stages of animals (Mentch and Locasale, 2016; Zhang, 2015). Therefore, nutritional challenges during the early development stage might have a dramatic impact on the DNA methylation, the growth and health of animals (Zhang, 2015). Besides, epigenetic variations may happen in the "dietary transition" period, in which animals are exposed to a nutritionally imbalanced diet that may affect the DNA methylation (Zhang, 2015). There are many studies that have reported the effects of nutritional supplementation, e.g., dietary methionine deficiency or excess, during pre- and post-natal stages on the global or locus specific alterations in DNA methylation, and the long-term impacts on the gene expression and consequently growth and health of animals (Del Vesco et al., 2015; Rolland et al., 2015; Waterland and Jirtle, 2003).

3. Methionine and one-carbon metabolic substrates

Previous studies have indicated a firm connection between dietary and hepatic methionine levels (Parrish et al., 2015). Feeding very high levels of dietary methionine (1.5%) in weanling rats for 2 weeks resulted in a 4- to 30-fold accumulation of methionine in the intestine, liver, skeletal muscle, kidney and plasma compared with feeding a diet containing 0.61% methionine (Regina et al., 1993). Similarly, methionine injection (100 mg/kg) in adult epileptic rats caused a 7-fold increase in methionine of hippocampus compared to control rats (Parrish et al., 2015). However, the effect of dietary methionine intakes on the hepatic methionine is not always in distinct proportions. No change in hepatic methionine was observed in adult rates fed purified diets with different methionine contents (0.3%, 1%, 1.5%, 2%, or 3% methionine; wt/wt) lasting for 7 days, showing effectual metabolic equilibrium facing dietary methionine excess (Finkelstein and Martin, 1986). More interestingly, Aissa et al. (2014) found that hepatic methionine levels substantially decreased in mice fed either a methionine-deficient or methionine-supplemented diet. A decrease in hepatic methionine level in mice fed a methionine deficient diet was expected, but not in those fed the methionine-supplemented diet.

Generally, an increase in hepatic SAM is expected when dietary methionine intake is increased (Rowling et al., 2002). Consequently, SAM would be converted to SAH by DNMT. But the activity of DNMT can be inhibited by a high intracellular SAH concentration (Rowling et al., 2002; Rowling and Schalinske, 2001). The results of previous research have illustrated a tight association between dietary methionine intake and one-carbon metabolic substrates, e.g., SAM and SAH, in liver or other organs (Table 1). Hepatic SAM level was maintained, whereas SAH gradually increased with the increase of dietary methionine from 0.3% to 2.0% in the study of Finkelstein and Martin (1986), illustrating a tremendous reduction in the ratio of SAM to SAH. Similarly, brain SAH was doubled with the injection of methionine (2 g/kg per day) subcutaneously lasting for 15 days in male mice, whereas SAM was not affected, and the ratio of SAM to SAH was decreased (Tremolizzo et al., 2002). In another study, providing 0.5% methionine in the drinking water from 7 to 15 weeks, the hepatic and cerebral SAH in weanling mice increased, whereas SAM was not affected. Furthermore, the ratio of SAM to SAH decreased (Devlin et al., 2004). These results suggest that the elevated SAH should inhibit the SAM-dependent methyltransferase reactions (Ueland, 1982), including Glycine N-methyltransferase (GNMT) which functions to regulate SAM levels and the ratio of SAM to SAH (Rowling and Schalinske, 2001), and reduce the hepatic and cerebral capacity of methylation (Barbés et al., 1990; Cabo et al., 1995; Chen et al., 2001; Dayal et al., 2001). However, the hepatic SAM increased 10 and 20 times, while SAH increased 30% and 4 times in rats fed diets containing 1.3% and 2.3% methionine, respectively, compared with control rats pair-fed a diet containing

Table 1
Summary of relevant studies showing effects of methionine on one-carbon metabolic substrates in humans and model organisms.

Methionine condition, %	Species	Period of dietary input	Lasted	Tissue	One-carbon metabolites modification(s)	References
Diets, 0.3, 1.0, 1.5, 2.0, 3.0	Rat	250 g weight	7 d	Liver	SAM -, SAH ↑, SAM:SAH ↓	Finkelstein and Martin (1986)
Injection, 6.6 mmol/kg subcutaneously	Mice	60 d of age	15 d	Brain	SAM -, SAH ↑, SAM:SAH ↓	Tremolizzo et al. (2002)
Drinking, 0, 0.5	Mice	Weanling	From 7 to 15 wk,	Liver and brain	SAM -, SAH ↑, SAM:SAH ↓	Devlin et al. (2004)
Diets, 0.61, 1.5	Rat	Weanling	2 wk	Liver	SAM ↑, SAH ↑, SAM:SAH ↑	Regina et al. (1993)
Diets, 0.3, 1.3, 2.3	Rat	54 to 74 g weight	10 d	Liver	SAM ↑, SAH ↑, SAM:SAH ↑	Rowling et al. (2002)
Diets, 0, 1.7	Mice	6 wk of age	20 wk	Serum	SAM ↑, SAH ↑, SAM:SAH ↑	Yang et al. (2015)
Diets, 0.5, 1, 2	Rainbow trout	Brood stock	6 months	Oocytes	SAM ↑, SAH ↑, SAM:SAH ↓	Fontagné-Dicharry et al. (2017)
Diets, 0.3, 2	Rat	5 to 6 wk of age	6 wk	Kidney	SAM -, SAH ↑, SAM:SAH -	Amaral et al. (2011)
Diets, 0, 2	Mice	4 wk of age	10 wk	Liver	SAM ↑, SAH ↑, SAM:SAH -	Aissa et al. (2014)

-: no change, ↑: increased, ↓: decreased.

0.3% methionine (Rowling et al., 2002). Consequently, the hepatic ratio of SAM to SAH increased 8 times in both 1.3% and 2.3% methionine diets, respectively, compared with control diet (Rowling et al., 2002). Similarly, an 80% increase of the hepatic ratio of SAM to SAH was observed following the 7 times of SAM and 4 times of SAH increases caused by a 1.5% dietary methionine intake (Regina et al., 1993). The 1.7% methionine diet increased the serum SAM, SAH and ratio of SAM to SAH, especially the serum SAH concentration, which was elevated by 2.28 folds (Yang et al., 2015). Furthermore, the SAM, SAH concentration increased in oocytes of rainbow trout which were fed 2% methionine diets for 6 months, but the ratio of SAM to SAH was decreased (Fontagné-Dicharry et al., 2017). More interestingly, hepatic SAM and SAH levels were both increased in mice fed diets supplemented 0 or 2% methionine but the ratio of SAM to SAH seemed unaffected (Aissa et al., 2014). Similarly, both renal SAM and SAH levels increased but only SAH showed a significant difference, while the SAM to SAH ratio remained unchanged in rats fed both the methionine-supplemented (2% methionine) and methionine-deficient (0.3% methionine) diets (Amaral et al., 2011).

Enzyme-catalyzed reaction is determined by substrate supply and production removal. Therefore, the ratio of SAM to SAH was called 'methylation index' in the context of methylation reaction, including hypo-methylation and hyper-methylation. However, methylation reaction is much more complex as besides the substrates (SAM) and production (SAH), and there are other factors affecting the transmethylation efficiency, e.g., animal age, breed and diet. Furthermore, there is more and more evidence that methylation reaction is tissue specific (Herzog et al., 2013; Wan et al., 2015). Relevant studies showing the effects of methionine on one-carbon metabolic substrates in humans and animals are summarized in Table 1, suggesting different methylation patterns that occur in different tissues with dietary methionine supplementation. More importantly, recent genome wide methylation analysis revealed that DNA hyper-methylation does not always repress gene expression (Zhang, 2015).

4. Methionine and DNA methylation

Theoretically, supplementation of methionine would increase the DNA methylation of genes and down-regulate gene expression (Waterland, 2006). Indeed, hypermethylation in the reelin promoter and down-regulated reelin gene expression were observed in cortex of mice by methionine supplementation (6.6 mmol/kg) (Tremolizzo et al., 2002). Reelin is a secreted extracellular matrix protein that is reduced in brains of patients with schizophrenia (Tremolizzo et al., 2002). Consistent with the hypermethylation in specific CpG sites, the 15-d methionine treatment facilitated the connection of methyl CpG binding protein 2 (MeCP2) and the reelin promoter (Dong et al., 2005). However, it does not happen on control genes, e.g., glutamic acid decarboxylase 65 (*GAD65)* and β-globin.

Brain derived neurotrophic factor (*Bdnf*), which presents in the central nervous system, is one member of the memory-permissive genes. Methionine administration significantly caused hyper-methylation of *Bdnf* DNA and, consequently, repressed gene expression of *Bdnf* mRNA in the epileptic hippocampus. Interestingly, the most significant DNA methylation of *Bdnf* occurred at CpG sites 5, 8, and 12, showing the importance of these sites in *Bdnf* transcription (Parrish et al., 2015).

As one of the key enzymes of transmethylation reaction from SAM to SAH in one-carbon metabolism, GNMT is present in large amounts in the liver, and in smaller amounts in other tissues such as kidney and intestinal mucosa (Lu and Mato, 2012), and is vital in avoiding drastic fluctuations of intracellular SAM concentrations to prevent aberrant methylation reactions (Lu and Mato, 2012). As an index of liver methylation capacity, the hepatic SAM to SAH ratio is increased 100-fold, resulted from a 40-fold increment of SAM and a 4-fold reduction of SAH in GNMT knock-off mice (Luka et al., 2006). Consequently, there is global DNA hypermethylation (Luka et al., 2006).

Protein phosphatase 2A (PP2A) is a conserved protein phosphatase that regulates growth processes by dephosphorylating specific target proteins, including the target of rapamycin complex 1 (TORC1) (Laxman et al., 2014; Sutter et al., 2013). S-adenosylmethionine- and methionine-treated hepatocytes showed increased PP2A methylation that influences TORC1 activity through modulating the phosphorylation state of Npr2p, which is a negative regulator of TORC1 (Sancak et al., 2010). Excess methionine caused high SAM and PP2A hypermethylation in hepatocytes of GNMT-knockout mice, resulting in lipid accumulation (Zubiete-Franco et al., 2016). Normalization of methionine and SAM levels using a methionine deficient diet normalized the methylation capacity, PP2A methylation, and ameliorated liver steatosis (Zubiete-Franco et al., 2016).

In practice, however, the effect of a methionine-supplemented diet on the DNA methylation is not always as expected. Methylenetetrahydrofolate reductase (Mthfr) has an important function for the re-methylation of homocysteine to methionine in the methionine cycle (Finkelstein, 1990). Surprisingly, a difference of genome wide DNA methylation was not observed in Mthfr-knockout mice feeding a methionine-supplemented diet, although a huge alteration happened in hepatic ratio of SAM to SAH (Devlin et al., 2004). It should be noted that this result does not

diminish the role of DNA methylation existing only at specific loci. Theoretically, high dietary methionine levels could change the DNA methylation pattern. However, methionine supplementation (2% methionine) enhanced the renal SAH concentration, but the ratio of SAM to SAH was not affected. At the same time, no significant difference was found in the *p53* gene promoter methylation pattern (Amaral et al., 2011).

Fatty acid binding proteins (FABP) can reversibly bind ligands of hydrophobicity, e.g., long-chain fatty acids and other high affinity lipids, and be involved in metabolic pathways in almost all tissues (Thumser et al., 2014). The FABP4 is expressed in adipose tissues and is important to the lipid metabolism (Parra et al., 2014). Recently, in an experiment determining the relationship between transmethylation reaction and dietary methionine, significant FABP4 promoter hypo-methylation was observed in ApoE$^{-/-}$ mice with supplementary feeding 1.7% methionine for 20 weeks. The methylation levels of FABP4 promoter region were 47% lower than the control group. Conversely, up-regulation of *FABP4* gene and protein expression was observed, consisting with the DNA methylation pattern (Yang et al., 2015). In the same study, a high level of dietary methionine enhanced the serum SAH and Hcy levels which inhibited *DNMT1* mRNA. The *DNMT1* mRNA expression decreased 52% in the methionine group compared with that in the control group. At the same time, the DNMT1 protein expression parallel altered with the mRNA expression. Conclusively, accumulation of SAH, decreased *DNMT1* gene, and protein expression caused by enhanced Hcy level might have an important impact on the FABP4 promoter demethylation.

Although the importance of DNA methylation in the context of epigenetics has been explored (Burdge and Lillycrop, 2010; Singh et al., 2012), the mechanisms governing global and region-specific DNA methylation are poorly understood, specifically in the context of nutri-epigenomics. Methionine as a dietary methyl donor is the major driver of DNA methylation because it is essential for the synthesis of SAM. The expression of specific genes (e.g., *PPARA*) might be silenced or repressed depending on global DNA-hyper or DNA-hypomethylation (Wang et al., 2013, 2014). For instance, mice fed a betaine-enriched diet had hypermethylation of hepatic DNA while the *PPARA* promoter region was hypomethylated, consequently up-regulating the expression of *PPARA* (Wang et al., 2013). But Holstein cows fed a methionine-supplemented diet had a lower global DNA methylation and hypermethylation of a *PPARA* specific-DNA region in the promoter (Osorio et al., 2016). The DNA sequence variations within specific regions, species, the onset of lactation of cows, and the down-regulation in expression of *DNMT1* possibly are partly responsible for the different outcomes in both global- and region-specific DNA methylation in response to dietary methyl donors. Alterations of methylation condition in a specific CpG site, rather than the whole promoter region, might be enough to change the gene expression (Ferreira et al., 2012).

5. Methionine and gene expression

High-methionine diets contribute to the enhancement of target of rapamycin (*TOR*) and insulin-like growth factor-I (*IGF-I*) gene expression, reduction of eukaryotic initiation factor 4E binding protein 1 (*eIF4EBP1*), forkhead box O4 (*FOXO4*) and atrogin-1 gene expression in chicks. Correspondingly, the growth performance, breast muscle production and serum IGF-1 content were increased with high methionine diet in young chicks (Wen et al., 2014). Furthermore, the expression of *IGF-I* and *GHR* was promoted, while that of atrogin-1 and cathepsin L2 (*CTSL2*) was inhibited with supplementation of methionine in the diet of acutely heat stressed broilers (Del Vesco et al., 2015), demonstrating that methionine promoted protein deposition characterized by increased gene expression for protein synthesis and decreased gene expression for protein breakdown. Similarly, the dietary methionine supplementation increased hepatic gene expression in somatotropic axis, e.g., *GHR-I* and *IGF-I*, and decreased gene expression in protein turnover, including the *TOR*, proteasome 20 delta (*Prot 20D*), calpains (*Capn*) 1 and 2, and calpastatin (*Cast*) long and short isoforms. Consequently, the growth performance of juvenile rainbow trout was increased (Rolland et al., 2015).

Aberrant methionine cycle may provoke molecular changes of one-carbon metabolism that results in deregulation of cellular hemostasis and health problems. Indeed, methionine deficient diets resulted in liver injury (Caballero et al., 2010). The alterations of gene expression included in the methionine cycle network should be the indicators to determine the mechanisms of abnormal dietary methionine supply on the aberrant development and health of animals. Specifically, the expression of SAH hydrolase (*Ahcy*) and cystathionine-synthase (*Cbs*), which are 2 key genes in hepatic one-carbon metabolism, was decreased in mice fed a methionine-deficient diet (Aissa et al., 2014), suggesting an aberrant gene expression profile of hepatic one-carbon. This suggests that a tight connection exists between the elevated hepatic SAH and homocysteine contents and the methionine-deficient diet. As the only enzyme controlling the reversible catabolism of SAH to homocysteine, *Ahcy* is encoded by the *Ahcy* gene in mammals (Tehlivets et al., 2013). Cystathionine beta-synthase is encoded by the *Cbs* gene and responsible for the transsulfuration pathway, which controls the removal of homocysteine (Tang et al., 2010).

There is growing evidence that the lipid metabolic pathway is affected by methionine (Pogribny et al., 2013; Walker et al., 2011). Therefore, the expression of key genes in lipid metabolism requires attention. Indeed, dietary methionine deficiency or excess up-regulated lipogenic transcription factors, mainly *SREBP1* and *CEBPα*, is usually followed by enhanced expression of fatty acid synthase gene *(Fasn)*, and inhibited lipid catabolism gene expression including *Adipor2*, *Cpt1*a, and *Chrebp* genes (Aissa et al., 2014). Either methionine deficiency or excess inhibited hepatic lipid catabolism and contributed to lipid accumulation in the liver of mice, but it was substantially greater in the liver of mice fed a methionine-deficient diet. Furthermore, methionine deficiency enhanced lipid turnover efficiency characterized by a substantially increase in gene expression of glucose transporter type 4 (*GLUT4*), malic enzyme 1 (*ME1*), *FASN*, hormone sensitive lipase (*HSL*) gene, and adipose triglyceride lipase (*ATGL*) in subcutaneous and perirenal adipose tissues in pigs (Castellano et al., 2015). Recently, an up-regulation of the expression of *PPARA* and its target genes, including *ANGPTL4*, *FGF21*, and *PCK1*, were observed with methionine supplementation in peripartal dairy cattle (Osorio et al., 2016), indicating that supplemental methionine activated *PPARA*-regulated signaling pathways, improved lipid metabolism and immune function.

6. Conclusions

Either methionine deficiency or excess in diets can have massive impacts on hepatic methionine cycle, which may result in marked alterations of metabolic substrates, e.g., SAM, SAH, and homocysteine. Therefore, a change in the dietary methionine level could easily alter the status of CpG methylation, gene expression related to hepatic lipid metabolism, and growth performance. DNA methylation patterns are tissue specific, and thus it is not always negatively correlated with gene expression, challenging the traditional view that DNA methylation represses gene expression. Further work on the mechanistic connections between hepatic global and region DNA methylation with gene expression and functional proteins is required. Nutritional epigenomics, as an

emerging and multi-disciplinary field of research, will help advance our understanding of the relationship between genes, molecules, and tissues in which methionine alteration influences DNA methylation and gene expression.

Conflicts of interest

The author declares no conflicts of interest.

Acknowledgments

This work was supported by the Special Fund for Agro-scientific Research in the Public Interest (Grant No.: 201303143) from the Ministry of Agriculture of the People's Republic of China.

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