Short communication: Relationship of blood DNA methylation rate and milk performance in dairy cows

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ABSTRACT

The objective of this study was to investigate the global methylation rate in blood DNA and its relationship with lactation performance. A total of 196 mid-lactation dairy cows were fed the same diet under the same management. Milk yield was recorded and blood samples were collected from the jugular vein before morning feeding. The blood global DNA methylation rates were quantified using a methylation quantification kit. Overall, the average blood global DNA methylation rate of all cows was 12.4%. When DNA methylation rates were compared between cows with high ($n = 40$; 37.0 to 42.0 kg/d) and low ($n = 33$; 24.0 to 30.0 kg/d) milk yield, DNA methylation rates in the lower-yield cows ($14.1 \pm 0.7\%$) were significantly higher than those in the higher-yield animals ($11.6 \pm 0.7\%$). Our results indicated an association of milk and protein yields with global DNA methylation rates in lactating dairy cows. However, further research is needed to determine whether this association reflects the true influence of epigenetic mechanisms on yield or whether other factors, such as different proportions of blood cell types in high- and low-yielding cows, affect apparent global DNA methylation levels.

Key words: blood DNA methylation, milk yield, milk protein yield, dairy cow

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Milk production in dairy cows can be affected by many factors, including heredity (Kocak, 2006), nutrition (Sejrsen and Purup 1997), health (Windig et al., 2005), stress (Wolfenson et al., 1988), and management practices (Stelwagen et al., 2013). Epigenetics is a chemical modification change to the genome (or its

surrounding chromatin) without altering the underlying DNA sequence; epigenetic changes can modify the function of the targeted genes and can be passed to the offspring (Jaenisch and Bird, 2003). Therefore, epigenetic modifications respond to external or internal environmental and nutritional stimuli that may alter gene expression and specific phenotypes in an animal or human. DNA methylation is the most extensively studied epigenetic modification important in gene regulation and genome stability and is typically characterized by the addition of a methyl group to the fifth carbon of cytosine in CpG dinucleotides to form 5-methylcytosine (Costello and Plass, 2001). As a result, DNA methylation plays a role in the regulation of chromatin structure and promoter activity. It may silence the promoters of imprinted genes showing monoallelic expression, as well as the promoters of transposons, and can contribute to gene silencing on the inactive X chromosome (Niller et al., 2017).

Alterations in global DNA methylation patterns have been reported to be associated with age-related skeletal muscle differences in swine (Jin et al., 2014) and reprogramming of pluripotency genes in mice (Farthing et al., 2008). In rodents, epigenetic changes in the mammary gland have been reported to regulate expression of milk protein genes, mammary gland development, and functional differentiation (Rijnkels et al., 2010). Increasing evidence also indicates that epigenetic mechanisms play a role in the molecular regulation of milk production in dairy cows (Singh et al., 2012; Osorio et al., 2016). DNA methylation inhibited bovine α_{S1} -casein gene expression during bovine mammary involution and disease, which further affected milk yield (Singh et al., 2012). When rumen-protected methionine was supplemented, milk yield (Osorio et al., 2013) and methylation in hepatic global DNA and peroxisome proliferator-activated receptor (PPAR)- α promoter were altered in periparturient dairy cows (Osorio et al., 2016). However, information on DNA methylation rates in lactating dairy cows is limited.

In our previous study, milk yield and milk protein yield varied even when lactating cows were fed the

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same diet and under the same environment (Wu et al., 2018). We hypothesized that the methylation-based epigenetic mechanism differed among these cows and could be one of the factors resulting in the varied lactation performance. Therefore, the objective of this study was to investigate the blood DNA methylation rate in a large cohort of dairy cows ($n = 196$) and to identify whether methylation is related to milk yield and milk protein yield.

The experimental procedures were approved by the Animal Care Committee at Zhejiang University (Hangzhou, China). In total, 196 dairy cows were housed at Hangjiang Dairy Farm (Hangzhou, China). Basic animal characteristics (parity, days in milk, milk yield, milk protein content, milk protein yield) are shown in Supplemental Table S1 (<https://doi.org/10.3168/jds.2018-15869>). All dairy cows were kept in a tiestall barn with good ventilation, had free access to water, and were fed an identical TMR with a concentrate-to-forage ratio of 57:43 (DM basis). The animals were milked and fed daily at 0630, 1400, and 2000 h. Milk samples were collected in a proportion of 4:3:3 corresponding to the morning, noon, and evening milkings. Blood samples (10 mL) were collected into two 5-mL pro-coagulation tubes each from the jugular vein at around 0400 to 0600 h, before the morning feeding. The methods for collection and storage of blood samples were as described previously (Wu et al., 2018). Milk yield was recorded for 3 consecutive days and samples of milk and blood were collected on the third day. Milk protein yield was calculated as milk protein content multiplied by milk yield measured on the same day.

The percentage of DNA containing 5-methylcytosine (**5-mC**) was used as an indicator of the global DNA methylation level. The DNA concentrations from blood were standardized. Global DNA 5-mC in blood was quantified using the MethylFlash Methylated DNA Quantification Kit (Epigentek Group Inc., Farmingdale, NY) with the same amount of DNA, and read with the spectrophotometer. According to the manual, the kit uses a unique anti-5-mC monoclonal antibody that is sensitive and specific for 5-mC detection across a wide range of starting DNA amounts (Cramer et al., 2017). The 5-mC percentage was quantified based on a standard curve, generated by combining synthetic unmethylated (contains 50% cytosine) and methylated DNA (contains 50% 5-mC) at different concentrations. Pearson analysis was performed between blood DNA methylation and milk yield or milk protein yield. All data were analyzed using SPSS Statistics 20 (SPSS/IBM Corp., Chicago, IL), with $P < 0.05$ defining the significance threshold and $P < 0.01$ regarded as a highly significant difference.

The mean value of blood global DNA methylation rate of 196 lactating cows was 12.4%, with a range from 3.5 to 22.6% (Figure 1), indicating variation in methylation among dairy cows managed under the same environmental conditions. DNA methylation can differ greatly among studies, with reports of 27.2% methylation in human blood (Yang et al., 2004) and 54.2% in rat blood (Kim et al., 2007). However, differences in DNA methylation rates may also arise from varied testing methods, which aim to capture the methylated DNA at different component units such as 5-mC or DNA repetitive element. The amount of 5-mC in genomic DNA can be measured using a wide range of methods designed to yield quantitative and qualitative information on genomic DNA methylation. The method used in our study aimed to detect the global amount of 5-mC, whereas some other studies focused on methylation of specific DNA repetitive elements. For example, methylation of identifier elements and of CpG sites in Alu elements was measured in pigs (Jin et al., 2014) and human studies (Yang et al., 2004), respectively. Therefore, the testing method should be taken into account when comparing methylation rates among species. Our work only described methylation rates at the global DNA level, thus future studies on methylation rates of different repetitive elements may reveal specific epigenetic patterns in dairy cows.

Our statistical analysis showed relatively weak but significant correlations between blood global DNA methylation rates and milk yield or milk protein yield (Figure 2), suggesting that some internal relationships existed between global DNA methylation rates and milk yield or protein yield. To further validate this relationship, we compared the methylation rate between cows of similar parity and DIM with higher (**H**) and

Figure 1. Distribution of blood global DNA methylation rates of 196 lactating cows.

Table 1. Difference in production variables and DNA methylation rates between dairy cows with higher and lower milk yields

Item	Group ¹		SEM	P-value
	H (n = 40)	L (n = 33)		
Milk yield, kg/d	36.1	27.5	0.70	<0.01
Milk protein content, %	2.97	3.19	0.03	<0.01
Milk protein yield, kg/d	1.16	0.88	0.19	<0.01
Parity	2.88	2.97	0.16	0.69
DIM	151	164	0.12	0.10
DNA methylation rate, %	11.6	14.1	0.50	0.01

¹H = cows with higher milk yield (37–42 kg/d); L = cows with lower milk yield (24–30 kg/d).

lower (**L**) milk yields (Table 1). Forty cows were defined as the H group, with milk yield of 37.0 to 42.0 kg/d, whereas 33 others were defined as the L group, with milk yield of 24.0 to 30.0 kg/d. As shown in Table 1, milk and milk protein yields in the H group were higher ($P < 0.01$) than those in the L group. The DNA methylation rate was lower ($P = 0.01$) in the H group than in the L group, indicating that high-milk-yield cows have lower DNA methylation rates. These results suggest that global DNA methylation is a factor that may contribute to varied milk and protein yields in dairy cows under the same environment, and increased DNA methylation rate plays a role in the reduced milk production.

To our knowledge, this is the first report to assess a global methylation rate of blood DNA in a large cohort of mid-lactation dairy cows. Although the molecular mechanism linking global DNA methylation rates and milk yield remains unknown, our results provide information on the potential relationship between blood global DNA methylation rates and milk and protein yields in lactating dairy cows. This preliminary study suggests that DNA methylation in blood cell DNA may affect milk and protein yields.

Milk production is a complex trait that can be affected by many biological factors. For the same cohort of cows, we found that blood metabolites (Wu et al., 2018) and rumen microbiota (Xue et al., 2018) also

Figure 2. Plots of milk yield (Y1) and milk protein yield (Y2) with blood DNA methylation rates (X). $Y1 = -0.286 (\pm 0.100) X + 37.3$ ($R^2 = 0.036$, $P = 0.005$); and $Y2 = -0.009 (\pm 0.003) X + 1.15$ ($R^2 = 0.044$, $P = 0.002$).

differed between cows with high and low milk and milk protein yields, and some metabolites and microbial taxa had moderate relationships with these traits. The observed variation in blood DNA methylation in the current study further confirmed the complexity of milk production traits and suggested the need for future studies with a more systematic approach to understand how varied biological factors could affect milk production. It is notable that we used total DNA isolated from whole blood in this study without considering different cell types in the blood; the DNA methylation rate variables come from different cell types in blood components. Further study is needed to verify whether different blood cell types with various methylation patterns exist between cows with high and low milk and milk protein yields, which would provide in-depth and holistic understanding of the relationship between blood DNA methylation level and milk and milk protein yields in cows under the same nutritional and management conditions.

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