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DNA METHYLATION PROFILE OF DNA METHYLTRANSFERASE 3B (*DNMT3B*) GENE AND ITS INFLUENCE ON GROWTH TRAITS IN GOAT

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ABSTRACT

The objective of this study was to identify the DNA methylation profile of *DNA methyltransferase 3b*（*Dnmt3b*） gene and its association with growth traits between the overweight and underweight groups of adult goats. Herein, hypermethylation pattern in *Dnmt3b* gene 5' region was firstly reported in adult goats and eleven polymorphic methylation loci from all expected CpG-dinucleotide positions were revealed. In the analyzed population, methylation percentages (MPs) varied from 77.69% to 93.94%. Intriguingly, methylation share rate (MSR) with a range from 0.787 to 0.975 for different individuals was firstly created to evaluate the methylation level. Furthermore, a significant association between DNA methylation of the 11th CpG-dinucleotide locus and body weight was found in adult goats ($^{*}P$=0.023), as well as a significant relationship between the average DNA methylation level and body weight ($^{*}P$=0.049). The methylation level of overweight group was significantly less than that of underweight group, suggesting that hypomethylation of *Dnmt3b* gene was significantly associated with the overweight of adult goats. Therefore, DNA methylation profile of the *Dnmt3b* gene was recommended to be a novel epigenetic marker which would benefit for selection and breeding through marker-assisted selection (MAS) in goat industry.

Keywords: Goat, DNA methyltransferase 3b (*Dnmt3b)* gene, DNA methylation, body weight, Association.

INTRODUCTION

As we know, it is not easy to culture outstanding goat breeds by the traditional breeding methods, hence currently major breeders mainly focus on genetic markers for animal selection and breeding through marker-assisted selection (MAS), but few consider epigenetic markers. As the most important epigenetic marker, DNA methylation changes drastically during early development, and are maintained throughout adulthood (Reik *et al*., 2001), which plays an important role in epigenetic gene regulation (Robertson and Wolffe, 2000). Therefore, it is novel angle for breeders to study DNA methylation profile of potential candidate genes and their effects on growth traits (e.g. body weight and other growth traits) in animals.

The recent findings had showed that the etiology of obesity is multifactorial (Herrera and Lindgren, 2010), and epigenetics has emerged as a very critical determinant (Campión *et al*., 2010; Milagro *et al*., 2009). Methylation of Cytosine at the C5 position is essential for normal development in animals and etiology of obesity (Bird, 2002; Li *et al*., 1992; Okano *et al*., 1999; Robertson and Wolffe, 2000). Because normally hypermethylation in the promoter regions of the genes involves in down-regulating the gene expression as well as affecting different gene pathways including apoptosis (Gopisetty *et al*., 2006; Kremenskoy *et al*., 2006). Hence, identification of the individuals who present with changes in epigenetic profiles could help to predict their susceptibility to gain or lose weight. In goat breeding, studies on methylation mechanisms affecting weight homeostasis may play a role in the promotion of excessive fat deposition, the prediction of the most appropriate weight increasing plan, and the implementation of newer feeding, which will contribute to culture goat breeds with excessive fat deposition (Campión *et al*., 2010). Thus, analyzing DNA methylation and its related genes falls in our interest in goat breeding and genetics.

Intriguingly, in DNA methylation process of growth development and adiposis in animals, DNA methylation in mammals was methylated by DNA methyltransferases (Dnmts) mainly including *Dnmt1*, *Dnmt2*, *Dnmt3a* and *Dnmt3b* (Bestor *et al*., 1998; Chen *et al*., 2010; Okano *et al*., 1998; Xie *et al*., 1999). Among them, Dnmt3b demonstrates equal methylation activities toward unmethylated and hemi-methylated DNA, so it is essential for de novo methylation during early mouse development (Okano *et al*., 1998, 1999). Furthermore, the expression pattern of Dnmt3b changes dynamically during early embryogenesis in mice and the expression is thereafter drastically downregulated (Watanabe *et al*., 2002). Interestingly, mutations in the *Dnmt3b* gene have

been revealed to be responsible for the human ICF (immunodeficiency, centromere instability and facial anomalies) syndrome, which is characterized by hypomethylation of pericentromeric repeats (Hansen *et al*., 1999; Okano *et al.*, 1999; Xu *et al*., 1999). In addition, Dnmt3b was necessarily required for DNA methylation of centromeric minor satellite repeats (Okano *et al*., 1999). Therefore, the developmental and adiposis regulation of Dnmt3b has been thought to play an important role in the control of a wave of de novo methylation after the implantation of blastocysts (Okano *et al*., 1999; Watanabe *et al.*, 2002). Considering DNA methylation strongly inhibiting gene expression, we hypothesized that aberrant DNA methylation of *Dnmt3b* gene may disturb itself and other genes expressions and then affect body weight and other growth traits of goat.

However, to date, DNA methylation profile of *Dnmt3b* gene and its influence on production traits is unknown in adult goat. Hainan black (HNB) goat is a well-known meat breed in southeast China, having characteristic of delicious meat flavor. However, this breed has slight body weight, slow growth speed and short status, which dramatically restricting its decreasing of rearing size, so taking the effective step through MAS was considered to enlarge the size of this breed by improving its shortcomings. Therefore, the objective of this study is to figure out the DNA methylation profile of *Dnmt3b* gene and its association with growth traits between overweight and underweight groups in adult HNB goats, which would possibly provide useful epigenetic markers for selection and breeding through MAS in the goat industry.

MATERIALS AND METHODS

The classification of overweight and underweight groups of adult HNB goats and tissue collection: To obtain the irrelative HNB individuals, a total of 291 HNB goats were investigated and measured in seven growth traits, which were reared in native breeding farms, in Zanzhou County (East Longitude 109.5 degree; North Latitude 19.5 degree), Hainan province, China. All HNB goats were 2 to 3 years old. Measurements including body weight (BW), body height (BH), body length (BL), chest circumference (ChC), chest depth (ChD), hucklebone width (HuW) and cannon circumference (CaC) were obtained by the standard measurements of meat goat. The detailed description involving the above-mentioned goat growth traits were shown in Table 1. Moreover, a total of 291 ear tissues were collected for further analysis.

According to the statistical probability (95.45%) of beyond two standard deviation (SD) for body weight and other growth traits, the overweight (OW) and underweight (UW) groups were classified. According to sample size, eight individuals are qualified and then were chosen in this study. For Table 1, each individual's body weight from overweight group was significantly more than average, as well as each individual's body weight in underweight group. To confirm the significant difference of body weight and other growth traits between overweight and underweight groups, the t-test was performed by SPSS software (version 18.0). The statistical results validated that there were significant differences in BW ($p<0.001$), BH ($p<0.001$), BL ($p<0.001$), ChC ($p<0.001$), HuW ($p<0.001$) and CaC ($p<0.001$) indexes between overweight and underweight groups (Table 1).

Genomic DNA extraction and bisulfite treatment: Genomic DNA samples from overweight and underweight groups were extracted from ear tissues using a standard phenol/chloroform protocol (Lan *et al*., 2007), followed by quantization using NanoDropTM ND-1000 spectrophotometer (Thermo Finnigan, America), then they were diluted to the final concentration with 50 ng/uL for next experimental use. The diluted genomic DNA samples were carried out finally by Sodium bisulfite treatment with EZ DNA Methylation-Gold^{TM} Kit (ZYMO RESEARCH, California, US) strictly following the manufacture's instruction.

Searching the 5' region of Dnmt3b genes for bisulfite analysis: Considering the high similarity and conservation of the *Dnmt3b* gene between bovine and goat, two pairs of nested PCR amplification primer sequences from bovine *Dnmt3b* gene were used to amplify goat *Dnmt3b* gene (Liu *et al.*, 2008): Outside forward (OF): 5'-GTTGGAGTAGATGAGGAATATGT-3';Outside reverse (OR): 5'-CTCAAA AACCAACACCCAAAACA-3'; Inside forward (IF): 5'-TTTG GGATTTTGAGTTTTAGTTGG-3'; Inside reverse (IR): 5'–CTCAAAAACCAACACCCAAAACA-3'.The nested PCRs were used to amplify 402 bp region using touch-down PCR protocol for the first amplification (OF and OR) and second amplification (IF and IR).

PCR amplification, cloning and DNA sequencing of goat *Dnmt3b* gene 5' region: A total of two PCRs were performed using one bead containing bisulfite-treated DNA in the first amplification (25 uL total volume) and 1 uL of this PCR product was used as template in the second amplification (50 uL total volume). The first amplification PCR reaction was performed in a 25 uL final volume, containing 50 ng bisulfite-treated DNA, 0.5 uM of each primer, 1xBuffer [including 1.5 mM $MgCl_2$], 200 uM dNTPs and 0.625 units of *Taq* DNA polymerase (MBI, Vilnius, Lithuania). The second amplification PCR reaction was performed in a 50 uL final volume, containing 1 uL of first PCR amplification product, 0.5 uM of each primer, 1xBuffer [including 1.5 mM $MgCl_2$], 200 uM dNTPs and 1.25 units of *Taq* DNA polymerase

(MBI, Vilnius, Lithuania). The PCRs were carried out on a Peltier Thermal Cycler-100 (MJ Research, USA) using the touch-down PCR program: 5 min at 95 ^{o}C; 2 cycles of 94 ^{o}C for 30 s, annealing from 68 ^{o}C to 52 ^{o}C by 2 ^{o}C decrease for 30 s, respectively, 72 ^{o}C for 30 s; 20 cycles of 94 ^{o}C for 30 s, 51 ^{o}C annealing for 30 s, 72 ^{o}C for 30 s; a final extension at 72 ^{o}C for 10 min; subsequently cooling to 4 ^{o}C. The PCR products obtained from the second round of amplification were gel-purified using the gel purification system (Tiangen, Beijing, China). Purified fragments were cloned into the pGM-T easy vector (Promega, WI, USA). The 10-15 inserts were verified using colony PCR and sequenced using an external sequencing service. Each experiment was repeated at least four times. Scores for the methylation of each CpG were obtained by sequencing PCR clones derived from bisulfite-treated genomic DNA and only completely converted sequences were used.

Population genetic indexes and statistical test of DNA methylation patterns of goat Dnmt3b gene 5' region: In order to analyze the methylation profile effectively, DNA methylation or unmethylationin different CpG-dinucleotide positions was scored as 1/0. Locus methylated frequencies (L-MF) were directly calculated. Observed methylation CpG-dinucleotide numbers (OMCN), observed polymorphic methylation CpG-dinucleotide numbers (OPMCN), methylated percentages (MP) were also calculated.

According to population genetics, genetic indexes including locus heterozygosity (L-He) and locus homozygosity (L-Ho), and genetic diversity index (H) were calculated by Nei's method (Nei and Li, 1979). Specially, Shannon's genetic diversity index (H)=- $\pi_i \ln\pi_i$, π expresses the frequency one amplified methylation to appearing in a population, π_i stands for a distribution frequency of one amplified methylation in the general population.

According to the data analysis model of random amplified polymorphic DNA (RAPD) marker (Welsh and McClelland, 1990; Williams *et al.*, 1990), methylated-DNA-fingerprint similarity is generally defined as methylated share rates (MSR). The genetic similarity (S) or methylated share rates (MSR) was determined by Nei and Lynch's method (Nei and Li, 1979; Lynch, 1990): MSR= $2N_{AB}/(N_A + N_B)$, where "N_{AB}" is the number of methylation that colony A and B shared in common, "N_A" is the number of methylation in colony A and "N_B" is the numbers of methylation in colony B. The mean MSRs and their standard deviation, standard error was carried out by C^{++} protocol which was designed by our group.

The mean comparisons of values obtained from bisulfite sequencing and the differences in methylation rates between adult overweight and underweight groups were analyzed by independent population *t*-test with significance level of $P<0.05$ (Liu *et al.*, 2008).

RESULTS

DNA methylation profile of goat Dnmt3b gene 5' region: The fragment size of PCR amplification product including goat *Dnmt3b* gene 5' region was 402 bp. Then the purified PCR amplification products were cloned into the pGM-T Easy vector; the insert colonies were selected. The positive rate of inserts was 95.83%, and about 10 inserts were verified using DNA sequencing by forward or reverse directions. The comparisons among these sequences and nt -617 to -216 (GenBank accession No. NC007311) revealed different methylated and unmethylated patterns of 11 different CpG-dinucleotide loci (namely, CpG-dinucleotide No.1-11 or C1-C11) in different adult goats (Fig.1).

DNA methylation pattern analysis of goat Dnmt3b gene 5' region: The detailed information including locus methylated frequencies (L-MF), locus homozygote (Ho), loucs heterozygote (He) and locus Shannon's genetic diversity index (H) in different CpG-dinucleotide locus of goat *Dnmt3b* gene 5' region was shown in Table 2. In overweight group, the methylated percentages (MPs) of different CpG-dinucleotide locus of individuals waved from 0.182 to 1.000. In the underweight group, MPs of locus of individuals ranged from 0.333 to 1.000.

From Table 3, there were eleven polymorphic methylation numbers in the total of all expected eleven CpG-dinucleotide dinucleotide loci. The numbers of 100% methylation for different CpG-dinucleotide locus ranged from 2 to 10. The average of methylation number was from 8.546 to 10.330. The range of mean methylated percentages varied from 77.69% to 93.94%. The average of methylation percentage for all analyzed samples was 83.24%.

In Table 4, the average heterozygote waved from 0.040 to 0.271, as well as average heredity diversity index (H) from 0.033 to 0.168. MSRs for different individual ranged from 0.787 to 0.975. The wave of minimum of MSR was from 0.333 to 0.952. The average of MSR for all analyzed samples was 0.870.

Significant DNA methylation difference of goat *DNMT3b* gene 5' region between overweight and underweight groups: As can be seen from Table 5, there was a significant difference of methylation in the 11th CpG-dinucleotide locus between overweight and underweight groups ($^{*}P=0.023$). The methylation level of overweight group (38.3%) was significantly less than that of underweight group (71.6%). However, there was no significant difference of methylation in other CpG-dinucleotide loci ($P>0.05$).

For the whole methylation level, there was significantly different DNA methylaion ($^{*}P=0.049$). The

methylation level of overweight group (78.8%) was significantly less than that of underweight group (87.7%).

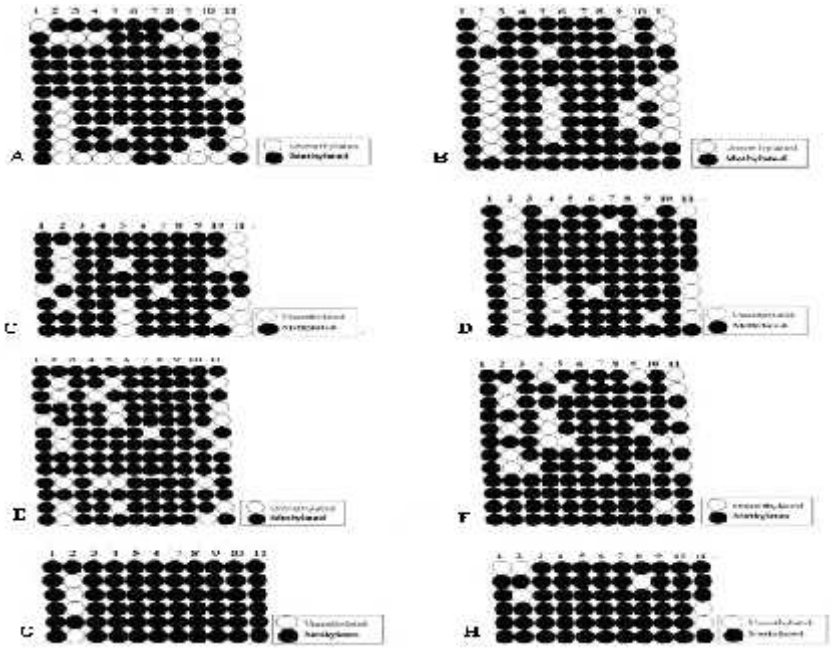

Figure 1: Methylation patterns of goat *Dnmt3b* gene 5' region from OW and UW group
Note: A: G134 individual (OW1); B: G190 individual (OW2); C: G136 individual (OW3); D: G231 individual (OW4); E: G8 individual (UW1); F: G219 individual (UW2); G: G236 individual (UW3); H: G247 individual (UW4).

Table 1. Body weight and other growth traits of adult overweight and underweight groups and individuals in HNB goats

Types	BW(kg)	BH(cm)	BL(cm)	ChC(cm)	ChD(cm)	HuW(cm)	CaC(cm)
Sample size	291	291	291	291	291	291	291
Mean± SD	28.38	52.63	55.88	71.96	26.43	13.66	7.78
	±6.94	±4.16	±4.53	±6.45	±2.38	±1.36	±0.73
Data range	13.50-	40.10-	43.80-	54.00-	14.50-	9.00-	6.00-
(Min-Max)	51.00	64.70	68.30	91.00	33.00	17.20	10.00
G134(OW1)	45.00	59.90	62.70	85.00	28.00	14.90	10.00
G190(OW2)	51.00	56.90	67.90	82.50	31.30	14.20	9.50
G136(OW3)	50.00	55.50	71.80	83.50	30.0	15.30	9.00
G231(OW4)	42.50	63.20	67.0	87.00	33.00	14.00	8.50
G8 (UW1)	13.50	44.20	43.80	56.50	23.00	9.00	7.00
G219(UW2)	13.50	43.70	51.00	54.00	22.00	11.00	6.50
G236(UW3)	14.50	44.20	46.00	62.00	22.50	12.00	6.20
G247(UW4)	14.00	46.70	51.00	58.00	22.50	23.00	6.00
Mean±S.D.	47.13^{a}	58.87^{a}	64.85^{a}	84.50^{a}	30.58^{a}	14.60^{a}	9.25^{a}
(OW group)	±2.02	±1.71	±1.52	±0.98	±1.06	±0.30	±0.32
Mean±S.D.	13.88^{c}	45.20^{c}	47.95^{c}	57.63^{c}	22.50^{c}	10.50^{c}	6.43^{c}
(UW group)	±0.24	±0.74	±1.82	±1.68	±0.20	±0.50	±0.22
P values	P<0.001**	P<0.001**	P<0.001**	P<0.001**	P<0.001**	P<0.001**	P<0.001**

Note: OW = Overweight (High body weight); UW = Underweight (low body weight); S.D. = Standard Deviation; BW=Body weight; BH=Body height; BL=Body length; ChC=Chest circumference; ChD=Chest depth;ChW=Chest width;HuW=Hucklebone width; CaC=Cannon circumference.
Means superscription with different letters mean significant statical difference (P<0.05 or P<0.01); "*" means P<0.05; ** means P<0.01;

Table 2. DNA methylation patterns in different CpG loci of goat *Dnmt3b* gene for OW group and UW groups

No.	Types	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
G134	L-MF	0.917	0.417	0.833	0.833	0.833	**1.000**	**1.000**	0.833	0.750	0.750	0.417
(OW1)	L-Ho	0.847	0.514	0.722	0.722	0.722	1.000	1.000	0.722	0.625	0.625	0.514
	L-He	0.153	0.486	0.278	0.278	0.278	0	0	0.278	0.375	0.375	0.486
	L-H	0.080	0.365	0.152	0.152	0.152	0	0	0.152	0.216	0.216	0.365
G190	L-MF	**1.000**	0.182	1.000	1.000	0.545	**1.000**	**1.000**	**1.000**	0.636	0.818	0.364
(OW2)	L-Ho	1.000	0.702	1.000	1.000	0.504	1.000	1.000	1.000	0.537	0.703	0.537
	L-He	0	0.298	0	0	0.496	0	0	0	0.463	0.298	0.463
	L-H	0	0.310	0	0	0.331	0	0	0	0.288	0.164	0.368
G231	L-MF	**1.000**	0.100	**1.000**	0.600	**1.000**	0.900	0.900	**1.000**	0.800	**1.000**	0.500
(OW3)	L-Ho	1.000	0.820	1.000	0.520	1.000	0.820	0.820	1.000	0.680	1.000	0.500
	L-He	0	0.180	0	0.480	0	0.180	0.180	0	0.320	0	0.500
	L-H	0	0.230	0	0.306	0	0.095	0.090	0	0.179	0	0.348
G136	L-MF	0.875	0.500	**1.000**	**1.000**	0.500	**1.000**	0.880	**1.000**	**1.000**	0.750	0.250
(OW4)	L-Ho	0.781	0.500	1.000	1.000	0.500	1.000	0.780	1.000	1.000	0.625	0.625
	L-He	0.219	0.500	0	0	0.500	0	0.220	0	0	0.375	0.375
	L-H	0.117	0.347	0	0	0.347	0	0.120	0	0	0.216	0.347
G8	L-MF	0.923	0.462	**1.000**	0.846	0.615	**1.000**	0.920	**1.000**	**1.000**	0.769	0.615
(UW1)	L-Ho	0.858	0.503	1.000	0.740	0.527	1.000	0.860	1.000	1.000	0.645	0.527
	L-He	0.142	0.497	0	0.260	0.473	0	0.140	0	0	0.355	0.473
	L-H	0.074	0.357	0	0.141	0.299	0	0.070	0	0	0.202	0.299
G219	L-MF	**1.000**	0.583	0.917	0.667	0.833	**1.000**	0.920	**1.000**	0.750	0.833	0.583
(UW2)	L-Ho	1.000	0.519	0.847	0.556	0.722	1	0.850	1.000	0.625	0.722	0.514
	L-He	0	0.486	0.153	0.444	0.278	0	0.150	0	0.375	0.278	0.486
	L-H	0	0.314	0.080	0.270	0.152	0	0.080	0	0.216	0.152	0.314
G236	L-MF	0.833	0.667	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	0.833	**1.000**	**1.000**	0.667
(UW3)	L-Ho	0.722	0.556	1.000	1.000	1.000	1.000	1.000	0.722	1.000	1.000	0.556
	L-He	0.278	0.444	0	0	0	0	0	0.278	0	0	0.444
	L-H	0.152	0.270	0	0	0	0	0	0.152	0	0	0.270
G247	L-MF	**1.000**	0.333	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	1.000
(UW4)	L-Ho	1.000	0.556	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	L-He	0	0.444	0	0	0	0	0	0	0	0	0
	L-H	0	0.366	0	0	0	0	0	0	0	0	0

Note: No.=Number; C=CpG-dinucleotide No., such as C1=CpG-dinucleotide No.1; L-MF=Locus methylated frequencies; L-Ho= Locus homozygote (Ho); L-He= Loucs heterozygote(He); L-H =Locus Shannon's genetic diversity index (H).

Table 3.DNA metylation analysis of *Dnmt3b* gene 5' region in OW and UW groups

No.	Groups	OMCN	Sample sizes	OPMCN	100% MCN	Total of MCN	Average of MCN	metylation percentages (MP)
G134	OW1	11	12	9	2	103	8.583	78.03%
G190	OW2	11	11	5	6	94	8.546	77.69%
G136	OW3	11	8	6	5	70	8.750	79.55%
G231	OW4	11	10	6	5	88	8.800	80.00%
G8	UW1	11	13	7	4	119	9.154	83.22%
G219	UW2	11	12	8	3	109	9.083	82.57%
G236	UW3	11	6	1	10	62	10.333	93.94%
G247	UW4	11	6	4	7	60	10.000	90.91%

Note: OMCN= Observed methylation CpG-dinucleotide number; OPMCN=observed polymorphic methylation CpG-dinucleotide number; MCN=methylation CpG-dinucleotide nmber; MP=methylation percentage.

Table 4. Methylated share rate (MSR) and index of epigenetic diversity of methylaton pattern of *Dnmt3b* gene 5' region in OW and UW groups

Sample No.	Methylated share rate (MSR)				groups	Average homozygote	Average heterozygote	mean H
	Mean±SE	SD	Min	Max				
G134 (OW1)	0.787±0.021	0.169	0.333	1.000	OW	0.729	0.271	0.168
G190 (OW2)	0.870±0.009	0.065	0.778	1.000	OW	0.817	0.183	0.133
G136 (OW3)	0.852±0.016	0.082	0.625	0.947	OW	0.833	0.167	0.114
G231 (OW4)	0.886±0.011	0.072	0.750	1.000	OW	0.801	0.199	0.135
G8 (UW1)	0.857±0.009	0.079	0.625	1.000	UW	0.787	0.213	0.131
G219 (UW2)	0.830±0.013	0.104	0.462	1.000	UW	0.759	0.241	0.143
G236 (UW3)	0.975±0.006	0.025	0.952	1.000	UW	0.869	0.131	0.077
G247 (UW4)	0.904±0.014	0.056	0.842	1.000	UW	0.960	0.040	0.033

Note: MSR= Methylated share rate; Min=Minimum; Max=Maximum.

Table 5. Independent t-test analyses of methylation pattern of *Dnmt3b* gene between overweight and underweight group in HNB goats (mean%±SE)

Group	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	mean
OW	94.8± 3.1	30.0± 9.5	95.8± 4.2	85.8± 9.5	72.0± 11.9	97.5± 2.5	94.5± 3.2	95.8 ±4.2	79.7 ±7.6	83.0± 5.9	**38.3^{b}± 5.2**	**78.8^{b}± 0.6**
UW	3.9± 3.8	51.1± 7.3	97.9± 2.1	87.8± 7.9	86.2± 9.1	100± 0	96.0± 2.3	95.8± 4.2	93.8± 6.3	90.1± 5.9	**71.6^{a} ±9.6**	**87.7^{a}± 2.8**
t value	0.178	1.771	0.450	0.162	0.950	1.00	0.380	0	1.433	0.851	3.046	3.067
df	6	6	6	6	6	6	6	6	6	6	6	3.245
P value	0.864	0.127	0.668	0.877	0.379	0.356	0.717	1.000	0.202	0.427	**0.023***	**0.049***

Note: Means superscription with different letters mean significant statically difference (P<0.05).

DISCUSSION

It is well known that epigenetic modifications of gene control region regulate gene expression (Aapola *et al*., 2004; McCarrey *et al*., 2005; Wu *et al*., 2006). DNA methylation is the most prominent epigenetic mark associated with compact and inactive chromatin structure (Bird and Wolffe, 1999). DNA hypomethylation is associated with gene reactivation (Smet *et al*., 2004) and chromosomal instabilities (Eden *et al*., 2003). While, hypermethylation in the promoter regions of the genesis led to gene silence (Jones and Baylin, 2002; Rivenbark *et al.*, 2006). DNA hypermethylation mediated gene silencing affects many genes involved in molecular events, a partial list of which include tumor suppressors, DNA repair, hormonal response, cell adhesion, drug metabolism (Teodoridis *et al*., 2004). The biological consequences of DNA hypomethylation or hypermethylation are mediated by methyl-CpG binding proteins, such as MeCP2 and MeCP2, which recruits histone deacetylases, resulting in gene silencing (Bleich *et al.*, 2006; Fuks *et al.*, 2003; Jones *et al*., 1998). Form the above-described documents, Dnmts methylating DNA methylation play a critical role in DNA methylation and they may directly or indirectly affect numerous physiological process including growth, development and reproduction. Therefore, herein, *Dnmt3b* gene was selected to studies DNA methylation profile in overweight group and underweight groups, in order to study whether methylation level or possible specific CpG-dinucleotide-methylated locus of gene influence the growth traits.

In this study, methylation share rate (MSR) was firstly created to evaluate methylation level, which was enlightened by the fraction of brands share rate (BSR) of the RAPD model. **Firstly,** the C-methylation and C-unmethylation of the specific CpG-dinucleotide locus is random, which similarly complied with the character of the RAPD marker. These phenomena were listed as follows: **(1)** methylation or unmethylation status randomly occurred in different positive colonies from the same individual in all analyzed samples (Fig.1); **(2)** For the CpG-dinucleotide loci, 100% methylation loci randomly presented in different loci, such as C1, C3-C11 dinucleotide loci; **(3)** there were significant differences of CpG-dinucleotide methylation in different positive colonies from the same individual, such as colony-4, 5 (100% methylation) **versus** colony-11 (36.36%) in G134 individual; colony-11 (100%) **versus** colony-6,7,8,9 (63.64%) in G190 individual; colony-1 (90.91%) **versus** colony-3 (63.64%) in G136; colony-4 (100%) **versus** colony-1,7,9 (63.64%); colony-8,9,11 (100%) **versus** colony-10 (63.64%) in G8 individual;colony-9,10,12 (100%) **versus** colony-8 (54.55%) in G219 individual; colony-1,5 (100%) **versus** colony-2,3,4,6(90.91%) in G236 individual; colony-6 (100%) **versus** colony-2,3,4,5 (90.91%) in G247 individual. **Secondly,** the C-

methylation or C-unmethyatlin in cytosine is co-domination, which was identical with the RAPD marker, because the single positive insert only bring one haplotype. **Thirdly,** the methylation of individuals being compared was run with adequate controls, to minimize the errors in assigning identity to methylation pairs. **Fourthly,** all individuals were to be relatively random members of the population. **Fifthly,** the marker loci are to be unlinked and in Hardy-Weinberg equilibrium within and between loci.

As we know, the methylation percentage (MP) has been widely used in DNA methylation analysis, which will miss a lot of potential and important information. While the MSR index will effectively evaluate the methylated information beyond the methylation percentage. Because MSR equation includes all types of comprehensive data sources, such as CpG-dinucleotide loci, colonies, individuals and populations. In this work, the MSR value of different individuals was slightly higher than methylation percentage (MP). Even the lower methylation percentage of individual may possess the higher MSR value, such as G190 individual. Therefore, MSR was strongly recommended to be a useful and effective index to evaluate the DNA methylation level as well as the MP index.

Based on genetic diversity, heterozygosity, percentage and MSR, it was considered that the adult HNB goat possessed hypermethylation level in *Dnmt3b* gene 5' region, which maybe comply with low expression of *Dnmt3b* gene in adulthood (She *et al*., 2009; Yaqinuddin *et al*., 2008).

The result of t-test analysis showed that DNA methylation of *Dnmt3b* gene 5' region may affect body weight and other growth traits in adult goats, which might be mainly explained by two hands. On the one hand, DNA hypermethylation of *Dnmt3b* gene was possibly associated with gene reactivation, chromosomal instabilities, gene repression. The documents had reported that DNA methylation of *Dnmt3L* gene promoter significantly decreases the transcriptional activity (Aapola *et al*., 2004; Carougea *et al*., 2010; Jones and Baylin, 2002). One the other hand, the DNA methylation of *Dnmt3b* gene may regulate itself express, and different expression of *Dnmt3b* gene will directly or indirectly affect DNA methylation of other genes which may directly or indirectly influence growth traits in animal, such as *GH*, *PRL*, *POU1F1* genes (Lan *et al*., 2007), IGFs and its binding protein genes.

Briefly, the current work firstly revealed eleven polymorphic CpG-dinucleotide loci of goat *Dnmt3b* gene 5' region, and induced MSR method. Moreover, hypomethylation was probably associated with the high body weight of adult goat, which will benefit for animal selection and breeding through MAS as a novel epigenetic marker.

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REFERENCES

Aapola, U., K. Maenpaa, A. Kaipia and P. Peterson (2004). Epigenetic modifications affect Dnmt3L expression. Biochem. J. 380: 705-713.

Bestor, T., A. Laudano, R. Mattaliano and V. Ingram (1998). Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. The carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases. J. Mol. Biol. 203: 971-983.

Bird, A. (2002). DNA methylation patterns and epigenetic memory. Genes Dev. 16: 6-21.

Bird, A. P. and A. P. Wolffe (1999). Methylation-induced repression—belts, braces, and chromatin. Cell 99: 451-454.

Bleich, S., B. Lenz, M. Ziegenbein, S. Beutler, H. Frieling, J. Kornhuber, and D. Bonsch (2006). Epigenetic DNA hypermethylation of the HERP gene promoter induces down-regulation of its mRNA expression in patients with alcohol dependence. Alcohol. Clin. Exp. Res. 30: 587-591.

Campión, J., F. Milagroaand J. A. Martínez (2010). Epigenetics and Obesity. Prog Mol Biol Transl Sci. 94: 291-347.

Carougea, D., L. HostLione, A. Dominique, Z. Jean and A. Patrick (2010). CDKL5 is a brain MeCP2 target gene regulated by DNA methylation. Neurobiol. Dis. 38: 414-424.

Chen, M. F., W. C. Chen, Y. J. Chang, C. F. Wu and C. T. Wu (2010). Role of DNA methyltransferase 1 in hormone-resistant prostate cancer.J. Mol-Med.88: 953-962.

Eden, A., F. Gaudet, A. Waghmare and R. Jaenisch (2003). Chromosomal instability and tumors promoted by DNA hypomethylation. Science 300: 455.

Fuks, F., P. J. Hurd, D. Wolf, X. Nan, A. P. Bird and T. Kouzarides (2003). The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. J. Biol. Chem. 278: 4035-4040.

Gopisetty, G., R. Kavitha and S. Rakesh (2006). DNA methylation and apoptosis. Mol. Immunol, 1729-1740

Hansen, R. S., C. Wijmenga, P. Luo, A. M. Stanek, T. K. Canfield, C. M. Weemaes and S. M. Gartler (1999). The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. Proc. Natl. Acad. Sci. USA 96: 14412-14417.

Herrera, B. M. and C. M. Lindgren (2010). The Genetics of Obesity.Current Diabetes Reports10: 498-505.

Jones, P. A. and S. B. Baylin (2002). The fundamental role of epigenetic events in cancer. Nat. Rev. Genet. 3: 415-428.

Jones, P. L., G. J. Veenstra, P. A. Wade, D. Vermaak, S. U. Kass, N. Landsberger, J. Strouboulis and A. P. Wolffe (1998). Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat. Genet. 19: 187-191.

Kremenskoy, M., Y. Kremenska, M. Suzuki, K. Imai, S. Takahashi, K. Hashizume, S. Yagi, and K. Shiota (2006). Epigenetic characterization of the CpG islands of bovine Leptin and POU5F1 genes in cloned bovine fetuses. J. Reprod. Dev. 52: 277-285.

Lan, X. Y., C. Y. Pan, H. Chen, C. L. Zhang, J. Y. Li, M. Zhao, C. Z. Lei, A. L. Zhang, and L. Zhang (2007). An AluI PCR-RFLP detecting a silent allele at the goat POU1F1 locus and its association with production traits. Small Rumin Res. 73: 8-12.

Li, E., T. H. Bestor and R. Jaenisch (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 69: 915–926.

Liu, J. H., X. W. Liang, J. Q. Zhu, L. Wei, Y. Hou, D. Y. Chen and Q. Y. Sun (2008). Aberrant DNA methylation in 5' regions of DNA methyltransferase genes in aborted bovine clones. J. Genet. Genomics 35: 559-568.

Lynch, M. (1990). The Similarity Index and DNA Fingerprinting. Mol. Biol. Evol. 7: 478-484.

McCarrey, J. R., C. B. Geyer and H. Yoshioka (2005). Epigenetic regulation of testis-specific gene expression. Ann. N. Y. Acad. Sci.1061: 226-242.

Milagro, F. I., J. Campión, D. F. García-Díaz, E. Goyenechea, L. Paternain and J. A. Martínez (2009). High fat diet-induced obesity modifies the methylation pattern of leptin promoter in rats. J. Physiol. Biochem. 65: 1-10.

Nei, M. and W. H. Li (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269-5273.

Okano, M., D. W. Bell, D. A. Haber and E. Li (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99: 247-257.

Okano, M., S. Xie, and E. Li (1998). Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat. Genet.19: 219-220.

Reik, W., W. Dean, and J. Walter (2001). Epigenetic reprogramming in mammalian development. Science 293: 1089-1093.

Rivenbark, A. G., W. D. Jones, J. D. Risher and W. B. Coleman (2006). DNA methylation-dependent epigenetic regulation of gene expression in MCF-7 breast cancer cells. Epigenetics1: 32-44.

Robertson, K. D. and A. P. Wolffe (2000). DNA methylation in health and disease. Nat. Rev. Genet. 1: 11-19.

She, X. W., C. A. Rohl, J. C. Castle, A. V. Kulkarni, J. M. Johnsonand R. H. Chen (2009).Definition, conservation and epigenetics of housekeeping and tissue-enriched genes. BMC Genomics10: N269.

Smet, D. C., A. Loriot and T. Boon (2004). Promoter-dependent mechanism leading to selective hypomethylation within the 5_ region of gene MAGEA1 in tumor cells. Mol. Cell. Biol. 24: 4781-4790.

Teodoridis, J. M., G. Strathdee and R. Brown (2004). Epigenetic silencing mediated by CpG island methylation: potential as a therapeutic target and as a biomarker. Drug Resist. Updat. 7, 267-278.

Watanabe, D., I. Suetake, T. Tada and S. Tajima (2002). Stage- and cellspecific expression of Dnmt3a and Dnmt3b during embryogenesis. Mech. Dev. 118: 187-190.

Welsh, J., and M. McClelland (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213-7218.