Original Article

Genetic Polymorphisms Of The DNMT3b Gene In Local And Imported Calves

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Abstract

summary

This study was conducted in the laboratories of the Department of Animal Production of the College of Agriculture - University of Basrah for the period from 1/11/2021 to 1/5/2021. The data for this study were collected from Dhi Qar Governorate included samples of blood and meat. These samples were taken from 30 imported and local (Jenoubi) calves aged (1 to 2) years. Ten ml of blood as well as 250 g of meat were taken from the thigh, shoulder and back area of all animals. DNA was extracted from blood samples and the primer of the DNMT3b gene was used in (PCR) technique to amplify the gene. the aim was to study the association of DMNT3b polymorphism with sensory and physical characteristics of meat. The result of amplification of DNMT3b gene with a size of 611 bp and three haplotypes were obtained in local and imported calves. The 2nd haplotype differed from the 1st and 3rd by the nitrogen base 409, while the 3rd haplotype differed from the 1st and 2nd by the nitrogen base 526. The statistical analysis did not show significant differences in the distribution of the three haplotypes of local and imported calves. The hot carcass weight was significantly affected ($P<0.05$) by calf breed as the imported calf's carcass weight (155.87 kg) exceeded those of local calves (131.71kg). The carcass weight was also influenced by the different haplotypes of the DNMT 3b gene, with the superiority of the 2nd and 1st types compared to the 3rd haplotype. The saturated and unsaturated fatty acids% in imported and local calves' meat were significantly affected by the haplotypes of DNMT3b gene, where the palmitic acid% of 1st and 2nd haplotypes were superior than that of 3rd haplotype (25.78, 26.61 and 24.69%, respectively). As for the total saturated and polyunsaturated fatty acids, the 1st haplotype outperformed the 3rd haplotype (48.23 and 44.9% for saturated fatty acids and 8.87 and 8.10% for polyunsaturated acids, respectively).

Keywords: DNMT3d gene, polymorphisms, meat quality, fatty acids, Jenoubi, cattle

Introduction

The primary genetic concern with regard to local animals is their genetic polymorphisms (Bastos et al, 2001). The technique of markers assisted selection is one of the techniques that increase the accuracy and quantity of genetic improvement of traits. In recent years, meat quality improvement has received increasing attention (Zeisel, 2004), and thus has become one of the main objectives of beef cattle breeding (Pariacote, et al., 1998). Quality traits of beef include the thickness of the marbling, thickness and color of fat, and the rib-eye muscle area (Kononoff, et al., 2005; Ayied and Al-Mossawi, 2017). Some of these traits have been reported to be associated with lipid deposition (Wood, et al., 2004 and 2008). The quality of the meat from beef cattle is assumed to be influenced by the expression of genes involved in lipid deposition, which may be influenced by the expression of DNMT3a and DNMT3b. Cow studies have always focused on growth efficiency while ignoring meat quality. The rise in living standards around the world caused many consumers to prioritize meat quality, which prompted plans to raise and improve cows in order to satisfy consumer and market demands to include meat quality (Nonneman et al., 2013).

Carcass and the meat quality has a significant impact on how profitable livestock husbandry is. Diet, nutrition, pre- and post-mortem care, as well as techniques for handling and storing meat, all have an impact on these characteristics (Adzitey, 2011; Guerrero et al., 2013; Njisane et al., 2017). In this context, carcass characteristics and meat quality were measured and integrated into global beef cattle breeding programs (Reverter et al., 2000; Yokoo et al., 2010; Berry et al., 2017; Gordo et al., 2018). Wang et al (2012) proposed a GWAS method based on an unbiased best monolinear genome predictor (ssGBLUP, Legarra et al, 2009; Aguilar et al., 2010; Legarra et al., 2014), which became the ideal method for GWAS called ssGWAS, that has the advantage of accurately calculating the effect of a SNP (Zhang et al., 2016; Faraj, et al., 2020).

The study aimed to reveal the relationship between genetic polymorphisms of DNMT3b gene and the quality of meat and the degree of marbling in the meat of cattle raised in Iraq (imported and local) to determine the genotypes that give the best quality of meat.

Materials and methods

For 30 animals, blood samples were taken and kept by freezing in test tubes containing an anticoagulant, and samples of meat were taken from various body areas (thigh, back, and shoulder) from 15 local and 15 imported calves. In the Molecular Genetics Laboratory at the College of Agriculture/University of Basrah, the DNA was extracted from the blood samples using a DNA extraction kit manufactured by the Korean Geneaid in accordance with the instructions provided in the kit. DNA samples were sent to South Korea to identify the sequence of selected segments of the DNMT3b gene, according to the primers (F: AATGCTTGCAGGAAAGAAGTC, R: ATTCTTGCACTTCCCACCAG) described by Liu et al., (2012). After obtaining the required sequences, they were analyzed with Bio Edit program (Hall, 1999) and then the genotypes were identified using DnaSP v5.10 (Librado and Rozas, 2009). The effect of breeds and genotypes within breed were analyze by the statistical packages of social sciences (SPSS, 2019). Means differences were tested by Revised Least Squares Test (Bonferroni) within the above statistical program.

Results and Discussion

A segment of the DNMT3b gene was amplified with a size of 611 bp for both domestic and imported calves. Three haplotypes were shown in Fig. (1). The second haplotype (Hap2) differed from the first and third haplotypes by the nitrogenous base of the sequence 409 and changed from the base A to the base G (A>G), while the third haplotype (Hap3) differed from the first and second haplotypes by the nitrogenous base of the sequence 526. The nitrogenous base C was converted to the base T (C>T). Genetic diversity, expressed as the diversity of haplotype was 0.3235.

a

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1 Hap 1  aGATCCCTACCCCCCGCCCAGTCTCTTAACTGGAAGCTCTGCCCCTCTCA
2 Hap 2  gGATCCCTACCCCCCGCCCAGTCTCTTAACTGGAAGCTCTGCCCCTCTCA
3 Hap 3  aGATCCCTACCCCCCGCCCAGTCTCTTAACTGGAAGCTCTGCCCCTCTCA
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b

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1 Hap 1  cTGCCTTTCCTTTCTCTCCTGGGAGCTAGCTAAGCTCTTCCTAAGCCTGA
2 Hap 2  cTGCCTTTCCTTTCTCTCCTGGGAGCTAGCTAAGCTCTTCCTAAGCCTGA
3 Hap 3  tTGCCTTTCCTTTCTCTCCTGGGAGCTAGCTAAGCTCTTCCTAAGCCTGA
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Fig. (1) a: the difference between the second haplotype (Hap2) and the other two haplotypes at base 409, b: the difference between the third haplotype (Hap3) and the other two haplotypes at base 526

The number of individuals representing the first haplotype was 14 individuals with a frequency equal to 0.47 (Table 1). The number of local calves representing this haplotype was 8 calves with a frequency equal to 0.53, while the number of imported calves representing this haplotype was 6 calves with a frequency of 0.47. While the second haplotype represented 9 calves with a frequency equal to 0.30, the number of local calves representing this haplotype was 4 calves with a frequency equal to 0.27, while the number of imported calves was 5 calves with a frequency equal to 0.33. The number of calves included in the third haplotype was 7 calves with a frequency equal to 0.23, three local calves represented this haplotype with a frequency equal to 0.20, while 4 imported calves represented it with a frequency equal to 0.27. The statistical analysis did not show significant differences in the distribution of the three haplotypes on local and imported calves. It is clear from these results that the genetic structures of this gene for both local and imported calves (which are cross of breeds specialized in beef production) are similar.

Table (1) Number and frequency of haplotypes in local and imported calves

Haplotypes	**Local calves**		**Imported calves**		**Total**		**Chi-squares**	**p- value**
	No.	**Frequency**	**No.**	**Frequency**	**No.**	**Frequency**		
Hap 1	**8**	**0.53**	**6**	**0.40**	**14**	**0.47**	**0.18**	**NS**
Hap 2	**4**	**0.27**	**5**	**0.33**	**9**	**0.30**	**0.005**	**NS**
Hap 3	**3**	**0.20**	**4**	**0.27**	**7**	**0.23**	**0.01**	**NS**
Total	**15**	**1.00**	**15**	**1.00**	**30**	**1.00**		

- **NS= not significant.**

The first to record the genetic diversity of this gene was Liu et al., (2012), whose results agreed with our results regarding haplotypes I, II and III. Liu et al., (2012) also showed that there are nine SNPs, of which SNP1-SNP6 were recorded for the first time, while the other three SNPs were identical to those in the GenBank. Those SNPs are SNP7 located within the fourth intron and registered under accession number rs134010442 in NCBI and both SNP8 and SNP9 belonging to the tenth intron that were previously registered under accession number rs135724333 and rs132930989, respectively. The wild type of allele (A or C) and genotype (AG or CC) were highly distributed in the two breeds of beef cattle Wagyu and Snow Dragon (frequency greater than 0.8) as well as Fuzhou yellow cows (frequency greater than 0.7), the lowest frequency was recorded in the Limousin breed was less than 0.3 (Liu et al., 2015).

Carcass weight

Due to the difference in breed and the improved in carcass weight of the imported calves (155.87 kg) over the local calves (131.71 kg), the hot carcass weight was significantly ($P< 0.05$) impacted by this factor (Table, 2). The carcass weight was also affected by the different haplotypes of the DNMT3b gene by the superiority of the second and first haplotypes (148.42 and 144.35 kg, respectively), compared to the third haplotype (137.33 kg). While there were no significant differences in carcass weight between the first and second haplotypes. These findings are in line with those of Liu et al. (2015), who discovered a significant difference in haplotypes of the same gene in 14 beef cattle breeds in China. They also discovered that the genes DNMT1, DNMT3a, and DNMT3b, which are related to DNA methylation, are significantly associated with carcass traits and meat quality in beef cattle. Studies have shown that DNMT3a and DNMT3b gene expression is linked to the accumulation of fat, which may lead to an increase in adipose tissue and, ultimately, a probable rise in carcass weight (Kamei et al., 2010). Additionally, it was found that the DNMT3a and DNMT3b genes strongly influence beef quality parameters (Liu et al., 2012 and Guo et al., 2012).

Table (2) Mean of carcass weight (kg) of calves belong to different haplotype of DNMT3b gene within local and imported breed

Breed	Haplotypes			Breed mean
	Hap1	Hap2	Hap3	
Local	131.27±3.42	145.00±3.45	127.50±3.54	131.71^{b}±3.71
Imported	160.33±3.34	149.00±4.19	157.00±3.20	155.87^{a}±4.21
Mean of haplotypes	144.35^{a}±3.21	148.42^{a}±4.23	137.33^{b}±3.21	
RLSD	Breed=7.23	Haplotype= 2.80	Haplotype within breed= Not significant	

- **Means with different superscript differ significantly at $p <0.05$**

The substantial relationship between the SNP of the DNMT3b gene and increased carcass weight in local and crossbred calves suggests that these loci could be exploited as possible genetic markers to increase the amount of meat that local or crossbred calves produce. It was revealed that DNMT3b gene is a noteworthy gene containing 5 SNPs significantly associated with many meat quality traits of beef cattle, the significant effect of SNPs in DNMT3b gene was on carcass weight and that this gene could be a potential candidate gene in future studies to improve Beef quality (Liu et al., 2015).

Fatty acid percentages

Table (3) shows the percentages of different fatty acids in the dorsal longissimus dorsi muscle meat of local and imported calves' meat of different haplotypes. The 1st and 2nd haplotype showed higher ($P<0.05$) percentages of palmitic acid compared to that of the 3rd haplotype (25.78, 26.61 and 24.69%, respectively). In terms of palmitoleic and stearic acids, the third haplotype exceeded the other two haplotypes ($P< 0.05$), with palmitoleic percentages of

4.62, 3.90, and 1.81% for the 3rd, 1st, and 2nd haplotype, respectively. Those of stearic were 16.33, 15.43 and 14.63%, respectively. While the second haplotype showed a significant ($P<0.05$) superiority in the percentage of oleic acid (41.60%), the 1st and 3rd haplotypes scored 37.89 and 38.11%, respectively. The percentage of linoleic acid was significantly ($P<0.05$) higher for calves within the third haplotype with a percentage of 0.71% compared to its percentage in the first haplotype (0.45%) and the second haplotype (0.40%). The 1st haplotype was significantly ($P<0.05$) superior in the proportion of arachidonic acid (1.76%) compared to the 2nd (1.12%) and 3rd haplotype (1.23%).

As for the percentage of total saturated and polyunsaturated fatty acids, the 1st haplotype outperformed the 3rd (48.23 and 44.96%, for saturated respectively, and 8.87 and 8.10% for polyunsaturated acids, respectively).

As a higher concentration of unsaturated fatty acids results in a lower fat melting point, it also influences the softness of the fat and the flavor of the meat, making it one of the commercially significant carcass characteristics impacting meat quality (Narukami et al. 2011). Numerous authors have demonstrated in their investigations that genetic variables, such as lipid synthesis and genes associated to fatty acid metabolism, may regulate the composition of fatty acids (Avilés et al. 2013; Urrutia et al. 2020). Zhang (2008) discovered a relationship between the C14:0, C16:0, C18:1, SFAs, and MUFAs in the longissimus dorsi muscle of Angus bulls and the g.17924A>G SNP of FASN gene, which results in an amino acid change from threonine to alanine. According to Yeon et al. (2013), SNP in Hanwoo's cattle was substantially correlated with C16:0, C16:1, C18:1, SFA, and unsaturated fatty acids.

Table (3) The percentages of fatty acids (%) in the dorsal region (Longissimus dorsi muscle) for the different genotypes

Fatty acids %	Haplotypes of MTDN3b		
	Hap 1	Hap2	Hap 3
Myristic acid (c14:0)	3.94±0.48	4.09±0.39	3.93±0.49
Palmitic acid (c16:0)	25.78^{b}±0.27	26.61^{a}±0.12	24.69^{c}±0.23
Palmitoleic acid (c16:1n-9)	3.90^{b}±0.26	1.81^{c}±0.09	4.62^{a}±0.23
Stearic acid (c18:0)	15.43^{ab}±0.77	14.63^{b}±0.48	16.33^{a}0.67
Oleic acid (c18: n1-9)	37.89^{c}±0.43	41.60^{a}±0.60	38.11^{b}±0.99
Linoleic acid (c18:2n-3)	6.45±0.08	5.78±0.57	6.15±0.56
Linoleic acid (c18:3n-3)	0.45^{b}±0.08	0.40^{b}±0.06	0.71^{a}±0.09
Arachidonic acid (c20:4n-6)	1.76^{a}±0.20	1.12^{b}±0.18	1.23^{b}±0.13
Eicosapentaenoic acid (C20:5n−3)	0.23±0.01	0.24±0.05	0.25±0.07
Docosahexaenoic acid (C22:6n−3)	0.28±0.07	0.25±0.09	0.35±0.10
Saturated fatty acids (SFA)	48.23^{a}±1.57	45.43^{ab}±1.46	44.96^{b}±1.22
Polyunsaturated Fatty acids (PUFA)	8.87^{a}±0.19	7.97^{b}±0.14	8.10^{b}±0.16

- **Means with different superscripts within each row differ significantly at 5%**

Liu et al. (2012) discovered that the g.17924A> G SNP was linked to a variety of SFAs, ranging from 10:0 to 20:0, as well as various PUFAs and one long PUFA chain. Barto et al. (2016) demonstrated that the aforementioned exon 39 SNP in Holstein cattle revealed that the ratios of C14:0, C16:0, C14:1n-5 and SFA in muscle fat were higher in animals with AA genotype than in animals with GG genotype, whereas the C18:1n-9, MUFA, and MUFA/SFA ratios were lower.

Conclusion

The DNMT3b gene sequence revealed three haplotypes in both local and imported calves. Saturated and polyunsaturated fatty acids, as well as carcass weight, were strongly impacted by these haplotypes. According to these findings, this gene may be utilized as a marker to improve the quantity and quality of meat.

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