Genotyping of Diacylglycerol Acyltransferase 2 Gene in Holstein Cattle Population

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Summary

This study was conducted to describe the single nucleotide polymorphism (SNPs) within exon 8 and its intron 9 flanking region within bovine Diacylglycerol Acyltransferase 2 (*DGAT2*) gene and the possible association of these SNPs with the milk productive traits. Blood samples were collected from randomly selected Holstein cattle. Genomic DNA was extracted, and a pair of specific PCR primers was designed to amplify a segment that consists of partial exon 8/partial intron 9 of *DGAT2* gene. SSCP experiments were optimized and performed for each amplified PCR fragment. Each set of SSCP resolved bands was sequenced and analyzed. Four SSCP patterns representing four genotypes (BD, BB, CD, and AD) were detected with four alleles. Several novel genetic polymorphisms were discovered. Three SNPs (157 C/A, 158 T/G, and 159 G/A) were found in genotype BD, while one SNP (94 G/T, 153 C/A, 154 T/A) were found in the genotypes BB, CD, and AD, respectively. The only non-synonymous SNP was found in genotype CD (344 D/Y), and the effect of this missense mutation on the protein three-dimensional structure was visualized. The sequence homology between the bovine *DGAT2* gene and other species was also analyzed. The significance of the correlation of each genotype with the productive traits of milk was observed. The Holstein cattle with BD genotype produced significantly more milk for all studied 90 days of lactation, with a significant effect on fat for only the last 30 days of lactation, whereas there was no effect on protein and lactose percentage for the entire lactation period. The results suggest a novel association between bovine *DGAT2* genetic variability and the milk yield in Holstein cattle. This opens interesting prospects for future *DGAT2* based selection programs and preservation strategies.

Key words

Holstein cattle, *DGAT2* gene, genotyping, protein structure, milk yield

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Introduction

Genetic markers provide potential powerful tools for identifying the favorite breed of cattle (Blott et al., 1999). The implications of genetic tools in the Holstein cattle have been found to effectively produce a rapid genetic gain for the intended industrial goals (Zenger et al., 2006). For more than two decades, DNA markers gave many benefits for genetic improvement in animals' breeds (Gelderman, 1997). Although the milk production traits are polygenic traits and all the genes affecting them are difficult to know, a few potential candidate genes have been recognized (Gupta et al., 2009). One of these candidate genes is *DGAT* gene (Cases et al., 2001). *DGAT* gene is involved in triglyceride synthesis that influences dairy production in bovines (Cases et al., 1998). Two genes are known as *DGAT*, *DGAT1*, and *DGAT2*. *DGAT1* gene was the first identified gene encoding a protein with *DGAT* activity. The *DGAT1* protein is an enzyme catalyzing the final step of triglyceride synthesis (Coleman et al., 2000). It was found that a missense mutation (232 Lys/Ala) in *DGAT1* is associated with variation in milk fat percentage in cattle (Winter et al., 2002). The *DGAT1* gene became a promising candidate gene for milk fat percentage in cattle (Hradecká et al., 2008). The genetic variation in *DGAT1* was found to be associated with a major effect on milk yield and composition (Laubscher, 2011). However, *DGAT*-like activity has also been shown in other enzymes encoded by other genes and led to the detection of Diacylglycerol Acyltransferase 2 (*DGAT2*), It is well demonstrated that *DGAT2* plays an essential role in catalyzing the final step of the triacylglycerol (TAG) biosynthesis of the Kennedy pathway (Wakimoto et al., 2003). *DGAT2* gene is closely related candidate for quantitative traits, and it's associated with lipid synthesis and storage in eukaryotes. Overexpression of *DGAT2* in glycolytic muscles of mice increases the content of triglycerides and unsaturated long-chain fatty acyl-CoAs (Levin et al., 2007). The analysis of genetic variation of *DGAT2* genetic loci can be used to evaluate some productive traits in buffaloes (Kale et al., 2014). Since cattle and buffalos have 99 to 100% of genetic similarity, it could potentially be considered as one of the best biological markers in cattle. It was found that this gene plays a significant role in "marker assisted selection" (An et al., 2011). Accordingly, several techniques were employed to exploit the portions of this gene to detect the extent of polymorphisms in a way that could be used in routine labs, such as single-stranded conformation polymorphism (SSCP) (Gasser et al., 2006). SSCP is a simple (Sunnucks et al., 2000), sensitive technique (Hayashi and Yandell, 1993), and its reliability was confirmed (Jaeckel et al., 1998). SSCP is based on the assumption that changes in the nucleotide sequence of a polymerase chain reaction (PCR) product affect its single-stranded conformation (Girman et al., 1996). Molecules differing by as little as a single base substitution should possess different conformers under non-denaturing conditions and migrate differently. Therefore, those differences could be detected as a shift in electrophoretic mobility (Hayashi, 1991). Once the utilization for SSCP polymorphisms could lead to the finding of useful genetic markers of agricultural populations (Bonifacio et al., 2001), much more attention was gained in *DGAT2* gene since the control and prediction of obesity is of a high commercial interest for the industrial Holstein cattle breed. We were led to research the *DGAT2* gene in this study because: (1) the polymorphism of bovine *DGAT2* gene is potentially related to some milk productive traits (Kale et al., 2014); (2) understanding the mechanisms of triglyceride synthesis that is encoded by *DGAT2* gene has potential importance for elucidating the molecular processes that contribute to obesity and other metabolic disorders (Cases et al., 2001); (3) the combination of the crucial effect of its genetic polymorphism and its intervening in triglyceride synthesis makes the *DGAT2* gene an excellent candidate gene that may impart animal genetic breeding, and (4) despite that the *DGAT2* gene may have the same *DGAT1* gene importance in relation with milk production traits, most of polymorphism studies were focused on *DGAT1* genetic polymorphism correlation with milk productive traits, such as Spelman et al. (2002), Grisart et al. (2004), Kuhn et al. (2004), Thaller et al. (2003), and Winter et al. (2002). So, the focus of this research was to observe potential role of *DGAT2* gene and its unknown genetic polymorphism in milk productive traits. On the other hand, there have been no sufficient studies in cattle with respect to *DGAT2*. The variations of *DGAT2* gene have been studied in very few domestic animals, and there is limited number of studies concerning the bovine *DGAT2* gene polymorphism till now. Very little genetic data is currently available about these genes' fragments in Holstein cattle. Thus, *DGAT2* gene could be considered as an interesting genetic marker that could give an insight into the nature of the desirable productive traits. Therefore, the main goal of our study was to identify sequence variation of the *DGAT* gene, and to characterize this potential variation and its associations with the main milk productive traits in this population.

Materials and methods

Blood sampling and DNA extraction

The study was conducted on a total of 60 individuals of Holstein cattle in Iraq. This breed is found mainly in the almost all regions of the Iraqi plain. All of the studied populations were reared in a privet station in Al-Qadisya Governorate. Both maintenance and feeding were similar for all animals and remained in accordance with the obligatory standards. About 3 – 4 ml of blood samples were collected from the jugular veins of 60 of the Holstein cattle and placed in anticoagulation tubes. Genomic DNA was isolated using a manual salting-out extraction method (Al-Shuhaib, 2017).

PCR analysis

Two pairs of primers were designed using the NCBI primer BLAST online software (GenBank Acc. No. AJ534372). The designed oligonucleotide primers pair was forward 5′-GAGCCCATTACCATCCCCAG-3′, and reverse 5′-TCCTCCCAGAAGCTGGCCCC-3′, which is designed to amplify 181 bp to partially cover exon 8 and intron 9 of cattle *DGAT2* gene. PCR reaction was performed using *AccuPower* PCR premix (Cat # K-2012, Bioneer, Korea). Each 20 µl of PCR premix was contained 1 U of Top DNA polymerase, 250 µM of dNTPs, 10 mM of Tris-HCl (pH 9.0), 30 mM of KCl, and 1.5 mM of MgCl2. The reaction mixture was completed with 10 pmol of each primer and 50 ng of genomic DNA. The optimum annealing temperatures were determined empirically in extracted DNA template using gradient PCR (ver. Mastercycler nexus, Eppendorf, 22331 Hamburg). The amplification started by initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C

for 30 sec, annealing at 60°C for 30 sec, elongation at 72°C for 30 sec, and it was concluded with a final extension at 72°C for 5 min. After performing PCR thermocycling, PCR products were verified by electrophoresis on a 1.5% (w/v) agarose gel in 1 × TBE buffer (2 mM of EDTA, 90 mM of Tris-Borate, pH 8.3), using a 100-bp ladder (Cat # D-1010, Bioneer, South Korea) as a molecular weight marker for confirmation of the length of the PCR products. Gels were stained with ethidium bromide (0.5 mg/ml). All SSCP non-suitable amplicon bands were eliminated.

SSCP analysis

SSCP experiments were performed according to Mustafa's protocol with some optimizations (Mustafa et al., 2018). Briefly, 7 µl of each amplification product was mixed with 7 µl of SSCP denaturing loading buffer (95% formamide, 20 mM EDTA pH 8, 0.05% xylene cyanol and 0.05% bromophenol blue). The samples were heat-denatured at 95°C for 10 min and chilled on ice for at least 5 min. Then, PCR amplicons were separated in vertical mini-gel format, gel size (W×L) cm: 10×10, and gel thickness: 1 mm (OmniPAGE, Cleaver Scientific, UK). Denatured PCR products were loaded into the wells of 8% acrylamide/bis (37.5:1), containing 7% glycerol, and 1x TBE buffer. The gel was run at constant conditions (200 V/100 mA/100 min) at room temperature. Gels were stained with silver nitrate (Byun et al., 2009).

DNA sequencing

Each unique SSCP samples' pattern for the amplified *DGAT2* gene (181 bp) fragment was purified and sequenced from both ends (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. The cattle reference sequences of exon 8/DGAT2 gene (GenBank Acc. No. AJ534372) were retrieved from the NCBI website (http://www.ncbi.nlm.nih.gov). The sequencing results of the PCR products of different SSCP patterns were edited, aligned, and compared with their reference sequences using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). Further, in order to know the sequence homology of the four detected genotypes of bovine *DGAT2* gene with other bovine and non-bovine species, the online basic local alignment search tool for nucleic acids (BLASTn suite) analysis was performed (www.blast.ncbi.nlm.nih.gov).

Protein Structure Designing

The primary structure designing of each SSCP genotype begun by mutating the available reference NCBI DNA sequences of exon 8/DGAT2 gene (GenBank Acc. No. AJ534372) by substituting each observed SNP into its reference sequences using *BioEdit* / Lasergene software to represent BD, BB, CD, and AD genotypes. The whole amino acid sequences of the bovine *DGAT2* protein was retrieved online from the protein data bank (www.rcsb.org). These four genotypes were translated into amino acids in a reading frame that corresponds to the reference *DGAT2* amino acid sequences using the *Expasy* online program (web.expasy.org). Multiple amino acid sequences alignment was made between the reference exon 8/DGAT2 amino acid sequences and it's observed four genotypes using *Seqman*/Lasergene software. The three-dimensional structure of the *DGAT2* gene was constructed from the online three dimensional model prediction software: protein homology/analogy recognition engine (Phere2), ver 2.0 (www.sbg.bio.ic.ac.uk/phyre2). The virtual proposed changes within its corresponding mutants were performed by using PyMol-v1.7.0.1 software (www.shrodinger.com).

Statistical analysis

Data were collected and categorized into 90 days of lactation to estimate their effect on the main milk productive traits of animals. Two types of statistical analysis were performed in this study: genetic diversity and associate analysis. Regarding genetic diversity, A χ^2 test was performed to verify possible deviations from Hardy-Weinberg equilibrium (HW) expectations for the distribution of genotypes. Average heterozygosity was employed to estimate genetic diversity within the population. In addition to χ^2 test, allele and genotype frequencies and Nei's heterozygosity were calculated with PopGen32 software, v. 1.31 (Yeh et al., 1999). Regarding associate analysis, the associations of the *DGAT2* genotypes with the following milk productive traits: milk yield, milk protein ratio (%), and milk fat ratio (%) was made. The association analysis was conducted over 90 days of lactation using the General Linear Model of Statistical Analysis System, SAS, v 9.0 (SAS Institute, NC, USA) for a completely randomized design with repeated measures. The model contained the effects of genotype, age, and the interaction of genotype and age. The overall effect of genotype was tested using cow within genotype as the error term. The model for the analysis was:

$$Y_{ij} = \mu + A_i + G_i + (A + G)_{ij} + e_{ij},$$

Where Y_{ij} was the phenotypic value of the trait, μ was the overall mean of each trait, A_i was the fixed effect of age, G_j was the fixed effect of genotype, $(A + G)_{ij}$ was the interaction between the age and the genotype, and e_{ij} was the random residual error that is distributed naturally in an average equal zero and constant variance, i.e. NID (0, σ_e^2). Significant differences between least-square means of different genotypes were determined using the Duncan's multiple-range test. The values are reported as least square means and standard error of the means; P values of 0.05 were considered to be statistically significant.

Results and Discussion

Two types of genetic data were performed, which included *DGAT2* gene SSCP based polymorphic sequences SNPs genotyping, and the relation of the observed SSCP-SNPs with the milk productive traits of Iraqi Holstein population.

PCR-SSCP Genotyping

The polymorphisms of bovine *DGAT2* gene were detected by PCR-SSCP and DNA sequencing methods. Undoubtedly, too small sized of the studied groups is still a shortcoming of our research. Nevertheless, the observed variations of SSCP gels indicate the detection of four different genotypes with four allelic variations (Fig. 1). It was found that the BD, CD, and AD genotypes, in which the ssDNA region constitutes two unique bands, whereas the ssDNA region in the BB genotype constitutes

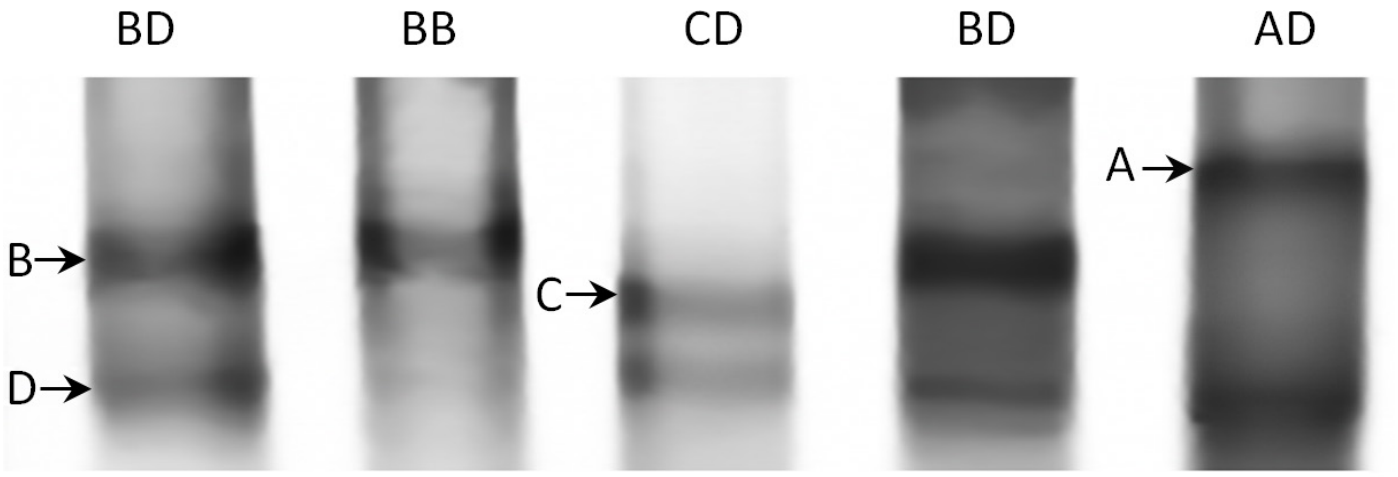

Figure 1. PCR-SSCP of the four genotypes (BD, BB, CD, and AD) and the four alleles (A, B, C, and D) that were observed in exon 8/partial intron 9 of the *DGAT2* gene fragment (181 bp) of Holstein cattle (*Bos taurus*) samples. The SSCP gel is silver stained. The color of the gel is converted into black and white for better resolution.

of only one unique ssDNA band. However, the multitude of SSCP bands that we observed in the *DGAT2* gene PCR fragment was usually evident in some SSCP configurations (Hayashi, 1991).

Nucleotide sequences from all four gel SSCP patterns revealed several novel SNPs. Sequencing results confirmed these four different electrophoretic SSCP patterns since several SNPs were detected between the four resolved genotypes and NCBI reference sequences (Fig. 2, A). The pattern and nature of each SNP that were detected by sequencing indicated that the genotype BD has three novel intronic SNPs (157 C/A, 158 T/G, and 159 G/A), the genotype BB has only one novel intronic SNP (153 C/A), and the genotype AD has also only one novel intronic SNP (154 T/A). The only observed exonic SNP was found in the genotype CD (Fig. 2, A). It was found that this SNP constitutes non-synonym mutation (Fig. 2, B), and the functional conformation of the produced *DGAT2* gene is modified with regard to exon 8 in the genotype CD (Fig. 3). When this exonic SNP (94 G/T) appears in the three-dimensional structure of the resulting proteins of AD genotypes, the positively charged/hydrophilic amino acid Asp (D) (Fig. 3, A), is converted into the uncharged/hydrophilic amino acid Tyr (Y), (Fig. 2, B). This, in turn, changes the conformational changes in the final phenotypic manifestation of the protein. Unfortunately, no previous studies were focused on bovine exon 8 of the *DGAT2* gene to compare our results with. Thus, these data provide the first

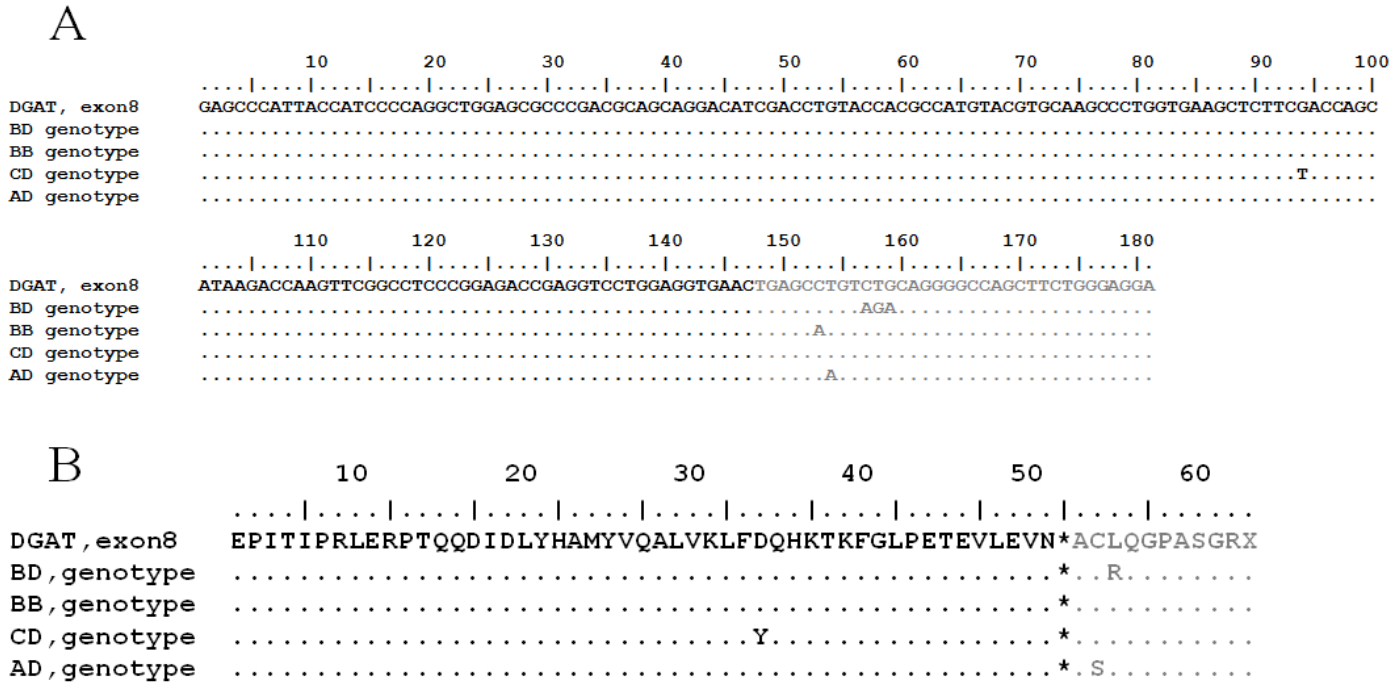

Figure 2. Sequences alignment results for *Bos taurus* exon 8/partial intron 9 of the *DGAT2* gene fragment (181 bp) four SSCP genotypes with their reference sequence (GenBank AJ534372) using DNA Star *EditSeq* software. (A) Multiple sequence alignment of nucleic acids. (B) Multiple sequence alignment of amino acids sequences. The "stars" refer to the stop codons.

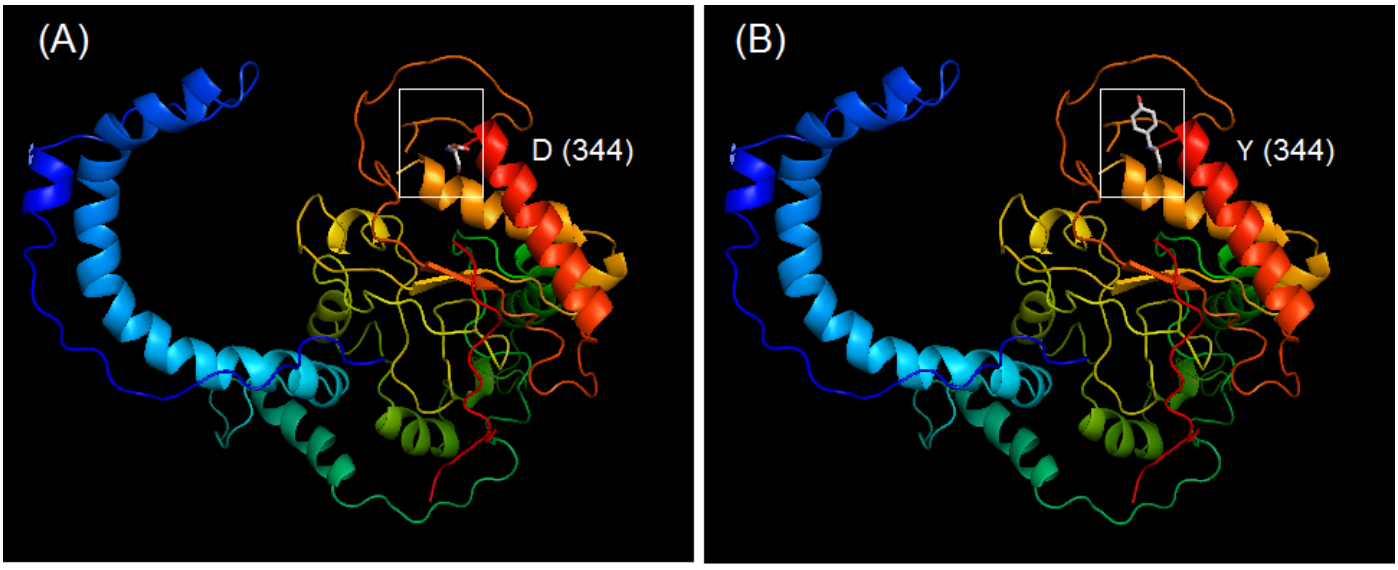

Figure 3. Postulated three-dimensional structure of the bovine *DGAT2* protein showing the change of amino acid "Asp" (in the position 344) of the reference protein (A), into amino acid "Tyr" of the CD genotype protein of Holstein cattle.

evidence about the nature of such missense mutation in this gene. In order to identify the sequence homology of Holstein cattle *DGAT2* gene with other species, the *BLASTn* suite was utilized. *BLASTn* suite results revealed a sequence identity of 100% with the other bovine species. With respect to small ruminants, the percent identity was 99% with goat, 96% with sheep, 95% with Tibetan antelopes, 94% with bighorn sheep, 93% with camels, and 90% with giant pandas, gray mice, and polar bears (Table 1). However, the observed SSCP genotypes of the current study are provided a beneficial and low-cost tool for the geneticists and breeders to identify the unknown SNPs that may be useful for establishing a possible association with productive parameters.

Association of SSCP genotypes with productive traits

In the marker-assisted selection of dairy animals, *DGAT2* gene is proposed as a potential candidate that is associated with dairy performance traits. It was known that some *DGAT2* gene may be correlated with the milk production trait, or at least be an effective DNA marker of the dairy animal genome. In this current study, it seems reasonable to start studies on associations between *DGAT2* genotypes and milk production traits in the highly economically important Holstein cattle in order to verify the obtained results before using them in dairy selection programs.

The observed frequencies of genotypes were 0.533 (n = 32), 0.316 (n = 19), 0.116 (n = 7), and 0.033 (n = 2) for BD, BB, CD, and AD genotypes, respectively. The observed frequency of: A allele was 0.333, B allele was 0.100, C allele was 0.025, and D allele was 0.541 (Table 2). Nei's expected heterozygosity has the same value of average heterozygosity, which was 0.5849. This indicates a high domination of allele D. The χ^2 test showed that the polymorphism of the *DGAT2* gene in Holstein cattle was at Hardy-Weinberg equilibrium for this locus in the studied population. The observed heterozygosity for *DGAT2* genotypes was higher than their expected values (Table 2). This refers to the high level of genetic variability in the studied population.

Table 1. BLAST results showing the highest homology of bovine Holstein *DGAT2* gene (exon 8/partial intron 9) with other bovine and other species.

Accession	Species	Query cover (%)	E-value	% identity
XM_006040164.1	*Bubalus bubalis*	100	2e-88	100
NM_205793.2	*Bos. taurus*	100	2e-88	100
HM566448.1	*Capra hircus*	100	2e-78	97
XM_012096078.2	*Ovies aries*	100	4e-75	96
XM_005954102.1	*pantholops hodgsonii*	100	2e-73	95
CP011900.1	*Ovis canadensis*	100	2e-72	94
XM_014556675.1	*Camelus ferus*	100	9e-67	93
XM_012744894.1	*Microcebus murinus*	100	9e-57	90
XM_008707435.1	*Ursus maritimus*	100	9e-57	90
XM_011219881.1	*Ailuropoda melanoleuca*	98	2e-57	90

Table 2. The observed, expected, average heterozygocities, χ^2 test for Hardy-Weinberg equilibrium, and genotype frequencies of *DGAT2* almost exon 8/ partial intron 9 for the Holstein cattle breed.

Obs-Het	Exp-Het	Avr-Het	Nei'-Exp -Het	χ^2	Allele A freq	Allele B freq.	Allele C freq.	Allele D freq.
0.9167	0.5898	0.5849	0.5849	42.1	0.333	0.100	0.025	0.541

Abbreviations: Obs-Het – observed heterozygocity, Exp-Het – expected heterozygocity, Avr-Het – average heterozygocity, Nei-Exp-Het – Nei's expected heterozygocity, freq. – frequency.

Based on the individual month data assessment (Table 3), significant differences (P> 0.05) in the daily rate of milk yield for all three months for genotypes BD, BB, CD, and AD were found. This study refers to the presence of significant differences (P> 0.05) in the fat ratio for four genotypes only in the last month. Regarding protein and lactose ratio, there are no significant differences (P> 0.05) for all three months for all four detected genotypes.

We noticed that the highest mean total milk yield was found in genotype BD followed by genotypes BB, CD, and AD respectively. The 90 days milk yield showed a significant variation. The genotype BD produced significantly more milk than other genotypes. Our results are in contrast with two data that revealed the absence of any significant association between the bovine *DGAT2* gene with the milk fat ratios in cattle (Winter et al., 2003), and with the main milk productive traits in water buffaloes (Kale et al., 2014). Nonetheless, the possibility of using the exon 8 PCR fragment of the *DGAT2* gene as a successful genetic marker in correlation with milk production traits was confirmed in our study at least in terms of milk yield. Besides, analysis of allelic variation of *DGAT2* gene in cattle can potentially be used to evaluate temporal changes in genetic diversity, and our analysis data showed that Holstein cattle breed can be differentiated using *DGAT2* gene SSCP based variations. In accordance with our results, it was observed that mutations in the *DGAT2* gene are associated with differences in some economically relevant traits, such as growth rate, carcass weight and lipid contents in the muscles in many breeds of cattle (Zhang et al., 2007; Dunner et al., 2013).

We noticed the presence of an effective correlation between *DGAT2* genotypes and milk yield. Though we found that the exonic SNP of CD genotype that is non-synonymous and induced a conformational change in the *DGAT2* protein doesn't have any association with the milk productive traits (Table 3). Alternatively, we found that the intronic SNPs of BD genotype is associated with the milk yield. Actually, it was found that it's not necessary for exonic polymorphism to be associated with milk productive traits; instead, intronic polymorphism may play this role in Holstein dairy cattle (Liefers, 2004). We found that BD genotype is characterized with a trend of better productive performance. The significant heterozygous BD genotype superiority over other genotypes in terms of milk yield trait was observed. Consequently, *DGAT2* gene polymorphism is significantly associated with milk yield traits in Iraqi Holstein cattle. Thus, it's possible to focus on the enhancing the production of the heterozygous BD genotype population over the most frequent homozygous BB genotype population. It might be rational to show that the identified *DGAT2* four genotypes were the main reason for the polymorphism in the productive traits of this population. Therefore, the polymorphism in *DGAT2* gene might be one of the important genetic factors that influence productive traits in Holstein cattle.

Table 3. Least square means and standrard errors of selected milk production traits in Holstein cattle with different almost exon 8/partial intron 9 *DGAT2* genotypes in three months of lactation.

Traits	Months of lactation	SSCP Genotypes				Level of significance
		BD (n 19)	BB (n 32)	CD (n 7)	AD (n 2)	
MY±SE	1st	9.06^a±0.712	7.42^b±0.342	7.40^b±0.981	6.00^b±1.981	*
	2nd	9.20^a±0.681	7.71^b±0.129	7.20^b±0.895	5.00^b±1.427	*
	3rd	9.06^a±0.801	6.71^b±0.468	6.60^b±1.030	5.00^b±1.931	*
F±SE	1st	3.57^a±0.592	3.33^a±0.215	2.83^a±0.994	1.77^a±1.341	NS
	2nd	3.52^a±0.671	3.52^a±0.198	3.42^a±0.950	4.10^a±2.901	NS
	3rd	3.51^a±0.551	3.41^a±0.309	2.44^b±1.002	3.29^b±1.562	*
P±SE	1st	2.87^a±0.509	2.73^a±0.287	2.85^a±0.979	2.11^a±0.996	NS
	2nd	3.35^a±0.561	3.54^a±0.422	3.84^a±0.896	3.22^a±1.844	NS
	3rd	3.79^a±0.821	3.68^a±0.611	3.41^a±1.021	2.13^a±1.038	NS
L±SE	1st	4.89^a±0.722	5.34^a±0.593	5.38^a±0.895	7.52^a±0.899	NS
	2nd	4.71^a±0.599	4.41^a±0.496	4.10^a±0.909	4.35^a±0.972	NS
	3rd	4.09^a±0.675	4.31^a±0.418	4.62^a±1.022	4.39^a±1.008	NS

Abbreviations: MY – daily milk yield (kg), F – fat content (%), P – protein content (%), L – lactose ratio, NS - not significant, * - (P<0.05), SE – standard error, and a, b, ab - mean values with the same superscripts letters within column differ significantly at P<0.05.

Conclusions

Our results indicated that the combination of both alleles (B and D) may potentially be associated with better productive traits in local Iraqi Holstein cattle. As long as the utilization of *DGAT2* gene is a suitable and informative genetic marker that is used to find the genetic variability association with the main milk yield traits, it's possible to apply this promising marker on larger samples to further scan cattle population. Besides, the powerful ability of the inexpensive SSCP technique can be invested to reveal further unknown genetic mutations in other genetic loci. In conclusion, the current PCR-SSCP genotyping technologies make the *DGAT2* gene an economically beneficial marker, and it would be a marker of choice at the moment in an efficient and cost-effective manner. However, further research is warranted to unmask many molecular markers that are still unknown to geneticists and breeders and that weren't given enough attention till now, which might enhance performance and productivity in the bovine industry.

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