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Research Article

Relationship between polymorphisms of growth hormone gene with milk yield and its components in Iraqi cattle bred of Janoubi, Cross and Holstein

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Abstract: This study aimed to identify the polymorphic SNPs of the growth hormone gene and their association with milk yield and its chemical components. A total of 24 cows were collected from local cattle belonging to Janoubi, cross, and Holstein breeds (8 cows each). Blood and milk samples were taken from each cow. The chemical composition of milk included moisture, protein, fat, solid not fat, and casein. PCR and sequencing of the growth hormone gene were performed to determine the polymorphic SNP. The results indicated that there is a polymorphic SNP at 172 bp (C>A), as it changed from the genetic code (AGG) to the genetic code (CGG), both of which encode the amino acid Arginine. Two genotypes were observed for Janoubi and three for the cross. AA genotype exceeded other genotypes in daily milk yield, total milk yield, adjusted milk yield, and fat%.

Keywords: Growth hormone gene, Milk yield, Composition, Cattle.

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Introduction

Growth hormone (GH) is a somatotropin/prolactin family of hormones secreted in the acidophilic cells of the anterior lobe of the pituitary gland of mammals. It plays an important role in growth, lactation, milk production, reproduction, and protein, fat, and carbohydrate metabolism (Dybus 2002; Wathes et al. 2012). It influences disease resistance by stimulating the immune system through several mechanisms. Recently, a role for growth hormone has also been revealed in mammalian aging (Vukasinovic et al. 1999). Because of these physiological effects, the growth hormone gene is considered a candidate marker for selection in livestock (Gordon et al. 1983; Singh et al. 2014). The bovine growth hormone (GH) gene (Gen Bank Acc. M57764) is located in BTA19. It contains five exons that encode several peptides, consisting of 217 amino acids (Hediger et al. 1990; Zhang et al. 1992). The different polymorphisms that characterize the GH site are of interest in many studies, and the single nucleotide polymorphism (SNP) C > G in exon 5, which changes the amino acid leucine to the amino acid valine at position 127, is of particular interest (Bartke & Quainoo 2018).

Mutations in the growth hormone gene also result in an amino acid variation. This ultimately affects the level of growth hormone and the phenotype, which is expressed in growth, reproduction and milk production. It also has a useful role in characterizing population studies and genetic diversity in cows (Hediger et al. 1990). Several studies have focused on this polymorphic locus, named GH p. L127V, to assess the allele frequency distribution in dualpurpose dairy and meat cattle breeds. Schlee et al. (1994) reported that the homozygous leucine amino acid genotype (p.127LL) was associated with a higher concentration of GH in the blood.

In his study, we emphasized the effects of polymorphisms of the growth hormone gene (GH p.L.127V) on production traits, particularly milk production, growth, or carcass traits in cattle. However, the effect of the GH p. L127V polymorphism on milk production traits is not considered a decisive effect, and it appears from the studies conducted that it has a strain-specific relationship (Lucy et al. 1993; Sørensen et al. 2002). The study of genetic polymorphisms is equally useful for revealing genetic markers related to economic traits, and many studies focused on the molecular characterization of the GH gene in *Bos taurus* cattle (Schenck & Brooks 2006) and the study of polymorphisms in crossbred cattle (CB) (Aravindakshan et al. 1997; Pal et al. 2005; Pal et al. 2014). Several studies have been conducted on *Bos taurus* cattle (Lee et al. 2013; Pal et al. 2004) which have to do with the association of growth hormone gene variants with growth, milk production, and reproductive characteristics. Some preliminary studies were also conducted to find the relationship between the growth hormone gene in the process of growth, expected milk production and reproduction (Pal et al. 2004; Lee et al. 2013).

Although many studies have been conducted on polymorphisms and their associated traits, the exact molecular mechanism of how and why the GH gene is mutated has not yet been revealed. Little and simple information is available about the appropriate structural and functional characterization of the GH gene, and so far, not adequately and clearly resolved how variation in growth hormone and peptide generated by a SNP leads to its functional alteration and how other genes are related to growth hormone functionally and biologically. Hence, the current study aimed to discover the SNP of the growth hormone gene and its association with milk production and its chemical components.

Materials and Methods

The study was conducted from 01/10/2021 to 01/08/2022, at the laboratory of Genetic Engineering, University of Basrah. The study included 24 cows (8 Holstein, 8 local and 8 crosses). The blood samples (5ml/cow) from the jugular vein of samples were collected.

Milk chemical contents including fat%, protein%, lactose% and solid not fat (SNF%) were estimated by Lactoflash device (Funke Gerber, Germany). Samples of milk (50ml) were collected every 15 days throughout the experiment period. Blood from each cow was sampled intravitally into sterile vacuum tubes containing K2EDTA (dipotassium ethylene diamine tetra acetic acid) anticoagulant. A fragment (929pb) of the GH gene in the reference cattle using the primer Forward 5'-CCCACGGGCAAGAATGAGGC-3' and Reverse5'-TGAGGAACTGCAGGGGCCC-3' was extracted (El-Nahas et al. 2018)

Data analysis: GH gene sequences were aligned using the BioEdit software (Hall 1999) and using DnaSP v5. 10 software (Librado & Rozas 2009), the haplotypes network was drawn using Network 5.0.0.0 software (Bandelt et al. 1999). Neighbor-joining (NJ) tree for testing cattle breed sequences and the phylogenetic tree of the three genotypes (Holstein, Local and crosses) were constructed using Mega version 7.0 software (Kumar et al. 2016). Two Way ANOVA and Least Significant Test using SPSS (2019) were performed to estimate significant differences between genotypes means of different parameters.

Results and Discussion

The results of electrophoresis on a 2% agarose gel showed the success of the DNA amplification process (Fig. 1), with a size of 298 bp, eight sequences for each of the domestic, cross and Holstein-Friesian cattle, respectively, were obtained. A genetic mutation was detected at position 172 of the studied segment of the growth hormone gene, in which C was changed to A (C>A). It changed from

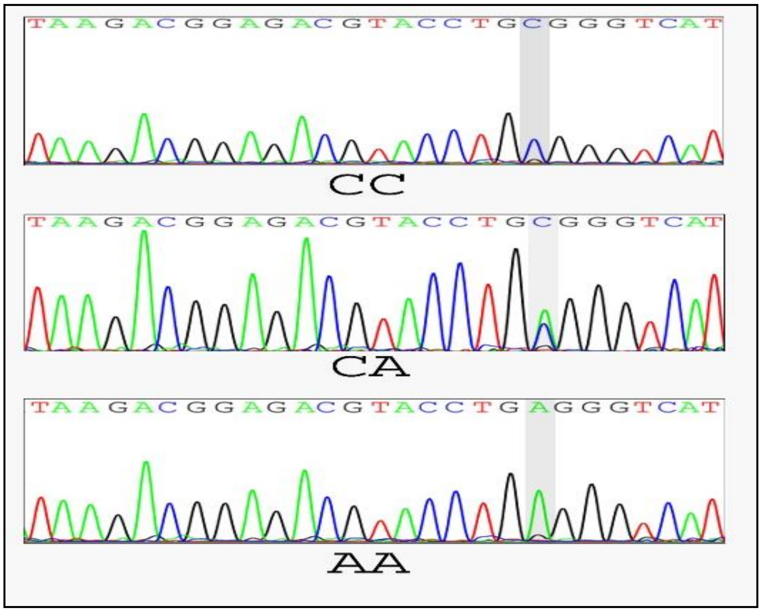

Fig.1. Polymorphic SNP at 172bp of the growth hormone gene.

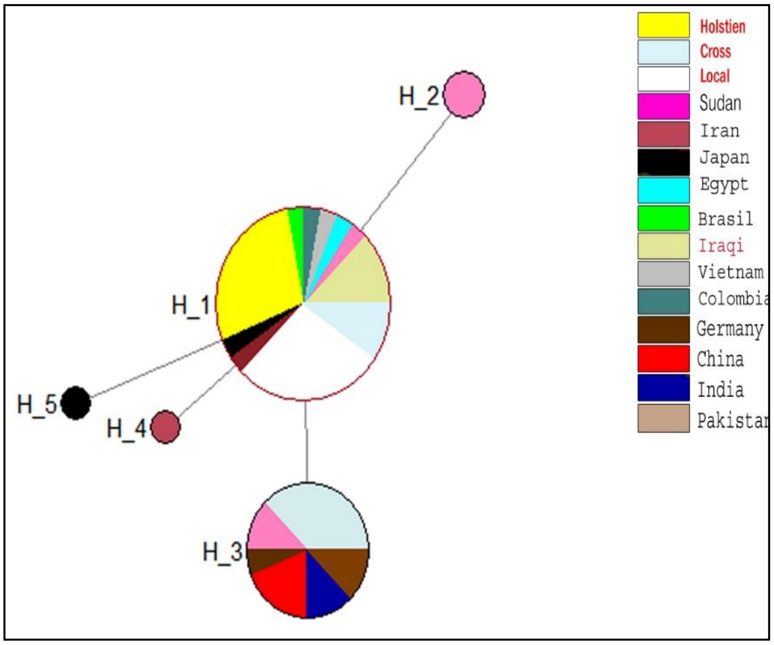

Fig.2. Haplotypes network of different cattle breeds.

the genetic code AGG to the genetic code CGG, both of which encode the amino acid Arginine. This mutation is considered one of the silent mutations because it did not change the structure of the amino acids in the studied piece, as the amino acid Arginine has five genetic codes, which is located at position 53 of the complete peptide chain of growth hormone. The change at position 2201 occurred in the fourth exon of the complete growth hormone gene recorded in the GenBank (JQ711182). In this study, three genotypes were obtained, which are AA, CC, and AC (Fig. 2). Where these structures were distributed among the three strains, the genotypes AA, AC and CC included 16, 5, and 3 individuals of the animals under study respectively.

The highest frequency of the A allele was in the Janoubi local cattle, where it reached 0.94, and its lowest frequency was in the crossed cattle (0.25). As

Table 1. Genotypic and alleles frequencies of Janoubi, Holstein Friesian and crosses cattle.

		All	Cross	Janoubi	Holstein
Polymorphisms	AA	15	1	7	7
	AC	3	2	1	0
	CC	6	5	0	1
Allele frequency	A	0.69	0.25	0.94	0.93
	C	0.31	0.75	0.06	0.07
Genotypes Frecuency	AA	0.62	0.13	0.88	0.88
	AC	0.13	0.25	0.12	0
	CC	0.25	0.62	0	0.12
X^2		0.16	0.89	0.04	0.06
P- Value		NS	NS	NS	NS

NS= not significant

Table 1. Milk yields of local, cross and Holstein cattle and its chemical components.

Traits	Genotypes	Holstein	Cross	Janoubi	Mean
Daily milk yield	AA	26.31	6.22	4.16	14.64^{a}
	AC	23.97	6.67	00	9.55^{b}
	CC	00	7.18	4.18	6.18^{c}
	Mean	26.31^{a}	6.74^{b}	4.17^{c}	00
Total milk yield	AA	6630.84	1566.18	1049.54	3688.59a
	AC	6036.91	1680.08		2406.22b
	CC	00	1808.23	1053.61	1556.69c
	Mean	6556.60a	1697.88b	1050.05b	
Adjusted milk yield for 305 days	AA	6564.53	1550.52	1039.05	3651.71a
	AC	5976.54	1663.28		2382.16b
	CC	00	1790.14	1043.08	1541.12c
	Mean	6564.53^{a}	1680.90^{b}	1039.56^{b}	
Protein%	AA	2.30	2.79	2.18	2.28
	AC	2.65	2.94		2.89
	CC		3.00	2.35	2.78
	Mean	2.35	2.93	2.20	
Fat%	AA	3.44	3.88	3.28	3.39b
	AC	3.49	4.09		3.99ab
	CC		4.56	3.32	4.15a
	Mean	3.44	4.18	3.29	
SNF%	AA	9.34	7.24	6.44	7.84
	AC	9.53	8.59		8.75
	CC		9.14	6.03	8.10
	Mean	9.36	8.56	6.39	
Lactose%	AA	5.91	4.48	4.20	5.02
	AC	5.37	4.97		5.04
	CC		5.12	4.06	4.77
	Mean	5.84	4.95	4.18	

Means with different superscripts of each trait different significantly at 0.05.

for the frequency of the C allele, it had the highest frequency in the crossed cattle (0.25), and its lowest frequency was 0.06 in Janoubi cattle (Table 1). These findings disagree with those of Aravindakshan et al. (1997) that discovered two breeds of the Polish Jersey and the Holstein breed, in which they assimilated three genotypes, BB, AA, and AB. BB recorded the highest frequency (0.583 and 0.541) followed by AB (0.339 and 0.427), while AA was the least common (0.078 and 0.032).

The total number of haplotypes that were obtained for the GH gene was five haplotypes (Fig. 2). The 1st haplotype included the Janoubi, Holstein bred in Iraq, Sudanese, Japanese, Egyptian, Brazilian Colombian, and German. While the haplotype H_2 included a part of the Sudanese cattle, haplotype H_3 included all of the Chinese cattle and a part of the Sudanese cattle, Indo-German, and a part of the local cattle. Part of the Iranian cattle and the Japanese cattle were isolated, each with its own haplotype, H_4 and H_5, respectively. These findings agree with those of Owaid et al. (2019), who analyzed three breeds of cows, local, Holstein, and cross, and discovered six distinctive haplotypes in each. These are also in agreement with those of Tarekegn et al. (2018), who evaluated the genetic structure of two indigenous Indonesian breeds (Kebumen & Brahma), and identified 8 and 6 haplotypes, respectively. The variation in the number of haplotypes may be attributable to the sample size (Tarekegn et al. 2018).

The findings revealed that the genotype AA of the Holstein breed exceeded the other genotypes (CC, and AC) in terms of both daily milk yield and adjusted (305) milk production. In terms of fat percentage, the cross-cattle genotype CC excelled over the other genotypes in both the Holstein and local breeds. Between the three breeds under study, there were no significant variations in the percentages of protein, lactose, and total solid not fat (SNF) (Table 2). These results agreed with what was found by Eattsh et al. (2016) when studying three breeds of cattle, the Egyptian Baladi, the Brown Swiss, and the Holstein, as the Baladi breed excelled in both fat and protein. Wdatcp (2010) stated that the average milk production that contains 3.7% fat and 3.02% protein in the Holstein breed is associated with breeds with higher milk production compared to other breeds such as the pure Brown Swiss breed. The results also showed that, in contrast to the Jersey, Guernsey, and Brown Swiss strains, which are distinguished by their low milk production, the protein content of their milk is higher than that of the Holstein strain (De Marchi et al. 2008). Additionally, it was suggested (Asian 2002) that the differences in milk production between the various breeds of milk cows were statistically significant.

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