Ferchichi MA, et al. J Anim Res Vet Sci 2022, 6: 032
DOI: 10.24966/ARVS-3751/100032
HSOA Journal of Animal Research and Veterinary Science

Research Article

The Association of the Genetic Polymorphism of the stat5a and gh/mspi Genes and the Incidence of Lameness in Holstein Cows in Tunisia

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Abstract

The objective of this study consists in determining the association between genetic polymorphism of the STAT5A and GH genes and the incidence of lameness in dairy cows in Tunisia. DNA was extracted from 412 blood samples from Holstein cows (lame and healthy). Genotyping was performed by the PCR-RFLP technique and the use of the BestEII and the MspI restriction enzymes, respectively for the Stat5A gene and the GH gene. The calculation of the association of lameness and its factors (parity, calving season, genetic polymorphism StAT5A and GH genes and levels of milk production) was done by the Logistic procedure. The genotypic frequencies of the Stat5A gene are of the order of 0.24, 053 and 0.23 respectively for the genotypes (GG), (GC) and (CC). For the two alleles (G and C), the allelic frequencies are equal; they are of the order of 0.5. For the GH-MspI polymorphism, the genotype frequencies are of the order of 0.55, 030 and 0.15, respectively for the genotypes (+/+), (+/-) and (-/-). The allelic frequencies are of the order of 0.7 and 0.3 respectively for the (+) and (-) alleles.

The logistic regression results reveal an association between the factors included in the model and the incidence of lameness. Multiparous cows are the most susceptible to lameness for all levels of milk production. The winter is the most favorable season for the incidence of feet pathologies. Similarly, the polymorphism of the Stat5A (production level) and GH (production level) genes seems to affect the incidence of lameness. The STAT5A G allele and the GH (+) allele increase the incidence of podal pathologies.

Keywords: Allele; Gene; GH; Lameness; Polymorphism; STAT5A

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Citation: Ferchichi MA, Jemmali B, Larbi MB, Gara AB (2022) The association of the genetic polymorphism of the STAT5A and GH/MspI genes and the incidence of lameness in Holstein cows in Tunisia. J Anim Res Vet Sci 6: 032.

Received: January 24, 2022; **Accepted:** February 07, 2022; **Published:** February 14, 2022

Introduction

Until the end of the 1990s, dairy cows breeding objectives' were on milk production and traits, fecundity and fertility parameters and some physiological aspects (teats and horns) without giving importance to the milk production, the cattle functional longevity and other robustness parameters' (mastitis, metritis and lameness resistances). These parameters are limited to the production level and they are essential for animals' welfare. Lameness is the third most common disease in dairy cattle and its heritability is relatively low [1]. This genomic selection and the study of QTLs related to the lameness resistance are the most efficient and the fastest solutions.

Several researchers have studied the effects of Stat5A and GH on milk production and traits as well as on the fertility parameters in dairy cows.

STAT5A is a gene coding for the anti-apoptotic protein (apoptosis is the programmed cell death mechanism) of neutrophils in cattle, located on chromosome 19 [2]. It is made up of 19 exonscoding, 794 amino acid chain [2]. This protein is a member of the STAT family of transcription factors. In response to cytokines and growth factors, members of the STAT family are phosphorylated by associated receptor kinases and then they form homo- or heterodimers that translocate to the cell nucleus where they act as transcriptional activators. It has been suggested that the anti-apoptotic mechanism is induced by an increase in Bcl-xL (B-celllymphoma-extra large) mRNA expression, dependent on the GM-activated STAT5 transcription factor. -CSF (Granulocyte-macrophage colony-stimulating factor). STATs act as signal transducers in the cytoplasm and activators of transcription in the nucleus.

Neutrophils, the most commonly represented granulocytic sub-population in the blood, have a high inflammatory potential and can induce tissue damage. Their death by apoptosis and their safe removal by phagocytic cells help to limit this damage during the resolution of inflammation. In the absence of cytokines or other pro-inflammatory agents (non-inflammatory context), the elderly neutrophils (half-life: 6 - 18h) spontaneously enter a programmed cell death process before being phagocytosed by macrophages [3]. It should be noted that cytokines play a major role in triggering and regulating the inflammatory response [4]. They can be produced by a large number of distinct cells (monocytes / macrophages, endothelial cells, keratinocytes, fibroblasts, etc.). They can be pleiotropic and several cytokines can have the same action on the same cell (redundancy). A cell type can produce several types of cytokine. Schematically, we can distinguish two classes of cytokines associated with two opposite types of action, either pro-inflammatory or anti-inflammatory.

We should cite that growth hormone influences reproductive functions [5] and assists the body's immune response, wound healing, and hematopoiesis [6]. Given the positive effects of this hormone, the cattle hoof could benefit enormously (healing of wounds, help with the immune response, hematopoietic growth).

[7] reported that bovine growth hormone (bGH) is a peptide with a molecular weight of about 22 kDa. [8] and Wallis et al. (1973) reported, respectively, that it is composed of 190 or 191 amino acids, containing Alanine or Phenyl-Alinine. In addition, Leucine or Valine amino acid substitutions at residue 127 exist due to allelic polymorphism [9]. [10] and [11] reported that it consists of five exons separated by introns.

Several polymorphisms have been identified in the GH gene. [12] and [13] detected a polymorphic site for the Msp I restriction endonucleasease enzyme, the polymorphism being localized in intron 3 of the GH gene at position 1547 [14]. [15] detected a polymorphic site for the restriction endonuclease Apa I. [16] and [17] detected a polymorphic site for Alu I, a restriction endonuclease.

It should also be remembered that bovine GH (bovine Somatotropin -STB) stimulates somatic postnatal growth and has diabetogenic, insulin-lactogenic and lactogenic effects in vivo. It coordinates the physiological processes affecting the nutrients involved in milk synthesis [18]. Similarly, the latter have reported that many studies have focused on the influence of bovine GH (long-term administration).) on the performance of lactation; recombinant GH increased milk production, corrected the fat material to a greater extent than the GH of pituitary origin. [19] reported that allelic variations in the bovine growth hormone gene were associated with variation in milk yield and also carcass characteristics, such as weight gain, meat quality, and so on [20] and reproductive characteristics [21]. Indeed, this hormone contributes to the growth of the body through rapid cell division and skeletal growth and metabolism [22], in mammogenesis, galactopoiesis, lipolysis, etc.

Several studies have been done on the association of these two genes with milk production and its traits, but few studies have been conducted on the incidence of lameness. The objective of our study is to study the genetic polymorphism of the STAT5A gene and the GH gene and their effects on the lameness incidence in Holstein cows in Tunisia.

Materials and Methods

Sample's description

Our sample was taken from a large dairy farm (Chargui), located in the delegation of Mateur-Bizerte (northeastern Tunisia) which belongs to the subhumid bioclimatic stage.

This region is one of the basins of dairy cattle in Tunisia. The dairy cattle herd in this region is about 49 000, mainly composed of cows of the Holstein breed. That's why, we have chosen to work on this breed. The total enrollment for four years 2013-2017 is about 412 cows. Sampling is done in December and January of each crop year. The period choice is not arbitrary, but it was related to the incidence of podal diseases during the winter season.

The sample covered three categories of cows: lame, not lame and lame cured bovines. The sorting on the lame has interested subjects with varied podal affection: cows with affection touching limbs and feet.

The identification of the affected category was not taken into consideration, for a clear registration failure in the health records and it has no influence in the results. Sampling consists of venous blood (jugular), the dose was approximately 5 ml, collected in EDTA (anticoagulant) tubes. The tubes are sent to Mateur higher school of agriculture and stored at -20 ° C.

Genotyping protocol

The DNA was extracted from a blood sample using the InnuPREP Blood DNA Mini Kit (analytikjena, Germany) according to the manufacturer's instructions. These DNA samples were examined by electrophoresis on a 1% agarose gel.

For genotyping the STAT5A (G / C) gene, an 820 bp fragment was amplified by PCR using the primer pair: 5 '- GAGAAGTTGGCG-GAGATTATATC-3' and 5'-CCGTGTGTCCTCATCACCTG-3 '[23]. The PCR amplification was carried out using approximately 200 ng of genomic DNA which corresponds to 4 µl of DNA, 2.5 µl of the PCR buffer, 2.5 µl for each primer (sense and antisense), 2.5 µl of dNTP, 0.2 µl of the Taq DNA Polymerase Recombinant (SEGMA-ALDRICH) and 10.8 µl of autoclaved water in a total reaction volume of 25 µl.

The PCR steps were as follows: a denaturation phase at 95 ° C for 5 minutes and then 35 cycles which contain a denaturation phase at 95 ° C for 40 seconds, an hybridization phase at 57 ° C for 40 seconds then an elongation phase at 72 ° C for 40 seconds and finally, a final extension period at 72 ° C for 10 minutes. The PCR products were revealed by 1.5% agarose gel electrophoresis.

The PCR products were digested with the BestEII restriction enzymes for the STAT5A gene in a reaction mixture containing 12µl of the PCR product, 0.3µl of restriction enzyme (BestEII), 2.5µl buffer (Buffer), 0.2 BSA and 10 µl of autoclaved water. The reaction mixture was incubated at 37 ° C for 12 hours.

For genotyping the GH (G / C) gene, a 329bp fragment was amplified by PCR using the primer pair: 5'-CCCACGGGCAAGAATGAG-GC-3 'and 5'-TGAGGAACTGCAGGGGCCCA-3' [24]. The PCR amplification was carried out using approximately 200 ng of genomic DNA which corresponds to 4 µl of DNA, 2.5 µl of the PCR buffer, 2.5 µl for each primer (sense and antisense), 2.5 µl of dNTP, 0.2 µl of the Taq DNA Polymerase Recombinant (SEGMA-ALDRICH) and 10.8 µl of autoclaved water in a total reaction volume of 25 µl.

The PCR steps were as follows: a denaturation phase at 95 ° C for 5 minutes and then 35 cycles which contain a denaturation phase at 95 ° C for 40 seconds, a hybridization phase at 60 ° C for 40 seconds and an elongation phase at 72 ° C for 40 seconds, and finally a final extension period at 72 ° C for 10 minutes. The PCR products were revealed by 1.5% agarose gel electrophoresis.

The PCR products were digested with the restriction enzymes MspI for the GH gene in a reaction mixture which contains 12 µl of the PCR product, 0.3 µl of restriction enzyme (MspI), 2.5 µl of buffer (Buffer), 0.2 BSA and 10 µl of autoclaved water. The reaction mixture was incubated at 37 ° C for 12 hours.

Statistical analysis

The database contains categorical data: (i) the genotypes of the two genes studied, STAT5A / BestEII and GH / MspI, (GG, GC, CC, ++, + - and -); (ii) calving season (fall, winter, spring and summer), and numerical data: (i) identification of the animal; (ii) parity (1, 2 and 3); (iii) age at calving; (iv) day milk yields test; (v) year of calving; (vi) country of animal seeds origin.

The analysis of the lameness incidence was done by the SAS logistic procedure (PROC LOGISTIC) using the following model:

$Y_{ijklmn} = \beta_i ST_i(Np_m) + \beta_j GH_j(Np_m) + \beta_k Nl_k(Np_m) + \beta_l sais_l(Np_m) + e_{ijklmn}$

Or

Y_{ijklmn}: *dependant variable* binaire of the lameness incidence.

ST: the fixed effect of the i^{th} genotype of the SATAT5A gene (i = 1, 2 or 3) with same level of production (Low, Medium, High)

GH: the fixed effect of the j^{th} gene genotype GH (j = 1, 2 or 3) with same level of production (Low, Medium, High)

Nl: the fixed effect of p^{th} parity (p = 1, 2 or 3) with same level of production (Low, Medium, High)

Sais: the fixed effect of the 1^{st} season of calving (l = 1, 2, 3 or 4) with same level of production (Low, Medium, High)

eijklmn : residuals error

Results

Detection of the genetic polymorphism

The separation of GH / MspI gene digests on the 2% agarose gel showed bands of 224 // 105 bp, 329 // 105 bp and 329 bp respectively for (+/+), (+/-) and (-/-) genotypes. The genotype frequencies are of the order of 0.55, 030 and 0.15 respectively for the genotypes (+/+), (+/-) and (-/-). The allelic frequencies of the GH / MspI gene are of the order of 0.7 and 0.3 respectively for the (+) and (-) allele (Table 1).

Similarly, digestion of the products amplified by the restriction enzyme (BestEII) revealed bands of 676 // 44 bp, 820 // 676 // 44 bp and 820 bp respectively for the GG, GC and CC genotypes. Genotypic frequencies are of the order of 0.24, 053 and 0.23 respectively for genotypes (GG), (GC) and (CC). And the allelic frequencies of the STAT5A / BestEII gene are equal for the two alleles (G and C), they are of the order of 0.5 (table 1).

Gene locus	Polymorphism	Allele		Genotypic Frequencies (%)			Allelic frequencies(%)	
STAT5A		G	C	GG	GC	CC	G	C
	153137	G	C	0.24	0.53	0.23	0.50	0.50
GH/ MspI		+	-	+/+	+/-	-/-	+	-
		G	C	0.55	0.30	0.15	0.70	0.30

Table 1: Genotypic and allelic frequencies (%) in the studied gene loci.

The effect of genetic polymorphisms on the prevalence of lameness

All the factors included in the statistical model; Stat5A genotypes (level of production), GH/MspI genotypes (level of production), parity (level of production), season of calving (level of production); significantly affect the incidence of lameness in Holstein cow (P< 0.01) (Table 2).

Effect	DF	Wald Chi-Square	Pr>Chisq
Stat5A (level of production)	6	145.02	<0.01
GH/MspI (level of production)	6	91.44	<0.01
Parity (level of production)	6	316.00	<0.01
Season (level of production)	9	118.78	<0.01

Table 2: Effect of factors included in the statistical model

DF: degree of freedom; p<0.01: signifiant

Table 3 represents the regression coefficients of the factors included in the multivariable logistic analysis hierarchical model. The highest regression coefficients of the Stat5A genotype factor (level of production) are of the order of 0.91, 1.46 and 1.04 for GG (Low), GG (Medium) and GG (High) respectively.

The highest regression coefficients for the genotype factor GH / MspI (level of production), are of the order of -0.83 and -0.49 respectively for the genotype +/- (Low) and +/- (High). At the level of the parity (level of production), the highest regression coefficients are of the order of 0.95, 1.36 and 1.49 respectively for the third parity (Low), third parity (Medium) and the third parity (High) . In addition we note that the highest regression coefficients are recorded at the winter for high and low production cows.

Parameter	DF	Estimate	Standard Error	Pr>Chisq	Exp(Est)
Genotypic Stat5A (level of production)					
CC (Low)	-	**Referent**	-	-	-
GC (Low)	1	0.5206	0.1506	<0.01	1.683
GG (Low)	1	0.9123	0.1912	<0.01	2.490
CC(Medium)	-	**Referent**	-	-	-
GC (Medium)	1	0.4611	0.0909	<0.01	1.586
GG (Medium)	1	1.6797	0.2357	<0.01	5.364
CC (High)	-	**Referent**	-	-	-
GC (High)	1	0.1593	0.1295	0.21	1.173
GG (High)	1	1.0287	0.1456	<0.01	2.798
Genotypic GH/MspI (level of production)					
+/+ (Low)	-	**Referent**	-	-	-
+/- (Low)	1	-0.8350	0.1546	<0.01	0.434
-/- (Low)	1	-0.6377	0.1726	<0.01	0.529
+/+ (Medium)		**Referent**			
+/- (Medium)	1	0.0760	0.1041	0.46	1.079
-/- (Medium)	1	-0.03914	0.1338	<0.01	0.676
+/+ (High)		**Referent**	-	-	-
+/- (High)	1	-0.4981	0.1171	<0.01	0.608
-/- (High)	1	1.2230	0.2710	<0.01	3.397
Parity (level of production)					
1 (Low)		**Referent**	-	-	-
2 (Low)	1	0.6196	0.1590	<0.01	1.858

3 (Low)	1	0.9504	0.1521	<0.01	2.587
1 (Medium)		**Referent**	-	-	-
2 (Medium)	1	0.5110	0.1039	<0.01	1.667
3 (Medium)	1	1.3643	0.1140	<0.01	3.913
1 (High)		**Referent**	-	-	-
2 (High)	1	0.9772	0.1532	<0.01	2.657
3 (High)	1	1.4960	0.1329	<0.01	4.464
Season (level of production)					
Autumn (Low)	-	**Referent**	-	-	-
Summer (Low)	1	0.3083	0.1510	0.40	1.361
Winter (Low)	1	0.5508	0.1474	<0.01	1.735
Spring (Low)	1	0.0791	0.1790	0.65	1.082
Autumn (Medium)		**Referent**			
Summer (Medium)	1	0.6939	0.1208	<0.01	2.001
Winter medium	1	0.4490	0.1002	<0.01	1.567
Spring medium	1	0.0788	0.1465	0.59	1.082
Autumn (High)		**Referent**			
Summer high	1	-0.0742	0.1284	0.56	0.928
Winter high	1	1.1328	0.1545	<0.01	3.104
Spring high	1	0.1075	0.1712	0.53	1.113

Table 3: coefficient of regression of polymorphism Stat5A (level of production, GH/MspI (level of production), Parity (level of production) and season (level of production).

Discussion

The genotypic frequencies, resulting from the digestion of the GH gene by the restriction enzyme MspI, are of the order of 0.55, 030 and 0.15 respectively for the (+ / +), (+/-) and (- / -) genotypes. The allelic frequencies of the GH / MspI gene are of the order of 0.7 and 0.3 respectively for the (+) and (-) alleles. The allelic frequencies agree with those found by [25] who studied the polymorphism of the GH / MspI gene in the native Turkish cattle breed. They found that the frequency of the (-) allele varies from 0.268 to 0.357. Similarly, our results agree with those of [26]. The frequency of the (-) allele has been studied in different countries and it varies from 0.09 to 0.26 [14, 27, 28, 19], Vukasinovic et al.,1998). On the other hand, the frequency of (-) in this study is lower than what had been found by [24, 29] and [30].

The genotype frequencies are of the order of 0.24, 053 and 0.23 respectively for the genotypes (GG), (GC) and (CC). And the allelic frequencies of the STAT5A polymorphism found in our current study agree with those of Oikonomou et al. (2011). The frequencies of the G and C alleles are of the order of 0.5 and 0.5, compared to 0.55 and 0.45 in the Oikonomou et al. (2011) study. But, there is no significant difference between these results and those of [23].

The association of STAT5A and GH / MspI polymorphisms and the incidence of lameness in Holstein cows is significant ($p < 0.01$). It is noted that GG animals are the most sensitive to the incidence of lameness for all levels of production (low, medium and high). [31] and [32] mentioned that high producing cows are the most susceptible to podal pathologies. According to Oikonomou et al. (2011), the G allele at the Stat5A locus is associated with the increase in milk production. These studies revealed that cows with GG genotypes produce 435.2 kg more milk than those with CC genotypes which can explains the affect ofthe G allele in the incidence of lameness. The incidence of lameness is high in homozygous animals (+ / +) for all level of production (low, medium and high).

[33] and [27] showed a significant association between the genetic polymorphism of the GH/ MspI gene and milk production. They mentioned that the (+) allele increases milk production in Holstein cows in China. This is consistent with our findings that the (+) allele favors the incidence of lameness because the most productive cows are the most sensitive.

According to Table 3, the incidence of lameness increases with the parity for all levels of production. The incidence of these podal pathologies in multiparas is higher than in primiparas for the three different levels of production (low, medium and high). According to [34], the best milk productions are recorded in third and fourth lactation. This leads to an imbalance between the intake of the ration and the production of the cows that should be accomplished by the concentrate. This excess of concentrate decreases animal's rumen ph that cause insufficient production of biotin [35, 36].

The latter which is synthesized by rumen bacteria is essential for the formation of skin, hair and horny envelopes such as the hooves of ruminants and pigs or hooves horses [37]. These bacteria are very sensitive to the low rumen pH caused by a diet rich in concentrates (in the first eight weeks of lactation concentrate intake is higher to meet production needs). Several researchers have examined this aspect of hoof health. It has been shown that the higher the proportion of concentrates in the cow ration, the less rumen microorganisms produce biotin [37] and [38].

The incidence of lameness is high in cows that calve during the winter season compared to those that calve during the spring, summer and fall seasons for all level of production. In addition, our study indicated that there is no difference in the incidence of this pathology for the fall, summer and spring seasons. Almost all of the studies mention the role of moisture and mild temperatures in the incidence of podal diseases of dairy cows, which are more common in free stalls, on straw areas (where contact between the animals' feet is tighter) than in cubicle buildings [39].

According to [40], cows' hooves absorb water quickly and hoof hardness decreases with increasing water content. These results suggest that even brief exposure of hooves to wet surfaces results in a decrease in their hardness. This thesis is confirmed by [41], who report that cows with softer hooves are more at risk for developing lameness problems. Also, [42-44] mention that when the hooves are softer, they become less resistant to microbiological and physico-chemical influences that can, among other things, be caused by exposure to excrement.

In Tunisia, the rainfall is winter type. It is thus accompanied by a drop in ambient temperature. During this period, there is a great disruption in the rationing of dairy herds because the access to fodder in green is often interrupted by the fall of the rains (inaccessible lands after the rain fall). Also, we have to note that the high calving rate is reached in the winter period. Thus, it is clear that many stressful

factors (change of rations, cold, soil moisture, calving) are not in favor of animal welfare but contrary, they are contributing factors to: (i) the disruption of the flora rumen (acidosis, alkalosis); (ii) disturbance of thermoregulation (increased lipolysis), (iii) friability of the foot horns, (iv) the negative affection of immunity, (v) the multiplication of pathogenic germs. This explains the increase in podal diseases in dairy cattle herds.

To conclude, the genetic polymorphism of the genes GH / MspI and Stat5A) is associated with the incidence of lameness in dairy cows ($p < 0.01$). The G and (+) alleles, respectively for the Stat5a and GH genes, seem to affect the incidence of lameness. The genotypes GG and (+ / +) are the most sensitive. Further studies must be done to confirm these results.

Thanks

The authors thank the Livestock and Pasture Office (OEP) and the Society of the Agricultural Development (Chergui).

References

1. Amstel SRV, Shearer JK, Palin FL (2006) The effect of parity, days in milk, season and walking surface on thin soles in dairy cattle, 14th International Symposium on Lameness in Ruminants, Colonia (Uruguay),142–3.
2. Seyfert HM, Pitra C, Meyer L, Brunner R.M, Wheeler TT, et al. (2000) Molecular characterization of STAT5A- and STAT5B-encoding genes reveals extended intragenic sequence homogeneity in cattle and mouse and different degrees of divergent evolution of various domains, J. Mol. Evol, 50:550–561.
3. Savill JS, Wyllie AH, Henson JE, Walport MJ, Henson PM, et al.(1989) Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages, J. Clin. Invest, 83:865-875.
4. Barrientos S, Stojadinovic O, Golinko M S, Brem H, Tomic-Canic M (2008) Growth factors and cytokines in wound healing, Wound Repair Regen, 16: 585-601.
5. Spiteri G J, Nieschlag E (1993) Paracrine factors relevant to the regulation of spermatogenesis-A review, J. Reprod. Fertil, 98:1-14.
6. Golde D W, Bersch N, Li CH (1977) Growth hormone: Species specific stimulation of erythropoiesis *in vitro*, Science., 196:1112-1113.
7. Wallis M (1973) The primary structure of bovine growth hormone. FEBS Lett., 35:11-14.
8. Lingappa VR, Ddevillers-Thiery A, Blobel G (1977) Nascent prehormones are intermediates in the biosynthesis of authentic bovine pituitary growth hormone and prolactin, Proc. Natl. Acad. Sci. USA, 74: 2432-2436.
9. Seavey BK, Singh RNP, Lewis UJ, Geschwind II (1971) Bovine growth hormone: evidence for two allelic forms. Biochem. Bioph. Res. Co., 43:189-195.
10. Gordon DF, Quick DP, Ewin CR, Donelson JE, Maurer RA (1983) Nucleotede sequence of the bovine growth hormone chromosomal gene. Mol. Cell. Endocrinol., 33:81-95.
11. Woychick RP, Camper SA, Lyons RH, Horowtts S, Goodwin E C, et al. (1982) Cloning and nucleotide sequencing of the bovine growth hormone gene, Nucl. Acids Res., 10:7197-7210.
12. Cowan CM, Dentine MR, Ax RL, Schuler LA (1989) Restriction fragment length polymorphism associated with growth hormone and prolactin genes in Holstein bulls: evidence for a novel growth hormone allele, Anim. Genet., 20:157-165.
13. Hilbert P, Marcotte A, Schwers A, Hanset R, Vassart G, et al. (1989) Aanalysis of genetic variation in the Belgian Blue Cattle bredd using DNA sequence polymorphism at the growth hormone, low density lipoprotein receptor, α-subunit of glycoprotein hormones and thyroglobin loci, Anim. Genet., 20:383-394.
14. Zhang HM, Brown DR, Denise SK, Ax RL (1933b) Nucleotide sequence determination of a bovine somatotropin allele, Anim Gen., 23:578.
15. Wang W, Huang L., Gao J, Ding J, Chen K, et al. (2003) Polymorphism of growth hormone gene in 12 pig breeds and its relationship with pig growth and carcass traits, Asian-Australasian J. Anim. Sci., 16:161-164.
16. Biswas TK, Bhattacharya TK, Narayan AD, Badola S, Kumar P, et al. (2003) Growth Hormone Gene Polymorphism and Its Effect on Birth Weight in Cattle and Buffalo, Asian-Aust. J. Anim. Sci., 16:494-497.
17. Aruna Pal A, Chakravarty K, Bhattacharya TK, Joshi BK, Sharma A (2004) Detection of Polymorphism of Growth Hormone Gene for the Analysis of Relationship between Allele Type and Growth Traits in Karan Fries Cattle, Asian-Aust. J. Anim. Sci., 17:1334-1337.
18. Bauman DE, Eppard PJ, Geeter MJD, Lanza G (1985) Responses of high-producing dairy cows to long treatment with pituitary somatotropin and recombinant somatotropin, J Dairy Sci., 68:1352-1362.
19. Sabour MP, Lin CY, Smith C (1997) Association of genetic variants of bovine growth hormone with milk production traits in Holstein cattle, J. Anim. Breed. Genet., 114:435-442.
20. Grochowska R, Lunden A, Zwierchowski L, Snochowski M, Oprzadek J (2001) Association between gene polymorphism of growth hormone and carcass traits in dairy bulls, Anim. Sci., 72:441-447.
21. Lechniak D, Machnik G, Szydlowski M, Switonski M (1999) Growth hormone gene polymorphism and reproduction performance of AI bulls, Theriogenology., 52:1145-1152.
22. Neathery MW, Crowe CT, Hartnell GF, Veensuizen JJ, Reagean JO,et al. (1991) Effects of sometribove on performance, carcass composition and chemical blood characteristics of dairy calves, J. Dairy Sci., 74:3933-3939.
23. Khatib H, Monson RL, Schutzkus V, Kohl DM, Rosa GJ,et al. (2008a) Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle, Journal of Dairy Science., 91:784–793.
24. Mitra A, Schlee P, Balakrishnan CR, Pirchner F (1995) Polymorphisms at growth hormone and prolactin loci in Indian cattle and buffalo, J Anim Breed Gene., 112:71–74.
25. Unal EO, kepenek ES, Dinç h, Ozer F, Sönmez G,et al. (2015) Growth hormone (GH), prolactin (PRL), and diacylglycerol acyltransferase (DGAT1) gene polymorphisms in Turkish native cattle breeds, Turkish Journal of Zoology., 39:1-15.
26. Baklacı CT (2005) Türkiye yerli sığır ırklarının büyüme hormon geni polimorfizmi, MSc, Çukurova University, Adana, Turkey.
27. Yao J, Aggrey SE, Zadworny D, Hayes JF, Kuhnlein U (1996) Sequence variations in the bovine growth hormone gene characterized by single-strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holsteins, Genetics, 144:1809–1816.
28. Falaki M, Gengler N, Sneyers M, Prandi A, Massart S, et al. (1996) Relationships of polymorphisms for growth hormone and growth hormone receptor genes with milk production traits for Italian HolsteinFriesian bulls, J Dairy Sci, 79:1446–1453.
29. Mattos KK, Lama SND, Martinez ML, Freitas AF (2004) Association of bGH and Pit-1 gene variants with milk production traits in dairy Gyr bulls, Pesq Agropec Bras, 39:147–150.
30. Sodhi M, Mukesh M, Prakash B, Mishra BP, Sobti RC, et al. (2007) MspI allelic pattern of bovine growth hormone gene in Indian zebu cattle (Bos indicus) breeds, Biochem Genet, 30:296–299.

31. Barkema HW, Westrik JD, Keulen KASV, Schukken YH, Brand A (1994) The effects of lameness on reproductive performance, milk production and culling in Dutch dairy farms, Prev. Vet. Med, 20:249–259.

32. Green LE, Hedges VJ, Schukken YH, Blowey RW, Packington AJ (2002) The Impact of Clinical Lameness on the Milk Yield of Dairy Cows, J Dairy Sci, 85:2250-2256.

33. Zhou GL, Zhu Q, Jin HG, Suo S L (2006) Genetic variation of growth hormone gene and its relationship with milk production traits in China Holstein cows, Asian-Aust J Anim Sci, 19:315–318.

34. Rekik B, Gara AB, Hamouda MB, Hammami H (2003) Fitting lactation curves of dairy cattle in different types of herds in Tunisia, Livestock Production Science, 83:309–315.

35. Davidson TM, Elliot R (1993) Response of lactating cows to grain based concentrate in Northern Australia, Trop. Grassl. Res*,* 27, 229–237.

36. Abel H, Immig I, Gomez, CDC, Steinberg W (2001) Effect of increasing dietary concentrate levels on microbial biotin metabolism in the artificial rumen simulation system (RUSITEC), *Arch. Anim. Nutr.*, 55:371–376.

37. Seymour MS (1999) Supplemental biotin for dairy cattle, Proceedings of Tri-State Dairy Nutrition Conference.

38. Midla LT, Hoblet KH, Weiss WP, Moeschberger ML (1998) Supplemental dietary biotin for prevention of lesions associated with aseptic subclinical laminitis in primiparous cows, Am. J. Vet. Res., 59:733-738.

39. Berry SL (2001) Diseases of the digital soft tissues, Veterinary Clinics of North America: Food Animal Practice, 17:129–14.

40. Borderas TF, Pawluczuk B, Passillé AMD, Rushen J (2004) Claw hardness of dairy cows: relationship to water 87:2085-93.

41. Higuchi N, Nagataha N (2001) Relationship between serum biotin concentration and moisture content of the sole horn in cows with clinical laminitis or sound hooves, Veterinary Record., 148:209-210.

42. Bergsten C, Petterson B (1992) The cleanliness of cows tied in stalls and the health of their hooves as influenced by the use of electric trainers, Prev. Vet. Med., 13:229-238.

43. SAS User's Guide Statistics, Version 8.0 Edition (1999). SAS Inst, Inc., Cary, NC.

44. Zhang HM, Brown DR, Denise SK, Ax RL (1993a) Rapid communication, polymerase chain reaction–restriction fragment length polymorphism analysis of the bovine somatotropin gene, J Anim Sci., 71:2276.

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