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Genetic characterization of indigenous Sudanese cattle using Growth hormone (GH) and Leptin (Lep) genes

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Abstract: One hundred and fourteen blood samples collected from Kenana (32), Butana (32) and Erashy (50) ecotypes from unrelated animals in different locations. DNA extracted following standard methods. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique used to screen for DNA polymorphisms of the *Growth hormone* (*GH*) and *leptin* (*Lep*) genes.

The *ALUI* Digestion of the211bp polymerase chain reaction (PCR) products in the *GH* gene atexon 5produced two alleles, namely (L) and (V). In *GH* gene, three genotypes LL, LV, and VV indicated in this study. The frequency of LL genotypes was 100.0%, 75.0% and 90.0% for Kenana, Butana and Erashy dairy cattle, respectively.

In the *leptin* gene, the digestion ofPCR productsbetweenexon2, intron2 using Sau3Al enzyme revealed the presence of allele A and allele B. Three genotypes observed and frequencies of AA genotypes were 96.86%, 96.86% and 80.0% in kenana, Butana, and Erashy cows, respectively.

Keywords: Growth Hormone, Leptin, Genes, Butana, Kenana, Erashy.

Introduction

Genetic characterization of populations, breeds and species allows the assessment of genetic variability, which wasa crucial element in determining breeding strategies and genetic conservation programs [1]. More recently, selection pressure for productivity has increased through the application of methods in quantitative genetics, with little regard for the preservation of genetic diversity [2]. Molecular markers have been widely used to access this variability since they provide information on every region of the genome, regardless of the level of gene expression. Variations at DNA level contribute to the genetic characterization of livestock populations and this may help to identify possible hybridization events as well as past evolutionary trends [3]. Such variations in DNA may also be associated with, or Linked to, economic traits, which governed by many genes each having a small effect [4]. Allelic variation in the regulatory and structural regions of candidate genes may influence diversification of milk yield and composition. For direct genotyping for candidate genes using polymerase chain reaction (PCR) [5], molecular markers that reveal polymorphism at the DNA level are now key players in animal genetics. Recently, a number of potential candidate genes have recognized.

Growth hormone is a peptide hormones synthesized by lactotropes of anterior pituitary. Well known that it plays an important role in biological processes such as mammary development, lactation, growth and metabolism regulation, being therefore a promising candidate gene marker for improving milk and meat production in cattle (citation). Bovine growth hormone gene *(GH)* is located in chromosome 19 [6] and consists of five exons separated by four introns [7, 8]. Several polymorphisms identified in the *GH* gene [9] and [10]found a polymorphic site for *Msp*I restriction endonuclease, while [11] localized the polymorphism in intron 3 of the gene *GH*.[8][12]reported that polymorphism of *growth hormone* gene occurred at.2141C>G (*Alu*I restriction, Leu/Val substitution in exon 5)of the sequence. Substitution of C for G nucleotide at that position caused an amino acid change from leucine to valine of the growth hormone polypeptide identified by *Alu*I restriction enzyme. Several polymorphisms were identified in the *growth hormone* gene and detected by restrictionfragment length polymorphism (RFLP) with the recognition site of *Alu*I restriction enzyme [13, 14, and 15].

Leptin is a protein, which is involved intricately in the growth and metabolism of animals it plays an important role in the regulation of feed intake, energy metabolism, growth, and reproduction *Leptin* gene is located on chromosome 4. It consists of 3 exons and two introns [16]. In cattle, *Leptin* is involved in regulation of feed intake, fetal growth, energy balance, fertility, and immune functions [17]. Leptin has a role in the onset of puberty and the sexual development [18]. It stimulates the reproductive system in both sexes through an increased release of the pituitary luteinizing hormone and the hypothalamic gonadotropin-releasing hormone

[19]. Leptin circulates in blood serum in both free and bound forms [20]. The free form is the biologically active form, while the other is bound to a carrier protein. The balance between free and bound leptin is a potential regulator of leptin bioavailability [21].

The aim of this Study was to investigate the allele and genotype frequencies of *Growth hormone* and *leptin* gene Polymorphism in Sudanese native cattle breeds.

Martials and methods

Sampling locations

One hundred and fourteen blood samples collected on filter papers from three local Sudanese cattle ecotypes(Kenana 32, Butana 32 and Erashy 50). The samples collected from homelands of the three ecotypes in central and eastern parts of the Sudan. Kenana cattle are found mainly in Sennar and Blue Nile States spreading in the area between the White and Blue Nile Rivers. This is roughly a triangular area bounded by Sennar, Singa and Kosti towns and lying approximately between latitudes 10º and 13º North longitudes 32º and 34º East. Butana cattle named after their homeland, the Butana plains of central Sudan lies between the Nile, Atbara and the Blue Nile Rivers. On the other hand, the Erashy cattle mostly found in Al-gash area in Gedaref, Kassala and Red sea States and raised by the Hadandowa tribe.

Isolation of genomic DNA

DNA isolated from the blood collected on the FTA papers (Whatman International Ltd, UK.) was done by (anon-enzymatic method – phenol precipitation) as described by [22].

Genotyping:

The *Growth hormone* (*GH*) locus analyzed using a 211 bp fragment covering the sequence containing the mutation site. Itamplified according to the procedure proposed by [23], carried out in the Central Laboratory of the Ministry of Science and Communication with forward primer: 5´- GCTGCTCCTGAGGGCCCTTCG -3´ and *Reverse*: 5´- GCGGCGGCACTTCATGACCCT -3`. The amplified PCR productdigested with *Alu*I restriction endonuclease at Temp 37 Cº for 16 hours to distinguish between L and V alleles. (The 20µl PCR reaction consisted of 2.5 µl genomic DNA, 10X PCR buffer, 0.75µl MgCl2, and 0.5µl dNTPs. A standard protocol was performed starting at 95 ºC for 4 min, followed by 1 cycles of 94 ºC for 20 sec, primer annealing 59 ºC for 30 sec, followed by 35 cycles 72 ºC for 30 s, and a final extension at 72 ºC for 4 min, followed by 1 cycles.)The nucleotide variation underlying the *Leptin* genediagnosed by an RFLP assay. Genomic DNA was genotyped for the locus responsible for the SNP in exon 5 according to [24] using forward primer: 5´-TGGAGTGGCTTGTTATTTTCTTC-3´ and reverse primer 5´- GTCCCCGCTTCTGGCTACCTAAT -3´.The PCR conditions were at 95°C for 5 min, followed by 30 cycles of94°C for 30 s, 62°C for 40 s and 72°C for 40 s. After 30 cycles,reactions completed by an extension at 72°C for 7 minutes.The PCR product for each sample digested with 10 units of*Sau3AI* at 37°C overnight. One RFLP in the intron between twoexons of the bovine leptin gene detected. There were two*sau3AI* sites in 422 bp fragments. The digested *AA* PCR productexhibited two fragments of 390 and 32 bp For the *BB* genotypeexhibited 303, 88 and 32 bp (only 303 bp fragments were visible inagarose gel)

Statistical analysis

The genotypic and allelic frequencies for the *GH* and *Lep* genescalculated based on the counting method [25]. To determine, if the population was in equilibrium for HardyWeinberg, theobserved and expected genotypic frequencies, was calculated using Chi-square test.

Result and discussion

The 211 bp DNA fragment of the *growth hormone* gene (*GH*) which is spanning from the forth intron to the five exon was amplified using the PCR method and digested with *AluI* restriction enzyme and identified polymorphism at this locus. The digestion with *AluI* restriction enzyme showed two types of restriction patterns. The first pattern was assigned as genotype LL which produced (159 and 52 bp fragments), and the second pattern was assigned as genotype LV which produced (211, 159, 52 bp fragments) as presented in Figure 1 and 2. Two genotypes LL and VV were observed in Butana (75% LL, 25% VV), while the Erashy cattle had 90% of the LL genotypes and 10% LV genotypes. Kenana cattlefound monomorphic for this locus and producing only LL genotypes (100%).

Figure 1

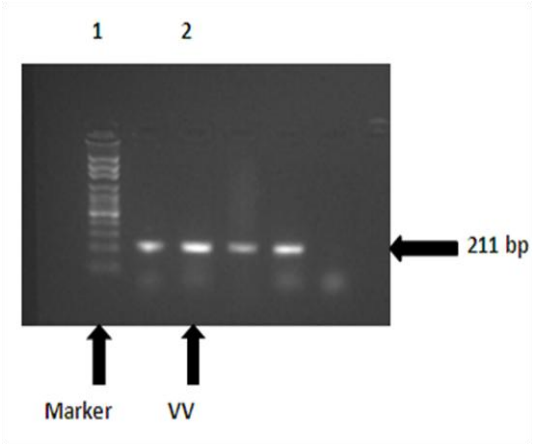
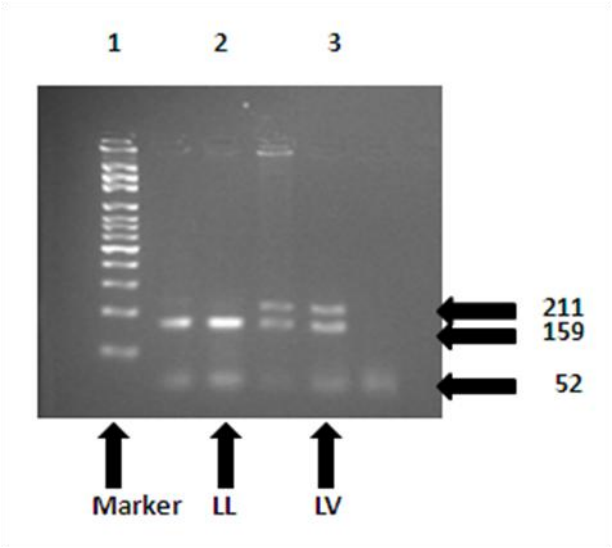


Figure 2



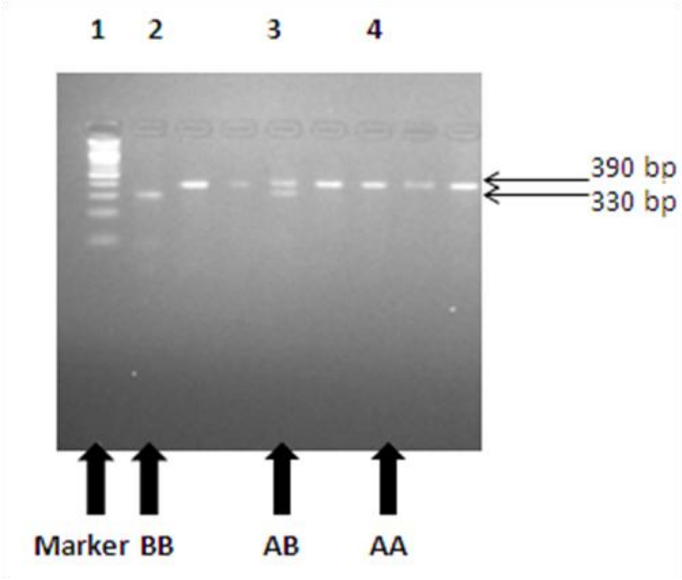
Allele Frequency			Genotype Frequency			
Breed	L	V	LL	LV	VV	NO of animal
Kenana	100%	0.00%	100%	0.00%	0.00%	32
Butana	75%	25%	75%	0.00%	25%	32
Ershay	95%	5%	90%	10%	0.00%	50
Total	91%	9%	89	4%	7%	114

Table 1: Genotype and allele frequencies of *GH* gene in Butana, Kenana and Erashy.

There was significant deviation From Hardy-Weinberg equilibrium for the *GH* – *Alu*ILocus[26].Showed that all Kenana and Butana cattle were homozygous for SNP g.2141C allele (Leu variant) in exon 5)the important SNP g.2141C>G (*Alu*I restriction, *Leu\Val* substitution in exon 5) as reported in the literature was genotypedby[27].This result is in contrast with ours because thegenotypes LL in the *GH* gene is the predominant genotype in allSudanese cattle ecotype, with frequencies ranging from 75% to 100%. Kenana's monomorphic genotype for GH gene is correlated with other zebu (*Bos indicus*) breeds (Nelore, Gyr, Guzerá) [28].The disequilibrium in the *GH* locus from Hardy Weinberg may reflect a series of events such as inbreeding, selection, genetic drift or population subdivision. The latter event could also attributed to the result from sampling few individuals from each location, although this expected to affect more than one locus. [1] concluded that it is difficult to indicate the most desirable *GH/Alu*I- genotypeand that introducing the information on *GH/Alu*I- genotype into dairy cattle marker-assisted selection (MAS) programs would probably be risky Based on the results presented here it is difficult to indicate the most desirable *GH/Alu*I-genotype. In relation to milk composition, *VV*/++ cows produced milk withthe highest F+P (fat and protein percent pooled) content. However, the small samplesize (only 23 *VV*/++ cows) does not allow drawing definite conclusions. It concludedthat introducing the information on *GH/Alu*I- genotype into dairy cattlemarker-assisted selection (MAS) programs would probably be risky. *A.* [29]results of the SNP g.2141C>G (AluI, Leu/Val substitution in exon 5) genotyping showed that all Kenana and Butana cattle were homozygous for g.2141C allele (Leu variant).

There is a considerable interest in leptin gene polymorphisms because of their potential use as genetic markers to improve the efficiency of selection for quantitative traits. [30]Suggested an association between leptin and feed intake.These results revealed that polymorphism detected in all studied herds and showed that *PCR-RFLP* is an appropriate tool for detecting genetic polymorphism. Digestion of polymerase chain reaction produce with Sau3Al/ leptin gene reveled presents of allele A was (390,32bp) fragments and allele B was (303, 88 and 31bp) only 303bp fragment visible on the gel Figure 3. Three patterns were observed and frequencies were AA (96.86%), BB (3.14%) in kenana ecotype and AA (96.86%), AB (3.14%) in Butana ecotype while Erashy ecotype was AA (80%) and AB (20%) genotypes.

Figure 3



Allele Frequency			Genotype Frequency			
Breed	A	B	AA	AB	BB	NO of animal
Kenana	96.9%	3.1%	96.9%	0.0%	3.1%	32
Butana	96.9%	3.1%	96.9%	3.1%	0.0%	32
Ershay	95%	5%	80.0%	20.0%	0.00%	50
Total	94.3%	5.7%	89.5%	9.6%	0.9%	114

Table 2: Genotype and allele frequencies of leptin gene in Butana, Kenana and Erashy.

[31]Stated that for the Mbo1-RLFP on the *lep* locus, the frequencies in allele A were 97.50% and 97.06% in Butana and Kenana breeds' respectively. However, both breeds showed a complete absence of homozygous BB carriers, while our present study found a 3.14% BB in Butana cattle.
Pvalue= 0.009
[32] verified that the frequency of a restriction fragment length polymorphism (*Sau*3AI) in bovine *LEP* gene was different between *Bos taurus* and *Bos indicus*, being possible that genotype differences in leptin could explain some of the phenotypic variation observed between –
breeds of cattle. [33] detected higher frequency of A allele in
Bos indicus breed group of cattle compared with *Bos taurus*.The study of [34]) on polymorphism within the intron region of the bovine leptin gene in Iranian Sarabi cattle (Iranian *Bos taurus*) showed that the *A* allele was frequent in the population.Other research on *leptin* gene polymorphism using single strand conformation polymorphism showed that Indian Sahiwal cattle exhibited a high genetic variability in the entire leptin gene [35]. Furthermore, [5] observed three genotypes: *AA* (60.71%), *AB* (37.5%) and *BB* (1.79%), in *leptin* gene polymorphism of Iranian Holstein cattle and suggested that this polymorphism could be further evaluated for marker assisted selection.
[24] reported that heifers with the Sau3AI-AB genotype produce 1.32 kg/d more milk and consume 0.73 kg/d more food compared with the Sau3AI-AA genotype [25].

Conclusion

It must be pointed out that, frequency of any allele can be altered simply by mating strategies in different herds based on economic and population demands. Unfortunately, phenotypic records were not available in our study to indicate desired allele and genotype**.**

References:

[1]. **Vasconcellos, L.P.M.K; Tambasco, D.D.; Talhari, A.; Pereira, P.; Coutinho, L.L. and Regitano, L.C.A. (2003).**Genetic charaterization of Aberdeen Angus cattle using molecular markers.Genet. Mol. Biol. 26:133-137.

[2]. **Marson**, **E.P., Ferraz, J.B. Meirelles, F.V. Balieiro, J.C. Eler, J.P. Figueiredo, L.G. and Mouro, G.B. (2005)**. Genetic characterization of European-Zebu composite bovine using RFLP markers. Genet. Mol. Res., 4: 496-505.

[3]. **Choudhary,V.; Kumar, P.; Bhattacharya, T. ;Bhushan, B.and Sharma, A. (2005**). DNA polymorphism of leptin gene in Bos indicus and Bos tauruscattle, Gen. Mol. Biol. 28(4): 740-742.

[4]. **Gelderman, H., (1997)**. Investigations on inheritance of quantitative characters in animals by gene markers 1 Methods. Theoretical and Applied Genetics, 46**,** 319–330.

[5]. **Sharifzadeh, A. and Doosti, A.(2010)**. Genetic polymorphism at the leptin gene in Iranian Holstein cattle by PCR-RFLP. African Journal of Microbiology Research, 4**,**1343–1345.

[6]. **Hediger, R.; Johnson, S.E.; Barendse, W.; Drinkwater, R.D.; Moore, S.S.,and Hetzel, J., (1990).** Assignment of the growth hormone gene locus to 19q26-qter in cattle and to 11q25-qter in sheep by in situ hybridization. Genomics8, 171-174

[7]. **Woychick, R.P.; Camper S.A.; Lyons, R.H.; Horwits, S.; Goodwin, E.C. and Rottmanf, M. (1982)** Cloning and nucleotide sequencing of the bovine growth hormone gene. Nucleic Acid Research 10, 7197-7210

[8]. **Gordon**, **D.F.; Quick, D.P.; Erwin, C.R.; Donelson, J.E., and Maurer. R.A.(1983).**Nucleotide sequence of the bovine growth hormone chromosomal gene. Mol. Cell. Endocrinol. 33: 81-95

[9]. **Cowan, C.M.; Dentine, M.R.; Axr, L. and Schuler, L.A. (1989)** Restriction fragment length polymorphism associated with growth hormone and prolactin genes in Holstein bulls: evidence for a novel growth hormone allele. Animal Genetics20, 157-165.

[10]. **Hilbert, P.; Marcotte, A.; Schwers, A., Hanset, R.; Vassart, G. and Georges, M., (1989).**Analysis of genetic variation in the Belgian Blue Cattle breed using DNA sequence polymorphism at the growth hormone low density lipoprotein receptor, á-subunit of glycoprotein hormones and thyroglobin loci. Animal Genetics20, 383-394.

[11]. **Zhang, H.M.; Maddock, K.C.; Brown, D.R.; Denise, S.K. and Axr, L. (1993)**.A novel allele of the bovine somatotropin gene detected by PCR-RFLP analysis. Journal of Animal Science71, 2276

[12]. **Lucy, M.C.; Hauser, S.D.; Eppard, P.J.; Krivi, G.G.; Clark, J.H.; Bauman, D.E. and Collier, R.J. (1993**). Variants of somatotropin in cattle: Gene frequencies in major dairy breeds and associated milk production. Domestic Animal Endocrinology, 10, pp 325-333.

[13]. **Biswas, T.K.; Bhattacharya, T.K.; Narayan, A.D.and Badola, S.**(**2003**). Growth hormone gene polymorphism and its effect on birth weight in cattle and buffalo. J. Anim. Sci. 16: 494-497.

[14]. **Aruna, P.; Chakravarty, A.; Bhattacharya, T.and Joshi, B.** (**2004).** Detection of polymorphism of growth hormone gene for the analysis of relationship between allele type and growth traits in Karan Fries cattle. J. Anim. Sci. 17: 1334-1337.

[15]. **Mu'in, M.A.; Astuti, M. ; Muladno, T.M.; Murti, dan Artama**, **W.T.** (**2007).** Polimorfisme Gen Growth Hormone dan Hubungannya dengan Sifat Pertumbuhan Sapi Silangan Peranakan Ongole dan Simmental. Anim. Prod. 9(2): 53-58.

[16]. **Zadworny, D.and Kuhnlein, V. (1990).** The Identification of the Kappa CaseinGenotype in Holstein Dairy Cattle Using Polymerase Chain Reaction,Theor. Appl. Genet., 80: 631-634.

[17]. **Glaum, S. R.; Hara, M. ; Bindokas, V. P., Lee, C. C.;Polonsky, . K. S.; Bell, G. I. and Miller,R. J.(1996)**. Leptin, the obese gene product, rapidly modulate synaptic transmission in the hypothalamus. Molecular Pharmacology, 50**,** 230–235.

[18]. **Hoggard, N.; Haggarty, P.; Thomas, L.and Lea, R. G. (2001)**. Leptin expression in placental and fetal tissues: Does leptin have a functional role? Biochemical Society Transactions, 29**,** 57–63

[19]. **Barash, I., Cheung, C., Weigle, D., Ren, H., Kabigting, E., Kuijper, J., Clifton, D. and Steiner, R. (1996).** Leptin is a metabolic signal to the reproductive system. Endocrinology, 137(7), pp.3144-3147.

[20]. **Mantzoros, C. S. and Moschos, S. J. (1998).**Leptin: In search of role(s) in human physiology and pathophysiology. Clinical Endocrinology, 49**,** 551

[21]. **Lahlou, N.; Clement, K.; Jean-Claude, C.; Vaisse, C.; Lotton, C.; Le Bihan, Y.; Basdevant, A.; Lebouc, Y.; Froguel, P.; Roger, M. and Guy-Grand, B. (2000)**. Soluble leptin receptor in serum of subjects with complete resistance to leptin. Diabetes, 49, 1347–1352.

[22]. **Lahiri DK and Nurnberger JI(1991).** A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nuc Acid Res, 19;5444.

[23]. **Schlee, P.; Graml, R.; Rottmann, O.and Pirchner, F.(1994).** Influence of growth hormone genotypes on breeding values of Simmental bulls. Journal of Animal Breeding and Genetics111, 253-256.

[24]. **Liefers, S.C.and Veerkamp, R.F. (2002).** Association between Leptin gene polymorphism and production, live weight energy balance feed intake and fertility in Holstein heifers, J. Dairy Sci., 85: 1633-1638.

[25]. **Falconer, D. S. and Mackay, T. F. C.** (**1996**).Introduction to Quantitative Genetics, Ed. 4.Longman, Harlow, Essex, United Kingdom.

[26]. **Musa L. M. A., Reissmann M., Ishag I. A., Rahamtalla Brockmann G. and PetersK.(2013).**Characterization of the Growth Hormone Gene(GH1) in Sudanese Kenana and Butana Cattle Breeds **J.** Anim. Pro, Adv 3(2):28-34.

[27]. **Yao, J.; Aggerey, S.E.; Zadworny, D.; Hayes, J.F. and 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, pp 1809-1816.

[28]. **Kemenes, P.A.; Regitano, L.C.A; Rosa, A.J.M.; Packer, I.U.; Razook, A.G.; Figueiredo, L.A.; Silva, N.A.; Etchegaray, M.A.L. and Coutinho, L.L. (1999).** k-Casein, b-lactoglobulin and growth hormone allele frequencies and genetic distances in Nelore, Gyr, Guzerá, Caracu, Charolais, Canchim and Santa Gertrudis cattle. Genet Mol. Biol. 22:539-541.

[29]. **Dybus, A.** (**2002**). Associations between Leu/Val polymorphism of growth hormone gene and milk production traits in Black-and-White cattle. Archives of Animal breedin. Tierz., Dummerstorf 45 5, pp 421-428.

[30]. **Lagonigro RPW, Pilla F, Woolliams JA(2003)**. A new mutation in the coding region of the bovine leptin gene associated with feed intake.Anim. Genet., 34(5): 371-374.

[31]. Rahamtalla, .S.A. (2010). Identification of genetic variants influencing milk production traits and somatic cell score in dairy cattle dissertation Humboldt, Universitatzu Berlin.

[32]. **Pomp, D. Zou T, Clutter A C, Barendse W (1997).** Mapping of leptin to bovine chromosome 4 by linkage analysis of a PCR-based polymorphism.J. Anim Sci 75:1427.

[33]. **Rasor C.C,Thomas M.G.,Enns R.M.,Williams G.L.,Stanko R.I.,Randel R.D.,Rios J.(2002).** Allelic and genotypic Ferquencies of the Leptin Gene Sau3AI-Restriction Fragment Length Polymorphism and Evaluation of its Association with age of puberty in cattle in the South Western United States and Northern Mexico .The professional Animal scientist, 18; 141-146.

[34]. **Javanmard, A., M. R. Mohammadabadi, G. E.Zarrigab Gharahedaghi, M. R.Nassiry, A. Javadmansh & N. Asadzadeh,(2008).** Polymorphism within the intron regionof the bovine leptin gene in IranianSarabi cattle (Iranian Bos taurus). RussianJournal of Genetics, 44,495–497.

[35]. **Dubey, P. P., A. Sharma, D. S. Gour, A.Prashant Jain, C. S. Mukhopadhyay, A.Singh, B. K. Joshi & D. Kumar, 2008.**Leptin gene polymorphism in Indian Sahiwalcattle by single strand conformation polymorphism (SSCP). South AfricanJournal of Animal Science, 38, 131–135.