

The Allele and Genotype Frequencies of Bovine Pituitary Specific Transcription Factor and Leptin Genes in Jordanian Cattle Population by Using PCR-RFLP

¹Khaleel I. Z. Jawasreh, ²Faisal Awawdeh, ³Ibrahem Rawashdeh, ¹Faiq Hejazeen and
¹Miassar Al-Talib

¹Director of Livestock and Range Land Research Directorate. National Center for Agricultural
Research and Extension (NCARE) /Al-Baqa, Jordan

²Director General of NCARE

Abstract: A total of 81 cows from Holstein Friesian and local breeds were genotyped for the *PIT-1* *HinfI* and Leptin *BFUC1* polymorphisms by the polymerase chain reaction and fragment length polymorphism (PCR-RFLP). *PIT-1* gene frequencies were 0.15 and 0.85 for A and B alleles, respectively. Genotypic frequencies of AA were low (0.04 and 0.00) in the two studied breeds while for BB frequencies were high (0.69 and 0.82). Leptin Gene frequencies for the two alleles were 0.775 for A allele and 0.225 for B allele. Heterozygosity percentages were low in the two studied genes ranged between 0.255 for Pit-1 gene and 0.348.

Key words: Holstein Friesian, Local cow Breed, Gene frequency and PCR- RFLP

INTRODUCTION

In Jordan, Holstein Friesian is the most dominant breed of cows because of its high milk production compared with the native breed, the total number of Friesian cows is 68245 heads compared with only 3199 heads of native cows (M.O.A., 2007). This number of dairy cattle covers only 50% of Jordan requirements from milk that obligate all the scientists to look for solutions for improving the productivity of dairy cattle.

Genetic progress in populations depends on statistical methodologies to reveal how the properties observed in populations are influenced by properties of the genes involved and by non- genetic circumstances that might affect a metric trait (Falconer, D. and F. Mackay, 1996).

Marker assisted selection (MAS) can be achieved by detection of polymorphisms and thereafter the alleles that can be identified and may related to production traits.

Pit-1 and *Lepin* genes are among the genes involved in mammalian productivity. The specific pituitary transcription coded by *Pit-1* gene was mapped to *Bos Taurus* chromosome1 (Moody, D.E., D. Pomp, 1995) and belongs to a group of genes that code for proteins involved in animals development, *Pit-1* gene, is required for the initiation of the expression of the growth hormone releasing factor gene, and is involved in the maintenance of the expression of the gene coding for the β subunit of thyroid stimulating hormone. Expression of prolactin and growth hormone genes are activated by *pit-1* gene.

The *leptin* gene is located on chromosome 4 (Pomp, D., T. Zou, 1997) and consists of three exons. Genetic differences in the *leptin* gene were first observed in mice; ob/ob mice lack functional *leptin* and are hyperphagic, obese, and infertile (Hamann, A. and S. Matthaai, 1996). Several alterations in the *leptin* gene have been found in cattle. Restriction fragment length polymorphism (RFLP) of the bovine leptin gene was reported (Lien, S., H. Sundvold, 1997).

Leptin gene influences milk performance in cattle and reproduction in beef cattle (Liefers, S.C., R.F. Veerkamp, 2002). *Sau3AI*-AB Cows produced 1.32 kg/d more milk and consumed 0.37 kg/d more food compared with the *Sau3AI*-AA genotype (Liefers, S.C., R.F. Veerkamp, 2002). Komisarek (Komisarek, J. and Z. Dorynek, 2005) reported that *Arg4Cys* TT genotype has a highly significant positive effect on milk and protein yield. In the Polish Black and White cattle the C allele (*Sau3AI*) was related to fat and protein content (Zwierzchowski, L., J. Krzyzewski, 2002). Buchanan (Buchanan, F.C., A.G. Van Kessel, 2003) demonstrated a strong influence of the *Kpn2I* alteration (the TT genotype) on milk and protein yield. Heravi (Heravi Moussavi, A., M. Ahouei, 2006) demonstrated that the RFLP B-allele of *leptin* gene can yield a higher 305-d milk production with a trend to better reproductive performance in Iranian Holstein cows.

Corresponding Author: Khaleel I. Z. Jawasreh, Livestock and Range Land Research Directorate. National Center for
Agricultural Research and Extension (NCARE) /Al-Baqa, Jordan
E-mail: tamkhajaw@yahoo.com

This the first study to be conducted in Jordan, the objectives were to estimate allele and genotype frequencies and heterozyosity after identification of *HinfI Pit-1* and *BFUCI leptin* Polymorphisms in Jordanian Local and Friesian cows.

MATERIALS AND METHODS

Blood samples were collected from Friesian and Local cows. The animals located in different governorates in Jordan (Irbid, and Madaba for the Friesian and Jordan Valley for Local Breed samples). A total of 81 (45 Friesian and 36 Local samples) samples were collected from tail vein of adult cows in 5 ml EDTA tubes and stored in the refrigerator (-20 °C) until Lab. work.

DNA Extraction:

The frozen blood was thawed under room temperature and EZ -10 spin column kit (Bio basic) was used for DNA extraction and according to the company procedure. Quality and quantity of DNA were measured by spectrophotometer by taking the optical density at wave length of 260-280 nanometer (n.m).

PCR- RFLP Analysis:

The sequences of the forward and reverse primers for the amplification of the *Pit-1* gene were:

Pit-1 F 5'- GAGCCTACAT GAGACAAGCATC-3'

Pit-1 R5'-AAATGTACAATGTGCCTTCTGA-3'

The reaction mixture of PCR was performed by using 12.5 µl of master mix (Promiga Madison WI USA) with 1 µl of each of forward and reverse primer and 1 µl of genomic DNA and the volume was completed to 25 µl (tube volume) by sterilized water. The mixture was placed in thermal cycler (*XP Cycler Bioer*) the conditions of the thermal cycler was: the initial denaturation temperature step at 95°C for 2 minutes followed by 30 cycles of 95°C for 45 second, 58°C for 1 minute, 72°C for 1 minutes and a final extension at 72°C for 3 minutes.

The polymerase chain reaction product was digested by 7 unites *Hinf I* (*BioLab*) at 37°C over night.

The sequences of the forward and reverse primers for the amplification of the *second intron*

Leptin gene were:

Lep F: 5'-TGGAGTGGCTTGTTATTTTCTTCT-3'

Lep R:5'-GTCCCCGCTTCTGGCTACCTAACT-3'

The reaction mixture of PCR was performed by using 12.5 µl of master mix (Promega Madison WI USA) with 1 µl of each of forward and reverse primer and 1 µl of genomic DNA and the volume was completed to 25 µl (tube volume) by sterilized water. The mixture was placed in thermal cycler (*XP Cycler Bioer*). The conditions of the thermal cycler were: the initial denaturation temperature step at 94°C for 2 minutes followed by 35 cycles of 94°C, 60°C and 72°C (each for 1 minute) and final extension at 72°C for 15 minutes. The polymerase chain reaction product was digested by 7 unites *BFUCI* (*BioLab*) at 37°C over night.

Statistical Analysis:

The allele and genotype frequencies were estimated by direct counting. The heterozygosities (as gene variation indicates) were calculated using the POPGENE software version 1.31 (Yeh, F.C., R. Yang, T. Boyle, 1999), according to Nei (1978) procedure.

RESULTS AND DISCUSSION

The amplification of *Pit-1* gene, in PCR produce 600 bp band while the digestion of this product by *HinfI* enzyme produce three genotypes, AA exhibited one fragment of 600bp and the PCR product cleaved into two fragment (600 bp and 357 bp) to produce AB genotype while the BB genotype appears in the gel in two fragments (357 and 243bp) (Figure 1).

The PCR product of *leptin* gene is 442bp. Results on *leptin* polymorphisms by *BFUCI* enzyme shows two alleles (A and B) and three genotypes (AA, AB and BB) the PCR product cleaved into two fragments of 390 and 303bp to produce AB genotypes, while AA and BB genotypes with one band of 390 & 303bp, respectively (Figure 2).

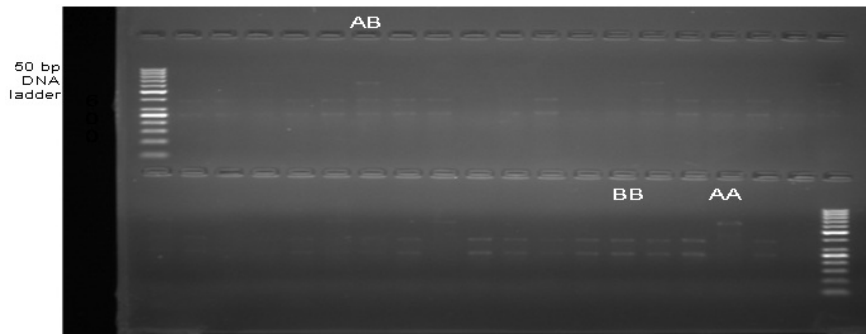


Fig. 1: Analysis of *HinfI* polymorphism at the bovine *Pit-1* gene: electrophoretic patterns of three genotypes separated on 2% agarose gel. L M is 50bp DNA marker.

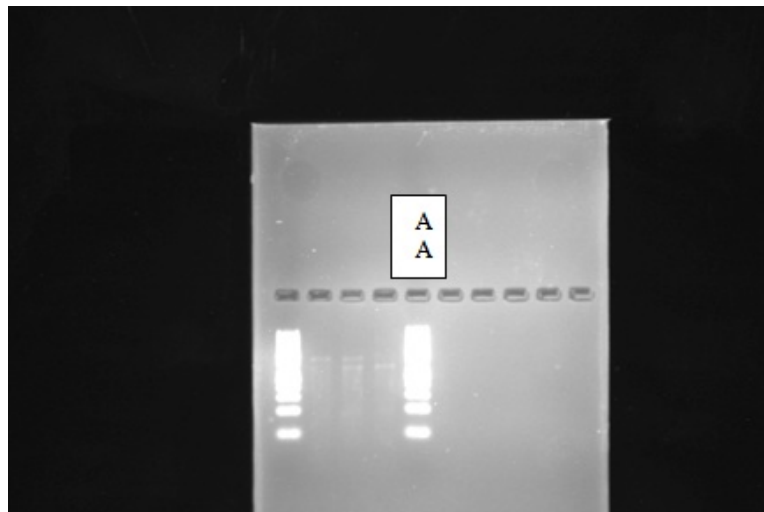


Fig. 2: Analysis of *HinfI* polymorphism at the bovine *Pit-1* gene: electrophoretic patterns of three genotypes separated on 2% agarose gel. L M is 50bp DNA marker.

Similar findings were obtained previously (Zwierzchowski, L., J. Oprzadek, 2001; Woollard, J., C.B. Schmitz, 1994; Javanmard, A., A. Nader, 2005) who observed those digested fragments in the gel for the two studied genes.

Similar findings were obtained by many workers who detect the fragments after digestion (Zwierzchowski, L., J. Krzyzewski, 2002; Woollard, J., C.B. Schmitz, 1994; Javanmard, A., A. Nader, 2005).

The overall mean of allelic frequency for *Pit-1* gene in the studied populations were the highest in BB (0.733) genotype, while it was too low (0.0333) for AA genotype (table 1) this figure was the opposite in *leptin* gene which was 0.623 and 0.07 for AA and BB genotypes (table 2).

The *pit-1* B allele frequency was observed to be the highest (0.85) in the studied population (table1). Friesian cows have frequency of 0.825 while it was 0.91 in local cows. The obtained results for *Pit-1*-B allele frequency were higher than the frequencies obtained (Zwierzchowski, L., J. Oprzadek, 2001) who studied different breeds of *Bos Taurus* (Angus (0.55), Holstein (0.74), Hereford (0.79), Brahman (0.75), Polish (0.75) and Gry cattle (0.05)) but lower than Glbvieh (0.9) breed. The frequencies of *Pit-1* B allele in Iranian cattle were estimated to be lower than the frequencies obtained through this study for Jordanian cattle (Javanmard, A., A. Nader, 2005).

Table 1: Allelic and genotypic frequencies of *Pit-1* Gene in Jordanian Friesian and Local Cows

Population	Genotypic Frequency			Allelic Frequency		heterozygosity
	AA	AB	BB	A	B	
Total population frequency	0.0333	0.233	0.733	0.15	0.85	0.255
Friesian	0.046	0.255	0.697	0.1744	0.8255	0.288
Local	0.000	0.176	0.8235	0.0882	0.9118	0.160

(Woollard, J., C.B. Schmitz, 1994) were the first who detect the RFLP polymorphisms within the bovine *Hnf1 Pit -1* gene and (Sabour, M.P., C.Y., Lin, 1996) found that A allele in *Pit -1* locus positively affected milk production traits in Friesian cattle and they indicate significant superiority of allele A over allele B for milk and milk protein yields and body confirmation traits.

Leptin gene genotypes were variable in the studied population; it ranged between 0.07 for BB and 0.623 for AA genotypes, respectively. Similar frequencies were found between the two studied Jordanian breeds (table 2).

Leptin B allele frequency was 0.225 in Jordanian cattle, which was lower than *leptin A* allele. Many Authors observed B allele to be lower than A allele (Buchanan, F.C., A.G. Van Kessel, 2003; Madeja, Z., T. Adamowicz, 2004; Choudhary, V., P. Kumar, 2005; Heravi Moussavi, A., M. Ahooui, 2006; Nassiry, M.R., A. Heravi Moussavi, 2005).

Table 2: Allelic and genotypic frequencies of *Leptin* Gene in Jordanian Friesian and Local Cows

	Genotypic Frequency			Allelic Frequency		heterozygosity
	AA	AB	BB	A	B	
Total frequency	0.623	0.303	0.07	0.775	0.225	0.348
Friesian	0.619	0.309	0.071	0.774	0.226	0.346
Local	0.629	0.296	0.074	0.777	0.223	0.3464

Pit-1 A allele is favorable (Sabour, M.P., C.Y., Lin, 1996), while *leptin B* allele is preferred over A allele (Pomp, D., T. Zou, 1997; Almeida, S.E.M., E.A. Almeida, 2003; Javanmard, A., A. Nader, 2005; Nassiry, M.R., A. Heravi Moussavi, 2005). The genotype and gene frequencies from six Iranian cattle populations were determined for *Pit-1 Hinf1* and *leptin Sau3A1* by PCR-RFLP, the highest frequencies of allele B (0.875) for the leptin gene and allele A (0.921) for the *Pit-1* gene were found in Dashtiyari and Sistani cattle, respectively. The highest AB genotype frequencies were found in the Taleshi and F1 Golpayegani x Brown Swiss cross for the leptin and *Pit-1* genes, (0.757, 0.769), respectively. The highest and lowest heterozygosities were found in Talehest and Dashtiyari cattle for the leptin gene and in F1 Golpayegani x Brown Swiss cross and Sistani cattle for the *Pit-1* gene, respectively (Javanmard, A., A. Nader, 2005).

The heterozygosity and genetic variability (tables 1 and 2) results in both cattle breeds for the two studied genes indicated the low variation that may result from high inbreeding rate. It's suggested to adopt some strategies such as migration, introduction of new diversity and cross breeding.

The low AB genotypes (the favorable genotype) frequency suggests to use cross breeding with known bull genotypes that increased heterozygosity in both cattle breeds, and to adapt the genotyping method for both sexes in early ages as Marker assisted selection (MAS) method and also for the imported bull semen that must be genotyped before using in artificial insemination (as mating for targeted genotype) process.

The above mentioned strategies should be adopted as a normal procedure that may produce high milk producers (cows) in the next generations.

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