DETECTING THE MOLECULAR ASSOCIATIONS OF PROLACTIN GENE GENOTYPES WITH MILK TRAITS IN FRIESIAN AND BALADI COWS USING *RFLP* TECHNIQUE

M.H. Khalil[1], M.M. Iraqi[1], M.H. Mohamed[2] and S.M. Zahed[2]

1-Department of Animal Production, Faculty of Agriculture at Moshtohor, Benha University, 13736 Tukh, Kalyoubia Governorate, Egypt. 2-Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. Corresponding author: maher.khalil@fagr.bu.edu.eg
ORCID profile: https://orcid.org/0000-0002-6929-3011

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SUMMARY

Four years of experimental work were conducted applying PCR-RFLP to detect the SNP polymorphic associations of the prolactin candidate gene (PRL) with milk traits in cattle. A total of 494 lactations for 180 milking cows of Friesian in Elkarda herd (FK) and Sakha herd (FS) and Baladi cows in Elserw herd (BS) were used. The PCR products were digested using *NaeI and SmaI restricted enzymes* to investigate the molecular associations of the PRL gene genotypes with milk traits.

The highest expected numbers of alleles (NE) in CC, CD and DD genotypes of the PRL gene were recorded in the DD genotype in both Friesian and Baladi herds, while the lowest expected numbers were recorded in CC genotype in all herds. The highest NE in GG, GT and TT genotype was in the TT genotype and the lowest NE was in the GG genotype in all herds. PRL gene was not in Hardy-Weinberg Equilibrium in all herds. The allelic frequency for the D allele was much higher than the frequency of the C allele, while the frequency of the T allele was higher than the frequency of the G allele.

The observed and expected values of heterozygosity for the PRL gene were mostly moderate in the three herds studied. The values of polymorphic information content (PIC) were mostly moderate in all herds. The values of fixation index (reduction in heterozygosity due to inbreeding, FIS) were high in the FK herd (0.82), FS herd (0.62) and BS herd (0.94).

The molecular associations between PRL gene genotypes and milk traits indicate that the CD genotype of the PRL gene has strong molecular associations with yields of milk, fat and protein relative to CC and DD genotypes. GG genotype was significantly associated with all milk traits relative to GT and TT genotypes.

Keywords: Friesian and Baladi cows, PRL gene, PCR-RFLP, polymorphic associations, milk yield and its compositions

INTRODUCTION

In the last two decades, some genes are used as potential candidate genes associated with milk traits in Marker Assisted Selection (MAS) and genomic selection (GS) in dairy cattle. With the advancing of molecular techniques in terms of availability and costs of genotyping, GS has become more affordable (Raschia *et al.*, 2018). According to genome-wide association studies (GWAS), milk traits are affected by several candidate genes and these genes could be used in GS. Among the various candidate genes, prolactin gene (PRL) seems to be promising, because it has diverse biological functions such as water and electrolyte balance, growth and development, immunity traits and reproductive functions and it plays crucial roles in mammary gland development, milk secretion and initiation and maintenance of lactation (Khatami *et al.*, 2005; Zhang *et al.*, 2008; César *et al.*, 2012; Ozdemir, 2020; Özcan-Gökçek and Işık, 2023; Samuel *et al.*, 2023).

In practice, most of the Quantitative Trait Loci (QTLs) studies have been supported the possibility to increase the efficiency of GS compared to traditional selection (Hu *et al.*, 2009; Yudin and Voevoda, 2015; Patel and Chauhan, 2017; Samuel *et al.*, 2023). Due to the importance of this PRL candidate gene, most of the studies suggested that the PRL variants could be useful in selection programs for improving milk yield and its composition in dairy cattle (e.g. Bukhari *et al.*, 2013; Deepika and Salar, 2014; Fontanesi*et al.*, 2014; Sonmez and Ozdemir, 2017; Thuy *et al.*, 2018; Dudule *et al.*, 2020; Ozdemir, 2020, and Shah *et al.*, 2021). Recently, Ilie *et al.* (2023) stated that the bovine PRL gene is essential for the initiation and maintenance of lactation and exerts multiple effects on mammary alveoli to promote the synthesis and secretion of major components of milk.

The Bovine *PRL* gene has been mapped to chromosome 23 at 43 *cM* close to QTL, about 10 *kb* in size, including 5 exons coding for 199 amino acids and 4 introns (Mahajan *et al.*, 2012; Patel and Chauhan, 2017, and Samuel *et al.*, 2023). Using polymerase chain reaction and the Rstriction Fragments Length Polymorphism technique (PCR-RFLP), these polymorphic structures of the PRL gene have been studied by many researchers, who confirmed significant associations between PRL

polymorphic variants and milk production traits in dairy cattle (He *et al.*, 2006; Hu *et al.*, 2009; Mehmannavaz *et al.*, 2009; Alfonso *et al.*, 2012; César *et al.*, 2012; Thuy *et al.*, 2018, and Ilie *et al.*, 2023).

Based on recent worldwide molecular studies, strong associations among the PRL gene genotypes and milk traits were detected, emphasizing the potentiality of the PRL gene as a candidate gene to be used in selection for improving milk traits in dairy cattle (Alipanah *et al.*, 2007; Lu *et al.*, 2010; Alfonso *et al.*, 2012; Cesar *et al.*, 2012; Das *et al.*, 2012; Mahajan *et al.*, 2012; Bukhari *et al.*, 2013; Patel and Chauhan, 2017; Thuy *et al.*, 2018, and Samuel *et al.*, 2023). But, the molecular studies practiced in developing countries concerning the associations of the PRL gene with milk traits in dairy cattle are limited and scarce. In Egypt, no systematic efforts have been undertaken to assess the status of PRL polymorphism in dairy cattle breeds. Therefore, the main aims of the present study were: (1) To characterize the PRL candidate gene of Friesian and Baladi cows, on SNPs molecular basis, in terms of Hardy Weinberg equilibrium, genotypic and allelic frequencies, observed and expected heterozygosity, polymorphic information content and fixation index, and (2) To investigate the molecular associations of the PRL gene genotypes with yields of milk, fat and protein in Friesian and Baladi cattle using *NaeI* and *SmaI* restriction enzymes and applying PCR-RFLP. Accordingly, this is the first attempt to report the associations between the PRL gene and milk production in dairy cattle in Egypt.

MATERIAL AND METHODS

Animals used and genomic DNA extraction:

A study was conducted on 494 lactations of 142 Friesian and 38 Baladi cows in herds of Elkarda Friesian (FK), Sakha Friesian (FS) and Elserw Baladi (BS)to characterize prolactin gene on molecular basis and to detect the polymorphic associations between SNP genotypes of this gene and milk traits applying PCR-RFLP method (Kumari *et al.*, 2008; Dudule *et al.*, 2020, and Samuel *et al.*, 2023). Cows in the first four groups were used, depending onavailable records. However, the polymorphism in exon 3 and 4 of the prolactin gene in dairy cattle was clarified and protocoled by several investigators (e.g. Zhang *et al.*, 2008; Hu *et al.*, 2009; Mehmannavaz et al., 2009; Das *et al.*, 2012; Karuthadurai *et al.*, 2019; Bayıl and Bozkurt, 2019; Dudule *et al.*, 2020; Shah *et al.*, 2021, and Samuel *et al.*, 2023).

Data on yields of milk, fat and protein of unrelated cows in Friesian herds were recorded for 10 months in FK and FS herds, while the data of unrelated cows in BS herd was available only for six months of lactation. These yields were collected over a period of four years from 2013 through 2016. Cows were milked twice a day at 7 am and 4 pm throughout the lactation period. Fat and protein yields were quantified by the automated method of infrared absorption spectrophotometry (Milk-o-Scan; Foss Electric, Hillerφd, Denmark) at the Dairy Services Unit, Animal Production Research Institute, Sakha, Kafr Elsheikh governorate, Egypt.

Blood samples were collected from the jugular vein of 180 cows in test tubes containing an anticoagulant of ethylenediamine tetraacetic acid tripotassium salt (K3EDTA). Genomic DNA extraction was performed and the RFLP method was applied to detect PCR products using *NaeI* and *SmaI* restriction enzymes (Kumari *et al.*, 2008; Sacravarty *et al.*, 2008; Dudule *et al.*, 2020, and Samuel *et al.*, 2023).

PCR amplification :

On chromosome 23, the PCR amplification was performed in a volume of 25 µl using 1.5 *mM* MgCl2, 200 *µM* dNTPs (deoxyribonucleotide triphosphate), 5 *pmol* of each primer, 50-100 *ng* of genomic DNA and 0.5 Units of Taq DNA polymerase (Matrix Scientific Trade CO., Egypt). Two fragments of 232 and 168 *bps* in length were used in the amplification process, based on the available sequences of the bovine PRL gene exon 10 (Gene Bank accession number: FJ901285, SNP location 486 and 828 *bps*). In the case of the 232-*bp* fragment containing a polymorphic *NaeI* restriction site, the Forward Primer was 5'-CCTATTTTCTGGCCAATGGA-3' and the Reverse Primer was 5'-TCTGACTCCCTCTGCTTGGT-3', while in the case of the 168-*bp* fragment containing a polymorphic *SmaI* restriction site, the Forward Primer was 5' AGATGGAGTGCTGGCTCTGT-3' and the Reverse Primer was 5'-GCCTTCTTGGCTGGTTCTTC-3'. Amplification reactions were carried out by a program starting with 5 minutes of denaturation at 94°C followed by 35 cycles of 94°C for 45 seconds, annealing at 60°C for 45 seconds and extension at 72°C for one minute, with a final extension of 10 minutes at 72°C.

PCR Products Digestion:

PCR products were separated by using an electrophoresis unit on 3.5 % agarose gel at a constant 75 V, stained with ethidium bromide and excised for digestion by specific enzymes. Since the DNA fragment of PRL gene is large and too long, therefore two restricted enzymes were used to ascertain the molecular analyses. Digestion using NaeI restriction enzyme was performed in 10 µl mixture containing 8.8 µl PCR products + 0.2 *µlNaeI* + 1 *µl* Buffer. The reaction system was performed under 37°C for three hours and the resulting fragments were separated on 3% agarose gel and detected by electrophoreses unit and U.V. unit to detect the number of bands for each genotype. Digestion by *SmaI* restriction enzyme was performed in a 10 *µl* mixture containing 4.9 *µl* PCR products + 0.1 *µlSmaI* + 5 *µl* Buffer. The reaction system was performed at 55°C overnight and the product of fragments was separated on 3 % agarose gel and detected by electrophoreses unit and U.V. unit to

detect the number of bands for each genotype. All the molecular investigations were carried out in the Laboratories of Molecular Genetics of Research Labs Park, Faculty of Agriculture, Benha University, Egypt.

Characterizing PRL gene in Friesian and Baladi herds:

The genetic molecular weights were detected for each genotype using POPGENE software, version 1.32 (Yeh *et al.*,1999; https://genepop.curtin.edu.au/). Hardy-Weinberg Equilibrium (*HWE;* https://genepop.curtin.edu.au/genepop_op1.html), the observed (*NO*) and expected (*NE*) numbers of alleles (https://genepop.curtin.edu.au/genepop_op4.html, and genotypic and allelic frequencies (https://genepop.curtin.edu.au/genepop_op5.html) of the PRL gene were estimated within each herd. The observed (*HO*) and expected (*HE*) heterozygosities were estimated using GENALEX software, version 6.5 (Peakall and Smouse, 2006; https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1471-8286.2005.01155.x. The polymorphic information content (PIC) was calculated using CERVUS software, version 3 (Kalinowski *et al.*, 2007). The reduction in heterozygosity due to inbreeding within each herd (i.e. fixation index, *FIS*) was calculated using POPGENE program, version 1.32 (Yeh *et al.*, 1999; https://genepop.curtin.edu.au/genepop_op5.html). The following equations were used in the estimation of the previous parameters:

$$H_o = No.heterozygosity / n$$

$$H_e = 1 - \sum_{i=1}^{n} p_i^2$$

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 2p_j^2$$

$$F_{IS} = (H_e - H_o) / H_e$$

Where *Pi* = the frequency of the ith allele, P_j = the frequency of the jth allele, n = the number of alleles (Peakall and Smouse, 2012), *FIS*= fixation index or the reduction in heterozygosis due to inbreeding.

Estimation of molecular associations between PRL gene genotypes and milk traits:

Test day (TD) records for milk, fat and protein yields were collected and data available was mostly for 10 months of lactation in the Friesian herds, while the data available in the Baladi herd were collected mostly for six months of lactation. For data analysis, TD records were used to predict 305 days of milk yield in Friesian herds using Fleishman method as mentioned by Kong *et al. (*2018). The polymorphic associations between PRL gene genotypes and yields of milk, fat and protein were analyzed by applying the "Mixed procedure" of SAS, package 9.0 (Kamp *et al.*, 2002) and using the following mixed model (in matrix notations):

$$\mathbf{y = Xb + Za\ a + Zp\ p + e}$$

Where: y = the vector of milk trait observations; X = the incidence matrix relating the fixed effects to y; b = the vector of the fixed effects of year-season of calving, parity and genotypes of PRL gene restricted by *NaeI* and *SmaI* restriction enzymes (three genotypes for each enzyme, genotypes CC, CD and DD for *NaeI enzyme and* genotypes GG, GT and TT for *SmaI* enzyme); Za = the incidence matrix relating the cow additive effects to y; a = the vector of the random additive effects associated with the incidence matrix Za; Zp = the incidence matrix relating the permanent environmental effects to y; p = the vector of random permanent environmental effects associated with the incidence matrix Zp; e = the vector of random residual effects.

RESULTS AND DISCUSSION

Molecular weights:

The amplified DNA fragment of 230 *bp* was digested using the *NaeI* restriction enzyme and three genotypes of CC, CD and DD of the PRL gene were detected. The banding patterns of the PRL gene yielded in PCR product were one band in the CC genotype (230 *bp*), three bands in the CD genotype (230, 150 and 80 *bps*) and two bands in the DD genotype (150 and 80 *bps*). Udina *et al.* (2001) using Black Pied, Russian Ayrshire and Gorbatov Red cattle reported that variation of the microsatellite of the *PRL* gene was monomorphic in Gorbatov Red cattle (164 bp) and two alleles were detected in Black Pied and Russian Ayrshire cattle (164 and 162 bps). Mahajan *et al.* (2012)with Frieswal cattleamplified a 156 *bp* of the PRL gene in the exon-3 segment by PCR-RFLP method using *Rsa*I restriction enzyme. Sonmez and Ozdemir (2017) using the PCR-RFLP method detected three fragments of the PRL gene in native EAR cattle raised in Turkey (GG genotype with 210 *bp*, AA genotype with 120 *bp* and AG genotype with 90 *bp*).

The DNA fragment of 180 *bp* was digested using the *SmaI* restriction enzyme and three genotypes of GG, GT and TT of the PRL gene were detected. The banding patterns of the PRL gene yielded in PCR product were one band in GG genotype (180 *bp*), three bands in GT genotype (180, 123 and 57 *bps*) and two bands in TT genotype (123 and 57 *bps*). These genotypes and bands were comparable with those obtained by Deepika and Salar (2014) who observed three genotypes of GG, GT and TT in indigenous Indian grey cattle. Similar RFLP patterns using *Rsa*I restriction enzyme were reported by Sacravarty *et al.* (2008) in Kankrej cattle. Mehmannavaz *et al.* (2009) detected a mutation in 294-*bp* fragment of PRL gene located in exon 4 in Iranian Holstein cattleusing PCR-RFLP technique and *RsaI* restriction enzyme. Karuthadurai *et al.* (2019) in Sahiwal cattle found that the fragments of genotype GA had 156, 84 and 72 *bps*, the genotype GG had 84 and 72 *bps* and the genotype AA had 156 *bp*.

The observed and expected number of alleles and Hardy-Weinberg equilibrium in each herd:

The observed (*NO*) and expected (*NE*) numbers of alleles and Hardy-Weinberg equilibrium (*HWE*) in the three genotypes of the PRL gene are presented in Table 1 for each separate herd. In case of using the *NaeI* enzyme, the highest *NE* was recorded in DD genotype in all herds (59.4, 37.0 and 12.9 for FK, FS and BS herds, respectively), while the lowest *NE* was recorded in CC genotype (9.8, 11.3 and 11.9 for FK, FS and BS herds, respectively). In *SmaI* polymorphism genotypes, the highest *NE* was recorded in TT genotype for all herds (56.1, 25.1 and 17.1 for FK, FS and BS herds, respectively), while the lowest *NE* was recorded in GG genotype (2.1, 8.2 and 4.1 for FK, FS and BS herds, respectively).

Table 1. The observed (*NO*) **and expected** (*NE*) **number of alleles and Chi-square values (χ2) for Hardy-Weinberg equilibrium (*HWE*) for the PRL gene genotypes in the three cattle herds studied**

Herd	No of cows	Observed number of PRL alleles (N_O)			Expected number of PRL alleles (N_E)			χ^2 value for HWE
Polymorphic genotypes in case of using *NaeI restriction enzyme:*								
		CC	CD	DD	CC	CD	DD	
FK	80	2	11	67	9.8	10.2	59.4	6.2*
FS	62	2	16	44	11.3	13.9	37.0	7.6*
BS	38	3	11	24	11.9	13.2	12.9	6.6*
Polymorphic genotypes in case of using *SmaI restriction enzyme:*								
		GG	GT	TT	GG	GT	TT	
FK	80	11	4	65	2.1	21.8	56.1	37.7**
FS	62	17	11	34	8.2	28.7	25.1	9.4**
BS	38	12	1	25	4.1	16.7	17.1	15.2**

FK= Friesian in Elkarada herd, FS= Friesian in Sakha herd, and BS= Baladi in Elserw herd.
*= P<0.05, **=P<0.01

In both *NaeI* and *SmaI* enzymes, Chi square values (χ2) of the PRL gene genotypes were significant in all herds studied (P<0.05 or P<0.01; Table 1). These significant values of χ2 indicate that all the herds were not in *HWE* for PRL gene. The result of Mahajan *et al.* (2012) in Frieswal cattle showed that the population was not in *HWE* for PRL gene. Dong *et al.* (2013) found that the haplotype frequencies of the *PRL* gene in Chinese Holstein cows were not in *HWE* (P<0.01). Akyuz and Cinar (2014) using East Anatolian Red, Zavot, Brown Swiss and Simmental breeds reported that the Zavot breed was the only breed in *HWE*. Patel and Chauhan (2017) found that the Chi-square values for Gir and Kankrej cattle in India were 13.46 and 12.58, indicating that the populations were not in *HWE* for PRL gene. Sonmez and Ozdemir (2017) applying PCR-RFLP in native East Anatolian Red cattle found that the population was in *HWE* for PRL gene. Bayıl and Bozkurt (2019) reported that the Holstein cattle population was in *HWE* for PRL gene since the Chi-square value was not significant (χ2 = 3.74).

Genotypic and allelic frequencies in each herd:

In case of *NaeI*restriction enzyme, the highest genotypic frequency in both Friesian and Baladi herds was recorded in DD genotype of the PRL gene (0.837, 0.709 and 0.631 in FK, FS and BS herds, respectively; Table 2), while the lowest frequency was in CC genotype (0.025, 0.032 and 0.079 in FK, FS and BS herds, respectively). The allelic frequency for D allele was much higher than for C allele (0.906, 0.838 and 0.776 vs 0.093, 0.161 and 0.223 in FK, FS and BS herds, respectively; Table 2). The allelic variation in the sequences of the PRL gene in dairy cattle would be of interest because the PRL gene has a possible direct or indirect effect on milk production (Hu *et al.*, 2009; Boleckova *et al.*, 2012; Thuy *et al.*, 2018). Udina *et al.* (2001) found that the frequencies of *B* allele of *PRL* gene detected by PCR–RFLP method were 0.141 and 0.869 in Russian Ayrshire and Gorbatov Red cattle, respectively. Mehmannavaz *et al.* (2009) found that the frequency of G allele (0.931) was much higher than A allele (0.069). Boleckova *et al.* (2012) reported that the frequencies in Holstein cattle were 0.12 for A allele and 0.88 for G allele. Thuy *et al.* (2018) stated that the allelic frequencies were 0.886 for L allele and 0.114 for V allele in Holstein Friesian dairy cows. Results of Mahajan *et al.* (2012) using PCR-RFLP method and *Rsa*I restriction enzyme in Frieswal cattle revealed that the allelic frequencies were 0.630 for A allele and 0.370 for B allele and the genotypic frequencies were 0.315 for AA genotype, 0.629 for AB genotype and 0.056 for BB genotype.

Table 2. The genotypic and allelic frequencies for genotypes of PRL gene in the case of using *NaeI* and *SmaI* restriction enzymes in the three cattle herds studied

In case of using *NaeI enzyme*:

Herd	Number of cows	Genotypic frequency			Gene frequency	
		CC	CD	DD	C	D
FK	80	0.025	0.137	0.837	0.093	0.906
FS	62	0.032	0.258	0.709	0.161	0.838
BS	38	0.079	0.289	0.631	0.223	0.776

In case of using *SmaI enzyme*:

		Genotypic frequency			Gene frequency	
		GG	GT	TT	G	T
FK	80	0.137	0.050	0.812	0.162	0.837
FS	62	0.274	0.177	0.548	0.362	0.637
BS	38	0.315	0.026	0.657	0.328	0.671

FK= Friesian in Elkarada herd, FS = Friesian in Sakha herd, and BS= Baladi in Elserw herd.

In case of *SmaI* restriction enzyme, TT genotype of the PRL gene was the highest frequency (0.812, 0.548 and 0.657 in FK, FS and BS herds, respectively) relative to GG genotype (0.137, 0.274 and 0.315) and GT genotype (0.050, 0.177 and 0.026) as presented in Table 2. The frequency of T allele was higher than G allele in all herds studied (0.837, 0.637 and 0.671 for T allele *vs* 0.162, 0.362 and 0.328 for G allele in FK, FS and BS herds, respectively). Accordingly, these allelic frequencies gave a noticeably higher incidence of allele G in genotypes of PRL gene as cited by Mehmannavaz *et al.* (2009) in Holstein (0.93) and by Singh *et al.* (2015) in Holstein crossbred (0.75). Khatami *et al.* (2005) in Russia using RFLP analysis and *RsaI* enzyme found clear differences in allelic and genotypic frequencies of PRL gene in Russian and German Black and White and Yaroslavl cattle. Thuy *et al.* (2018) found that only two genotypes were detected with genotypic frequencies of 0.780 and 0.220 for LL and LV genotypes, respectively.

The variation of PRL allelic frequencies obtained in both Friesian herds (FK and FS herds, Table 2) may be due to the differences in herd size and selection programs practiced in these herds, indicating also that PRL gene is considered as candidate gene potentially associated with milk performance traits to be used in marker-assisted selection program in dairy cattle in Egypt. However, the genotypic and allelic frequencies of GG, GT and TT genotypes of the *PRL* polymorphism reported here are similar to those findings reported in various regions of the world for different breeds (Mehmannavaz *et al.*, 2009; Lu *et al.*, 2010; Alfonso *et al.*, 2012; Boleckova *et al.*, 2012; Das *et al.*, 2012, and Sonmez and Ozdemir, 2017). Kumari *et al.* (2008) in India reported frequencies of AA, BB and AB genotypes to be 0.22, 0.13 and 0.65 in Jersey cattle with allelic frequencies of 0.55 for A allele and 0.45 for B allele. Karuthadurai *et al.* (2019) reported also in India that the frequencies of AA, GG and GA genotypes of Sahiwal cattle were 0.30, 0.45 and 0.25, respectively with allelic frequencies of 0.425 for A allele and 0.575 for G allele. In Croatia, Mauriæ *et al.* (2017) stated that most of the animals of Simmental and crossbred Holstein cattle were genotyped as GG homozygous genotype for PRL gene.

Heterozygosis in PRL gene genotypes:

In both *NaeI* and *SmaI* restricted enzymes, the observed values of heterozygosity (*HO*) for PRL gene (Table 3) were moderate and nearly similar in magnitude and trend in FK herd (0.47 and 0.36), FS herd (0.26 and 0.21) and BS herd (0.28 and 0.19). Similarly, Akyuz *et al.* (2012) reported moderate values of *HO*for PRL gene to be 0.28 in Black and White cattle and 0.43 in Jersey cattle. Sonmez and Ozdemir (2017) in native East Anatolian Red cattle found that the observed heterozygosity for PRL polymorphism was also moderate (0.34).

The expected values of heterozygosity (*HE*) were moderate and similar in the three herds studied (0.23 and 0.39; Table 3). Patel and Chauhan (2017) reported that the expected frequencies of heterozygosity of PRL gene obtained by PCR-RFLP technique in Gir and Kankrej cattle were high (0.83 and 0.70).

The values of fixation index (*FIS*) were high and nearly similar in magnitude and trend for both *NaeI* and *SmaI* restricted enzymes in FK herd (0.70 and 0.82), FS herd (0.75 and 0.62) and BS herd (0.70 and 0.94) (Table 3). Sonmez and Ozdemir (2017) applying PCR-RFLP in native East Anatolian Red cattle found that the value of FIS was of low rate (0.072).

The values of polymorphic information content (*PIC)* in both *NaeI* and *SmaI* restricted enzymes were moderate and nearly similar in magnitude in FK herd (0.22 and 0.23), FS herd (0.20 and 0.36) and BS herd (0.28 and 0.34) (Table 3). These moderate values of PIC measure the ability of a molecular marker to detect polymorphisms, and therefore have enormous importance in selecting markers for genetic studies in dairy cattle. These values can also serve as screening for genetic studies of the Egyptian herds with the objectives to investigate evolutionary and ecological

processes. Udina *et al*. (2001) in Russian Ayrshire, Gorbatov Red and Black Pied cattle found that the estimate of PIC applying PCR–RFLP in exon 3 of PRL gene was moderate (0.21). Dong *et al*. (2013) reported that the intron 3 and 4 of the *PRL* gene in Chinese Holstein cows indicated that the PIC values of the haplotype block (0.194) should be considered as slightly polymorphic information content.

Table 3. The observed (*HO*) and expected (*HE*) heterozygosities, fixation index (*FIS*) and the polymorphic information content (*PIC*) for genotypes of PRL gene in Friesian and Baladi cattle herds

Herd	No. of cows	H_o± SE	H_E± SE	F_{IS}	PIC
In case of using *NaeI restriction enzyme*:					
FK	80	0.47±0.03	0.23±0.03	0.70	0.22
FS	62	0.26±0.04	0.23±0.04	0.75	0.20
BS	38	0.28±0.02	0.23±0.02	0.70	0.28
In case of using *SmaI restriction enzyme*:					
FK	80	0.36±0.03	0.39±0.03	0.82	0.23
FS	62	0.21±0.04	0.39±0.04	0.62	0.36
BS	38	0.19±0.02	0.39±0.02	0.94	0.34

FK= Friesian in Elkarada herd, FS= Friesian in Sakha herd, and BS= Baladi in Elserw herd.
SE= Standard error.

Polymorphic associations between PRL gene genotypes and milk traits within each herd:

The molecular analyses in each separate herd revealed that three genotypes of the PRL gene of CC, CD and DD for *NaeI* enzyme and GG, GT and TT for *SmaI* enzyme were detected. However, there were abundant reports evidencing that the PRL gene is associated with milk production and/or composition traits in dairy cattle (e.g., Fontanesi *et al.*, 2014; Singh *et al.*, 2015; Mauriæ *et al.*, 2017; Raschia *et al.*, 2017; Shah *et al.*, 2021; Ilie *et al.*, 2023). Therefore, the results obtained in this study confirmed the molecular associations reported previously in the other populations of dairy cattle.

In the Friesian of Elkarada herd, the least square means for yields of milk, fat and protein were 3805, 52.8 and 32.2 kg in CC genotype, 3960, 54.8 and 39.6 kg in CD genotype and 3113, 50.5 and 35.8 kg in DD genotype in case of using *NaeI* enzyme, while the means were 2996, 59.8 and 41.5 kg in GG genotype, 2865, 47.5 and 27.6 kg in GT genotype and 2920, 53.6 and 40.1 kg in TT genotype in case of using *SmaI* enzyme, respectively (Table 4).

Table 4. The polymorphic associations of PRL gene genotypes with yields of milk, fat and protein in Friesian of Elkarada herd expressed as least square means (LSM) of 10 months lactation

Trait	LSM	SE	LSM	SE	LSM	SE	*p* value
In case of using *NaeI restricted enzyme*:	**CC Genotype (N= 10)**		**CD Genotype (N= 77)**		**DD Genotype (N= 143)**		
Milk yield, kg	3805^{b}	82	3960^{b}	85	3113^{a}	119	0.003
Fat yield, kg	52.8^{ab}	3.4	54.8^{a}	3.5	50.5^{b}	2.4	0.015
Protein yield, kg	32.2^{a}	2.8	39.6^{b}	2.3	35.8^{a}	1.6	0.019
In case of using *SmaI restricted enzyme*:	**GG Genotype (N= 52)**		**GT Genotype (N= 39)**		**TT Genotype (N= 139)**		
Milk yield, kg	2996^{a}	147	2865^{b}	170	2920^{a}	90	0.014
Fat yield, kg	59.8^{a}	4.2	47.5^{b}	4.8	53.6^{ab}	2.5	0.016
Protein yield, kg	41.5^{a}	2.8	27.6^{b}	3.2	40.1^{a}	1.7	0.001

a,b Means within each classification, not followed by the same letters differed significantly ($P < 0.01$), SE= Standard error.

The differences in yields of milk, fat and protein among CC, CD and DD genotypes of the PRL gene in Friesian of Elkarada herd were significantly in favour of CD genotype ($p<0.01$; Table 4). Also, the differences among the three genotypes of GG, GT and TT were significantly in favour of GG genotype relative to GT and TT genotypes. However, high means for CD and GG genotypes of the PRL gene indicate that there are strong associations of these genotypes with milk traits. Therefore, CD and GG

genotypes could be used in selection to improve milk traits of Friesian cows in Egypt (for milk, fat and protein yields). Singh *et al.* (2015) found that GG genotype had significantly greater milk yield in Indian Frieswal cattle (P<0.05) than AG genotype in crossbred Holstein cattle and this AG genotype had significantly higher milk yield compared to AG genotype in Simmental cattle (P<0.05). Mauriæ et al. (2017) stated that AG genotype of the crossbred Holstein cattle had significantly higher milk yield than GG genotype (P<0.05). Shah et al. (2021) reported that the differences in yield traits between RR and Rr genotypes in Jersey cows were significantly in favour of Rr genotype (p<0.05) relative to RR genotype.

Table 5. The polymorphic associations of the PRL gene genotypes with yields of milk, fat and protein in Friesian of Sakha herd expressed as least square means (LSM) of 10 months lactation

Trait	LSM	SE	LSM	SE	LSM	SE	*p* value
In case of using *NaeI restricted enzyme*:							
	CC Genotype (N= 14)		**CD Genotype (N= 25)**		**DD Genotype (N= 155)**		
Milk yield, kg	2920[a]	211	3093[a]	219	2757[b]	85	0.015
Fat yield, kg	63.2[a]	4.6	66.5[a]	5.9	56.6[b]	2.2	0.012
Protein yield, kg	50.4[a]	2.8	52.3[a]	4.0	38.8[b]	1.5	0.002
In case of using *SmaI restricted enzyme*:							
	GG Genotype (N= 40)		**GT Genotype (N= 10)**		**TT Genotype (N= 144)**		
Milk yield, kg	2852[b]	175	2670[a]	349	2795[a]	92	0.012
Fat yield, kg	70.9[b]	4.6	53.1[a]	9.2	54.5[a]	2.4	0.007
Protein yield, kg	44.7[b]	3.2	43.5[b]	6.5	39.2[a]	1.7	0.013

a,b Means within each classification, not followed by the same letters differed significantly (P<0.01), SE= Standard error.

The differences among the three genotypes of CC, CD and DD in yields of milk, fat and protein were significantly in favour of CD genotype of the PRL gene (p<0.01; Table 5). On the other hand, the differences among the three genotypes of GG, GT and TT in all milk traits were significantly in favour of GG genotype comparable to GT and TT genotypes. High least square means for CD and GG genotypes of the PRL gene indicate that these genotypes were considerably associated with milk traits in Friesian of Sakha herd. Therefore, CD and GG genotypes could be used in selection to improve milk traits of the Friesian cows in Egypt. He *et al.* (2006) using SNP in Chinese Holstein dairy cattle reported that BB genotype of the PRL gene was significantly associated with milk yield, fat yield, protein yield and protein percentage (p<0.05 or p<0.01), *i.e.* cows with BB genotype had significantly higher milk yield, fat yield and protein yield than that of AA genotype. Recently, Ilie *et al.* (2023) reported that AA genotype of the PRL gene was significantly associated with fat and protein percentages in the milk of the Romanian Brown cattle (p<0.05), *i.e.* AA genotype was associated with higher fat and protein percentages in milk compared to GG genotype.

In the Baladi cattle of Elserw herd, the molecular analysis of the PRL gene revealed that there are significant associations for CD and GG genotypes of the PRL gene with milk productivity parameters (Table 6), *i.e.* PRL gene is promising to be used as effective marker for milk productivity of Baladi cattle in Egypt. The least square means of yields of milk, fat and protein were 802, 25.0 and 22.0 kg in CC genotype, 815, 27.3 and 24.8 kg in CD genotype and 662, 22.7 and 20.3 kg in DD genotype in case of using *NaeI* enzyme, while the averages of yields of milk, fat and protein were 724, 25.7 and 22.9 kg in GG genotype, 294, 11.1 and 9.6 kg in GT genotype and 584, 19.2 and 17.2 kg in TT genotype in case of using *SmaI* enzyme, respectively (Table 6). The differences in yields of milk, fat and protein among CC, CD and DD genotypes of PRL gene in the local cattle of Elserw herd were significantly in favour of CD genotype (p<0.01). Also, the differences among the three genotypes of GG, GT and TT in the milk traits were significantly in favour of GG genotype (p<0.01). Therefore, CD and GG genotypes could be used in selection to improve the milk traits of Baladi cattle in Egypt.

Table 6. The polymorphic associations of the PRL gene genotypes with yields of milk, fat and protein in Baladi of Elserw herd expressed as least square means (LSM) of six months lactation

Trait	LSM	SE	LSM	SE	LSM	SE	*p* value
In case of using *NaeI restricted enzyme*:							
	CC Genotype (N= 5)		**CD Genotype (N= 17)**		**DD Genotype (N= 48)**		
Milk yield, kg	802[a]	68	815[a]	71	662[b]	40	0.013
Fat yield, kg	25.0[a]	2.2	27.3[a]	2.4	22.7[b]	1.4	0.015
Protein yield, kg	22.0[a]	1.8	24.8[a]	2.1	20.3[b]	1.2	0.015
In case of using *SmaI restricted enzyme*:							
	GG Genotype (N= 17)		**GT Genotype (N= 2)**		**TT Genotype (N= 51)**		
Milk yield, kg	724[a]	70	294[b]	206	584[c]	41	0.002
Fat yield, kg	25.7[a]	2.4	11,1[b]	6.9	19.2[c]	1.4	0.017
Protein yield, kg	22.9[a]	1.9	9.6[b]	5.6	17.2[c]	1.2	0.012

a,b Means within each classification, not followed by the same letters differed significantly (P<0.01), SE= Standard error.

The molecular associations between exon 3 and exon 4 of the PRL gene with milk production traits in cattle were significantly evidenced in the last two decades by several investigators (*e.g.,* Brym *et al.,* 2005; Alipanah *et al.,* 2007; Boleckova *et al.,* 2012; Mahajan *et al.,* 2012; Bukhari *et al.,* 2013, and Ilie *et al.,* 2023). Hu *et al.* (2009) in Chinese Holstein cows identified three genotypes within exon 4 of PRL gene (AA, AB and BB) using the SSCP technique of polymorphism where the cows with BB genotype showed higher milk yield in comparison to cows with AA and AB genotypes (P<0.05). Mahajan *et al.* (2012) in Frieswal cattle stated that the animals with AB genotype had higher milk yield relative to AA and BB genotypes, concluding that the PRL gene could be used as a considerable marker for genetic selection of milk traits in dairy cattle. Recently, Ilie *et al.* (2023) showed that PRL gene favored significantly higher fat and protein percentages in the milk of the Romanian Brown cattle compared to the Romanian Spotted breed.

CONCLUSION

Considering *NaeI*and *SmaI* as restriction enzymes, CD and GG genotypes of the PRL gene were strongly associated on the molecular basis with milk traits and therefore both genotypes could be the main target in selection to improve yields of milk, fat and protein in Friesian and Baladi dairy cattle in Egypt, *i.e.PRL* gene could be used as a powerful tool in gene selection.

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REFERENCES

Akyuz B., K.O. Agaoglu and O. Ertugrul, 2012. Genetic polymorphism of kappa-casein, growth hormone and PRL genes in Turkish native cattle breeds. International Journal of Dairy Technology, 65: 38-44.

Akyuz B. and M. U. Cinar, 2014. Analysis of PRL and kappa-casein genes polymorphism in four cattle breeds in Turkey. Annals of Animal Science, 14: 799-806.

Alfonso E., R. Rojas, J. G. Herrera, M. E. Ortega, C. Lemus, J. Ruiz, R. Pinto and H. Gómez, 2012. Polymorphism of the prolactin gene (PRL) and its relationship with milk production in American Swiss cattle. African Journal of Biotechnology, 11: 7338-7343.

Alipanah M., L. Kalashnikova, and G. Rodionov, 2007. Association of PRL gene variants with milk production traits in Russian Red Pied cattle. Iranian Journal of Biotechnology, 5(3): 158–161.

Bayıl O.S. and A. S. Bozkurt, 2019. A Study on the effect of prolactin gene variants on milk production traits of Holstein cattle. *Russian Journal of Genetics, 55(4), 480–486.*

Boleckova J., J. Matejickova, M. Stipkova, J. Kyselova and L. Barton, 2012. The association of five polymorphisms with milk production traits in Czech Fleckvieh cattle. Czech Journal of Animal Science, 57(2): 45–53.

Brym P., S. Kaminski and E. Wojcik, 2005. Nucleotide sequence polymorphisms within exon 4 of the bovine prolactin gene and its association with milk performance traits. Journal of Applied Genetics, 46(2): 179–185.

Bukhari S., N. N. Khan, P. Gupta, A. K. Das, G. A. Raher, D. Chakraborty and A. Pandey, 2013. Prolactin gene polymorphism and its associations with milk production traits in Frieswal cow. International Journal of Molecular Zoology, 3(3): 10-13. doi: 10.5376/ijmz.2013.03.0003

César C., A. Edy and R. Reyna, 2012. Polymorphism of the prolactin gene (PRL) and its relationship

with milk production in American Swiss cattle. African Journal of Biotechnology, 11(29): 7338–7343.

Das N.D., D. N. Hatkar, V. G. S. Hari, B.V. Srinivas, R. Kaliaperumal, O.A. Reddy and L. Krishnamurthy, 2012. Genetic polymorphisms of exons 3 and 4 of Prolactin (Prl) gene in Deoni cattle breed and their association with milk production traits. International Journal of Livestock Research 2: 120-126.

Deepika D. and R. K. Salar, 2014. Polymorphism studies of prolactin receptor (PRLR) gene indigenous grey cattle breeds of India. DHR International Journal of Biomedical and Life Sciences, 5(1): 314-321.

Dong C.H., Song X.M., Zhang L., Jiang J.F., Zhou J.P., Jiang Y.Q., 2013. New insights into the prolactin-RsaI (PRL-RsaI) locus in Chinese Holstein cows and its effect on milk performance traits. Genetics and Molecular Research 12 (4): 5766-5773.

DOI http://dx.doi.org/10.4238/2013.November.22.3

Dudule P. D., D. S. Kale, A. Sonwane, D. V. Patil, M. R. Jawale, and K. P. Kharkar, 2020. PCR-RFLP Polymorphism at Exon 3 and promoter region of prolactin gene in Gaolao cattle. Indian Journal of Animal Research, 54(4): B-3745.

Fontanesi L, D.G. Calò, G. Galimberti, R. Negrini, R. Marino, A. Nardone, P. Ajmone-Marsan, V. Russo, 2014. A candidate gene association study for nine economically important traits in Italian Holstein cattle. Animal Genetics, 2014, 45(4): 576–580.https://doi.org/10.1111/age.12164

He F., D. Sun, Y. Yu, Y. Wang and Y. Zhang, 2006. Association between SNPs within prolactin gene and milk performance traits in Holstein dairy cattle. Asian-Australian Journal of Animal Science, 19(10): 1384–1389.

Hu X., Lü A., Chen H., Gao X., Xu H., Zhang C., Fang X. and Lei C., 2009. Preliminary evidence for association of prolactin and prolactin receptor genes with milk production traits in Chinese Holsteins. Journal of Applied Animal Research, 36(2): 213-217, https://doi.org/10.1080/09712119.2009.9707062

Ilie D.E., A.E. Mizeranschi, C.V. Mihali, R.I. Neamt, L.T. Cziszter, M. Carabas, A.C. Gradinaru, 2023. Polymorphism of the prolactin (PRL) gene and its effect on milk production traits in Romanian cattle breeds.Veterinary Science,10(4),275. https://doi.org/10.3390/vetsci10040275

Kalinowski S. T., M. L. Taper and T. C. Marshall, 2007. Revising how the computer program CERVUS accommodates genotyping error increases successes in paternity assignment. Molecular Ecology, 16: 1099-1106.

Kamp R F, Koenen E P C and de Jong G 2002. SAS JMP Version 9.0 SAS Institute, Cary, NC, USA.

Karuthadurai T., A. K. Chakravarthy, A. Kumaresan, Aneet Kour, Nag B. S. Pradeep, E. Rana, D. Ravikumar and S. Yousuf, 2019. Identification of genetic marker for prolactin gene related to milk yield in pedigreed Sahiwal population. Indian Journal of Animal Research, 53(5): 566-571.

Khatami S. R., O. E. Lazebny, V. F. Maksimenko and G. E. Sulimova, 2005. Association of DNA polymorphisms of the growth hormone and prolactin genes with milk productivity in Yaroslav l and Black and White cattle. Russian Journal of Genetics, 41(2): 167–173.

Kong L., J. Li, R. Li, X. Zhao, Y. Ma, S. Sun, J. Huang, Z. Ju, M. Hou and J. Zhong, 2018. Estimation of 305-day milk yield from test-day records of Chinese Holstein cattle. Journal of Applied Animal Research, 46(1): 791-797. https://doi:10.1080/09712119.2017.1403918

Kumari R., K. Singh, K. Soni, R. Patel, J. Chauhan and S. Rao, 2008. Genotyping of the polymorphism within exon 3 of prolactin gene in various dairy breeds by PCR RFLP. Archives Animal Breeding, 51: 298-299. https://doi.org/10.5194/aab-51-298-299 , 2008

Lu A., X. Hu, H. Chen, J. Jiang, C. Zhang, H. Xu and X. Gao, 2010. Single nucleotide polymorphisms in bovine PRL gene and their associations with milk production traits in Chinese Holsteins. Molecular Biology Reports, 37: 547–551.

Mahajan V., S. N. S. Parmar, M. S. Thakur and G. Sharma, 2012. Association of prolactin gene polymorphism with milk production traits in Frieswal cattle. Journal of Animal Research, 2(2): 165-169.

Mauriæ M., T. Mmašek, M. Beniæ, M. Spehar and K. Starèeviæ, 2017. Effect of DGAT1, FASN and PRL genes on milk production and milk composition traits in Simmental and crossbred Holstein cattle. Indian Journal of Animal Sciences, 87(7): 859–863.

Mehmannavaz Y., C. Amirinia, M. Bonyadi and R. V. Torshizi, 2009. Effects of bovine prolactin gene polymorphism within exon 4 on milk related traits and genetic trends in Iranian Holstein bulls. African Journal of Biotechnology, 8: 4797–4801.

Özcan-Gökçek, E. and R. Işık, 2023. Genetic variations of fourth exon region of prolactin gene and its associations with growth traits in European Sea Bass *Dicentrarchus labrax* (Moroni dae). Journal Of Ichthyology,63, 572-578, https://doi.org/10.1134/S0032945223030128).

Ozdemir M., 2020. A Prl/RsaI polymorphism in exon 3 and 4 of prolactin gene in dairy cattle. *Pakistan J. Zool*.,52(1):393–396. https://doi.org/10.17582/journal.pjz/2020.52.1.sc7

Patel J. B. and J. B. Chauhan, 2017. Polymorphism of the prolactin gene and its relationship with milk production in Gir and Kankrej cattle. Journal of Natural Science, Biology and Medicine, 2017, 8: 167-170.

Peakall R. and P.E. Smouse, 2006. GenAlEx 6.5: Genetic analysis in Excel. Population Genetic Software for Teaching and Research. Molecular Ecology Notes, 6(1): 288-295.

Raschia M.A., J.P. Nani, D.O. Maizon, M. J. Beribe, A. F. Amadio and M.A. Poli1, 2018. Single

nucleotide polymorphisms in candidate genes associated with milk yield in Argentinean Holstein and Holstein x Jersey cows. Journal of Animal Science and Technology 60:31, https://doi.org/10.1186/s40781-018-0189-1

Sacravarty G., V. Vadodaria, C. Joshi, B. Brahmkhtri, R. Shah and J. Solanki, 2008. Prolactin gene polymorphism and its association with economic traits in Kankerj cattle. Indian Journal of Dairy Science, 61: 273-276.

Samuel B., H. Dadi, G. Dejene, M. Kang, C. Park and H. Dinka, 2023. Single nucleotide polymorphisms within exon four of the prolactin gene and their effect on milk traits in cattle populations of Ethiopia. Animal Biotechnology, 34(1), DOI: 10.1080/10495398.2023.2176867

Shah, R.M., N.A. Ganai, F.D. Sheikh, S. Shanaz, H.M. Khan, S. Alam, N. N. Khan, T. Sheikh, S. Bukhari, A. Hamadani and M. A. Rathe, 2021. Exon IV prolactin (PRL) gene polymorphism and its association with milk production traits in dairy cattle of Kashmir, India. Journal of Entomology and Zoology Studies, 9(2): 521-524.

Singh U., R. Deb, S. Kumar, R. Singh, G. Sengar and A. Sharma, 2015. Association of prolactin and beta-lactoglobulin genes with milk production traits and somatic cell count among Indian Frieswal (HF x Sahiwal) cows. Biomarkers and Genomic Medicine **7**(1): 38–42.

Sonmez Z. and M. Ozdemir, 2017. Prolactin-RsaI gene polymorphism in East Anatolian Red cattle in Turkey. South African Journal of Animal Science,47(2):124-129, http://dx.doi.org/10.4314/sajas.v47i2.3

Thuy N. T. D., N.T. Thua, N. H. Cuonga, L. V. Tya, T. T. B. Nguyenb and D. V. A. Khoac, 2018. Polymorphism of PIT-1 and prolactin genes and their effects on milk yield in Holstein Frisian dairy cows bred in Vietnam. Russian Journal of Genetics, 54(3): 346–352.

Udina I.G., S.O. Turkova, M.V. Kostyuchenko, L.A. Lebedeva, G.E. Sulimova, 2001. Polymorphism of bovine prolactin gene, microsatellite, PCR–RFLP. Russian Journal of Genetics, 37(4):407–411.

Yeh F. C., R. C.Yang and T. Boyle, 1999. POPGENE Version 1.32: Microsoft Window-Based Freeware for Population Genetics Analysis. University of Alberta, Edmonton, China.

Yudin N. S. and M. Voevoda, 2015. Molecular genetic markers of economically important traits in dairy cattle. Russian Journal of Genetics, 51: 506—517.

Zhang J. L., L. S. Zan, P. Fang, F. Zhang, G. Shen, and W.Q. Tian, 2008. Genetic variation of PRLR gene and association with milk performance traits in dairy cattle. Canadian Journal of Animal Science, 88: 33-39.

الكشف عن الإرتباطات الجزيئية للتراكيب الوراثية لجين البرولاكتين مع صفات اللبن في أبقار الفريزيان والبلدي باستخدام تقنية *RFLP*

ماهرحسب النبي خليل[١]، محمود مغربي عراقي[١]، محمد حمادة محمد[١،٢]، سميح محمد زاهد[٢]

١- قسم الإنتاج الحيواني، كلية الزراعة بمشتهر، جامعة بنها ٢ - معهد بحوث الإنتاج الحيوانى، مركزالبحوث الزراعية، وزارة الزراعة

أجريت هذه التجربة لمدة أربعة سنوات للكشف عن الارتباطات الجزيئية للتراكيب الوراثية لجين البرولاكتين مع صفات محصول اللبن والدهن والبروتين من خلال تطبيق تقنية اختلاف أطوال القطع العشوائية المعتمدة لتفاعل إنزيم البلمرة المتسلسل PCR-RFLP لإيجاد تعدد أشكال النيوكليوتيدات المفردة SNP . استخدم لذلك ٤٩٤ سجل إدرار لعدد ١٨٠ بقرة حلابة مرباه في ثلاث قطعان تجريبية هي قطيع الفريزيان في محطة القرضا (FK)، قطيع الفريزيان في محطة سخا (FS) وقطيع الأبقار المحلية في محطة السرو (BS). تم تسجيل محصول اللبن والدهن والبروتين في سلالة الفريزيان لفترة ١٠ شهور لكل موسم إدرار ولفترة ٦ شهور لكل موسم في الأبقار المحلية. تم الكشف عن التراكيب الوراثية CC ، CD، DD باستخدام انزيم القطع *NaeI* بينما تم الكشف عن التراكيب الوراثية GG، GT، TT باستخدام انزيم القطع *SmaI*. تم تحليل البيانات الجزيئية لكل قطيع على حده باستخدام النموذج المختلط Mixed Model.

كانت أنماط الحزم لجين البرولاكتين في التراكيب الوراثية DD، CD، CC هي حزمة واحدة للتركيب الوراثي CC (٢٣٠ زوج قاعدي bp)، ثلاث حزم للتركيب CD (٢٣٠، ١٥٠ ، ٨٠ زوج قاعدي bps) وحزمتين للتركيب DD (١٥٠، ٨٠ زوج قاعدي bps). في حين كانت أنماط الحزم لجين البرولاكتين في التراكيب الوراثية GT، GG، TT هي حزمة واحدة للتركيب GG (١٨٠زوج قاعدي)، ثلاث حزم للتركيب GT (١٨٠، ١٢٣، ٥٧ زوج قاعدي) وحزمتين للتركيب TT (١٢٣، ٥٧ زوج قاعدي).

كانت أعلى القيم المتوقعة للأليلات (*NE*) في التراكيب الوراثية DD، CD، CC لجين البرولاكتين موجودة في التركيب الوراثي DD في حين كانت أقل القيم المتوقعة للأليلات موجودة في التركيب الوراثي CC في جميع القطعان الثلاثة ، بينما كانت أعلى القيم المتوقعة للأليلات للتراكيب الوراثية GT ، GG ، TT موجودة في التركيب الوراثي TT لجين البرولاكتين وأقل القيم كانت في التركيب الوراثي GG موجودة في جميع القطعان الثلاثة. أظهرت كل القطعان الثلاثة عدم إتزان لهاردي فينبرج لجين البرولاكتين وكان التكرار الأليلي للأليل D أعلى بكثير عن الأليل C (٠,٩٣، ٠,٨٧، ٠,٧٨ مقارنة بالتكرار ٠,٠٧، ٠,١٣، ٠,٢٢ في قطعان الفريزيان بالقرضا وسخا وقطيع البلدي بالسرو على التوالي) بينما كان التكرار الأليلي للأليل T أعلى من الأليل G (٠,٨٤، ٠,٦٤، ٠,٦٧ مقارنة بالتكرار ٠,١٦، ٠,٣٦، ٠,٣٣ في قطعان الفريزيان بالقرضا وسخا وقطيع البلدي بالسرو على التوالي). وكانت معظم قيم محتوى معلومات التنوع المتعدد Polymorphic information content (PIC) متوسطة في قطعان الفريزيان وكذا في قطيع البلدي وكانت قيمة النقص في التراكيب الوراثية الخليطة نتيجة إتباع التربية الداخلية (FIS)عالية وموجبة في قطعان الفريزيان بمزرعة القرضا (٠,٨٢) وسخا (٠,٦٢) وقطيع البلدي بالسرو (٠,٩٤).

أظهرت دراسة تنوع الارتباطات الجزيئية للتراكيب الوراثية لجين البرولاكتين مع صفات اللبن وبإستخدام تقنية SNP فروقا معنوية بين التراكيب الوراثية DD ، CD ، CC في كل صفات اللبن وأن التركيب الوراثي CD لجين البرولاكتين أعلى من التركيب الوراثي DD ، CC موضحا بذلك إرتباطا قويا للتركيب الوراثي CD مع كل صفات اللبن وكانت أيضا الفروق بين التراكيب الوراثية GT ، GG ، TT معنوية وكان التركيب الوراثي GG أعلى التراكيب موضحا بذلك إرتباطا معنويا لهذا التركيب مع كل صفات اللبن في قطعان الفريزيان والبلدي.