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# Analysis association of milk fat and protein percent in quantitative trait locus (QTLs) on chromosomes 1, 6, 7 and 20 in Iranian Holstein cattle using ten micro satellite markers

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**Protein and fat percent as content of milk are high-priority criteria for financial aims and selection of programs in dairy cattle. In order to incorporate information on marker- quantitative trait locus (QTL) associations, QTL detection and parameter estimation should also be performed. Microsatellite markers are most valuable markers in association studies between different loci with the production traits. With the aim of analysis markers previously identified by other researchers in QTLs of milk fat and protein percent traits in Iranian Holstein dairy cattle sample population, we use ten microsatellite markers on chromosomes 1, 6, 7 and 20. All microsatellite markers were genotype in 156 dairy cattle which have at least one year data of milk fat and protein record using 6 to 8% polyacrylamide gel electrophoreses determined with silver nitrate staining. We find 7, 5 and 8 out of 10 markers showing significant effect on milk fat percent in 1 to 120, 120 to 240 and 240 to 360 milking days, respectively. Our results show 6, 8 and 9 out of 10 markers have significant effect on protein percent in 1 to 120, 120 to 240 and 240 to 360 milking days respectively.**

**Key words**: Fat percent, Iranian Holstein cattle, microsatellites, milking days, protein percent, quantitative trait locus (QTL).

## INTRODUCTION

Traits like milk fat and protein percent usually have a complex determinism. They are affected by an unknown number of genes and environmental factors. By the way, these traits are economically interesting. However, advances in molecular genetics in the last decade have made it possible to find out genetic variability of complex traits into quantitative trait loci. Moreover, even if the genes involved are still unknown, individual quantitative

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**Abbrviations: QTL,** Quantitative trait locus; **PCR,** polymerase chain reaction.

**Table 1.** Primer sequence microsatellite markers.

| Chr. Number | Microsatellite marker name | Annealing temperature (C°) | Primer sequence | |
|---|---|---|---|---|
| 1 | BM1824 | 57 | ARKMKR00006708 [1] | |
| | BM4307 | 57 | ARKMKR00006776 | |
| 6 | FBN13* | 56 | Forward | ACTTTCATTAGATTGCTGCAAATAG |
| | | | Reverse | AAATATGGAAACGACCTGTGG |
| | ILSTS97* | 56 | Forward | AAGAATTCCCGCTCAAGAGC |
| | | | Reverse | GTCATTTCACCTCTACCTGG |
| | BM143 | 57 | ARKMKR00006693 | |
| 7 | BMS1979 | 58 | ARKMKR00007053 | |
| | ILSTS006 | 60 | ARKMKR00007780 | |
| 20 | AGAL29* | 57 | Forward | AGGAAGCCGAGTGAGATATGTAAGC |
| | | | Reverse | TTACAGCCTGTGTGAATGTCCTCTA |
| | ILSTS72* | 52 | Forward | ATGAATGTGAAAGCCAAGGG |
| | | | Reverse | CTTCCGTAAATAATTGTGGG |
| | BM5004 | 57 | ARKMKR00006793 | |

[1] Marker ID in ArkDB. http://www.thearkdb.org, *Reinecke et al. (2005).

trait locus (QTL) information could enhance selection efficiency and is known to be particularly beneficial when the trait is difficult or expensive to measure, when each individual performance brings a few information or when the polygenic approach has a limited efficiency or a high cost.

Numerous studies on QTL mapping of milk production traits of dairy cattle have been reported. Zhang et al. (1998), Georges et al. (1995), Vittalla et al. (2003), Huglund et al. (2009) and Arranz et al. (1998) reported significant QTL for milk fat and protein on chromosome 20. Heyen et al. (1999), Boichard et al. (2003) and Mosig et al. (2001), find out QTL on chromosome 7 affected milk fat. Freyer et al. (2003), Szyda and Komisarek (2007), Chen et al. (2006), Zhang et al. (1998), Viitalla et al. (2003), Olsen et al. (2004), Nadesalingam et al. (2001), Ashwell et al. (2004), Mei et al. (2009), Ron et al. (2002) and many other researchers have shown most significant QTL on chromosome 6 for milk fat and protein. Nadesalingam et al. (2001), Bagnato et al. (2008) and Zhang et al. (1998) said some QTL on chromosome 1 affected milk fat and protein.

This research was carried out to check out if significant QTLs markers found by other researchers have the same results in Iranian Holstein cattle sample population which is not done yet.

## MATERIALS AND METHODS

### Animals and data

Blood samples were randomly collected from tail vein of 156 dairy cattle which have at least one year milking record in herd H125 of Astan-e Qods Razavi by salting out method. Four year data of milk fat and protein percent were collected daily, automatically for statistical analysis. Based on the positional information derived from the previously mentioned QTL regions, we select 10 microsatellite markers in 4 chromosomes of cattle with primer sequence mentioned in Table 1.

### Genotyping

Simple polymerase chain reaction (PCR) has been done for each primer at suitable situation mentioned in Table 1. All microsatellite markers were genotyped and PCR products were run on 6 to 8% polyacryamide gel electrophoreses to determine genotype of each sample using silver nitrate staining. The frequency of allele and genotype for each marker was determined by direct counting of alleles.

### Statistical design

Effect of each marker on milk fat and protein percent in different sections was calculated using a General Linear Model (GLM) program of SAS 9.1 software (SAS 9.1 software, SAS Institute Inc., Cary, NC). We only use cattle's data which have 1 to 6 lactation periods. Any lactation period which has more than 360 days of milking was omitted. We have classified the lactation period (360 days) to three sections: Section one (1 to 120 days), two (120 to 240 days) and three (240 to 360 days); and these sections were used for statistical analysis, separately.

To investigate the association of candidate microsatellite markers polymorphisms with percent of milk fat and protein, the following model was applied in the Statistical Analysis System (SAS):

$$Y_{ij} = \mu + G_i + L_i + S_i + (GL)_i + (GS)_i + e_{ij}$$

Where, $Y_{ij}$ is a vector of phenotypic observations (DYD) for cattle; μ is the overall mean; $G_i$ is the fixed effect of the marker genotype of

**Table 2.** Population analysis of genetic parameters for markers.

| Shannon index | Effective allele | Null allele Frequency | PIC | Expected He | Expected Ho | He | Ho | Obs. Allele | Total | Loci |
|---|---|---|---|---|---|---|---|---|---|---|
| 1.2897 | 3.3915 | -0.1854 | 0.651 | 0.7074 | 0.2926 | 1 | 0 | 4 | 312 | AGLA29 |
| 0.9588 | 2.3309 | -0.2918 | 0.479 | 0.5728 | 0.4272 | 1 | 0 | 4 | 312 | ILSTS72 |
| 1.5443 | 3.9623 | -0.1519 | 0.710 | 0.7501 | 0.2499 | 0.9808 | 0.0192 | 6 | 312 | BM143 |
| 1.4215 | 3.4865 | -0.1828 | 0.665 | 0.7155 | 0.2845 | 1 | 0 | 6 | 312 | FBN13 |
| 1.2581 | 3.1935 | -0.2039 | 0.635 | 0.6891 | 0.3109 | 1 | 0 | 4 | 312 | ILSTS97 |
| 1.2020 | 2.7722 | -0.2434 | 0.572 | 0.6413 | 0.3578 | 1 | 0 | 5 | 312 | BM4307 |
| 1.0731 | 2.5825 | -0.2422 | 0.535 | 0.6147 | 0.3853 | 0.9679 | 0.0321 | 4 | 312 | BMS1979 |
| 1.3269 | 3.2344 | -0.1995 | 0.636 | 0.6930 | 0.3070 | 1 | 0 | 5 | 312 | BM1824 |
| 1.2487 | 2.8927 | -0.2482 | 0.597 | 0.6564 | 0.3436 | 1 | 0 | 6 | 312 | BM5004 |
| 1.2691 | 3.2653 | -0.1935 | 0.637 | 0.6960 | 0.3040 | 1 | 0 | 4 | 312 | ILSTS006 |
| 1.2611 | 3.1141 | -- | 0.612 | 0.6739 | 0.3261 | 0.9949 | 0.0051 | 4.9 | 312 | Mean |
| 0.1639 | 0.4770 | -- | -- | 0.0526 | 0.0526 | 0.0112 | 0.0112 | 0.8756 | -- | SD |

**Table 3.** P-Values of loci and fixed effects on milk fat percent and their Interactions.

| Loci fixed effect | Fat (%) | | | Interaction between fat and lactation period (%) | | | Interaction between fat and season of giving Birth (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 -120 | 120 -240 | 240-360 | 1 - 120 | 120-240 | 240-360 | 1 - 120 | 120-240 | 240-360 |
| AGLA29 | 0.01 | ns | 0.0001 | 0.0001 | 0.0002 | 0.0001 | 0.0001 | 0.0002 | 0.0001 |
| ILSTS72 | ns | 0.008 | 0.0001 | ns | ns | 0.0001 | 0.01 | 0.04 | 0.0001 |
| BM5004 | 0.001 | ns | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| BMS1979 | ns | ns | 0.0001 | 0.003 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| ILSTS006 | 0.05 | ns | 0.003 | ns | 0.001 | 0.0001 | ns | 0.0003 | 0.0001 |
| FBN13 | ns | ns | ns | 0.05 | 0.0001 | 0.0001 | 0.001 | 0.0001 | 0.0001 |
| ILSTS97 | 0.02 | 0.0001 | ns | 0.0001 | 0.0001 | 0.0001 | 0.01 | 0.0001 | 0.0001 |
| BM143 | 0.002 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.001 | 0.0001 | 0.0001 |
| BM1824 | 0.004 | 0.0001 | 0.0001 | 0.001 | 0.0001 | 0.0001 | 0.0001 | 0.004 | 0.0001 |
| BM4307 | ns | 0.002 | 0.0001 | 0.0003 | 0.0001 | 0.0001 | ns | 0.0001 | 0.0001 |
| Season of giving birth | 0.0001 | 0.002 | 0.0001 | | | -- | | | |
| Lactation period | ns | 0.0003 | 0.0001 | | | -- | | | |

animal i; $L_i$ is the random effect of lactation period of animal i; $S_i$ is the random effect of calving season of animal i; $(GL)_i$ is interaction effect between genotype and lactation period of animal i; $(GS)_i$ is interaction effect between genotype and calving season of animal i; $e_{ij}$ is the random residual.

Analysis of population parameters was done by POPGENE 1.32 (Yeh et al., 1999) and CERVUS 2.0 (Marshall et al., 1998).

## RESULTS AND DISCUSSION

Analysis of association of candidate microsatellite markers polymorphisms with fat percent is shown in Table 3 and results for protein percent are shown in Table 4. Population analysis of genetic parameters for each microsatellite marker shows variety in our population (Table 2). Velmala et al. (1999) reported the effects of BM143 on protein and fat percent of milk. Ron et al. (2002) find significant effect of BM143 on milking traits except milk fat yield. Freyer et al. (2003) show the effects of FBN13 on protein and fat yield. Reinecke et al. (2005), Ron et al. (2001), Zhang et al. (1998) and Georges et al. (1995) confirmed that, chromosome 6 and 20 have more QTL loci affecting milking traits. Szyda and Komisarek (2007) reported significant QTL loci on chromosome 6 affecting milking traits such as protein and fat yield. Ashwell et al. (2004) stated that some QTL loci affected protein and fat yield on chromosomes 6, 7, 11, 14 and 17. Olsen et al. (2004) and Nadesalingam et al. (2001) confirmed that QTL loci affecting protein percent and fat yield is near BM143. They show significant QTL affecting fat yield and fat percent on chromosome 1. Olsen et al. (2004) find out significant effect of FBN13 and BM143 on protein and fat percent. Zhang et al. (1998) reported significant QTL loci on chromosome 3, 6, 20 and 28 affecting milk protein and on chromosome 6, 20, 26 and 28 affecting milk fat, respectively.

**Table 4.** P-Values of Loci and Fixed Effects on Milk Protein Percent and their Interactions.

| Loci fixed effect | Protein (%) | | | Interaction between protein and lactation period (%) | | | Interaction between protein and season of giving birth (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 - 120 | 120 - 240 | 240 - 360 | 1 - 120 | 120 - 240 | 240 - 360 | 1 - 120 | 120 - 240 | 240 - 360 |
| AGLA29 | ns | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.001 | 0.0001 | 0.0001 |
| ILSTS72 | ns | 0.0008 | ns | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| BM5004 | 0.0002 | 0.01 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0002 | 0.0001 | 0.0001 |
| BMS1979 | 0.0005 | 0.005 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.02 | 0.0001 | 0.0001 |
| ILSTS006 | ns | ns | 0.0001 | ns | 0.0001 | 0.0001 | ns | 0.0005 | 0.0001 |
| FBN13 | ns | 0.01 | 0.0001 | 0.0009 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| ILSTS97 | 0.0001 | ns | 0.0001 | 0.0005 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| BM143 | 0.0001 | 0.0002 | 0.0001 | 0.004 | 0.0001 | 0.0001 | 0.001 | 0.0001 | 0.0001 |
| BM1824 | 0.009 | 0.0002 | 0.0001 | 0.004 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| BM4307 | 0.05 | 0.0005 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Season of giving birth | ns | 0.0001 | 0.0001 | | | | -- | | |
| Lactation period | 0.001 | 0.0001 | 0.0001 | | | | -- | | |

We find out significant effects of all markers on fat and protein percent on chromosome 20. Arranz et al. (1998), Viitalla et al. (2003) and Georges et al. (1995) confirm that, AGLA29 has significant effect on protein percent. Olsen et al. (2004) and Hugland et al. (2009) show significant effect of AGLA29 and BM5004 on protein yield. Ashwell et al. (2004) and Viitalla et al. (2003) show significant effect of ILSTS97 and BM5004 on protein percent.

We find out significant effect of BMS1979 on protein yield on chromosome 7, the same as Heyen et al. (1999). Ehud et al. (1998) show effect of ILSTS006 on milking traits that is not the same with our result. Heyen et al. (1999) confirmed the effect of ILSTS006 on protein yield that is not found in our study. But Mosig et al. (2001) reported significant effect of ILSTS006 on protein percent that was reviewed in this research.

Significant effect of FBN13 on protein yield was found out by Freyer et al. (2003), Szyda and Komisarek (2007) and Olsen et al. (2004). Although, Chen et al. (2006), Szyda & Komisarek (2007), Reinecke et al. (2005), Velmala et al. (1999), Zhang et al. (1998) and Ehud et al. (1998) reported significant effect of ILSTS97 on milking traits, our study does not confirm it. Most studies such as Liu et al. (2004) show significant effect of BM143 on milking traits such as protein and fat percent as seen in this study.

Significant effects of milk protein and fat on chromosome 1 have been shown by Nadesalingam et al. (2001), Viitalla et al. (2003), Bagnato et al. (2008) and Zhang et al. (1998) researches.

Comparison of our results with other reports shows that most of the markers used in this study have significant effect on fat and protein percent. Because our data are separated in different lactation periods and seasons of giving birth to calves in each cattle, we need to find out the effect of these two parameters and in this way, data show significant effect of lactation period and season of giving birth to calves as seen in Tables 3 and 4. Interaction effects between lactation period and season of giving birth with each marker are also shown in Tables 3 and 4.

It seems that FBN13 has no significant effect on fat percent in any lactation days, as it has no effect on total kilograms of fat in our last investigation. Therefore, significant effects of interaction between FBN13 and lactation periods and season of giving birth must not be described by the effect of FBN13. Our results show that most of these markers have more significant effects on fat and protein percent on 240-360 days of lactation. Also it seems most of the markers used by this research have more significant effect on protein percent. According to our results, we can say that AGLA29, ILSTS006 and BMS1979 might have more significant effect on protein and fat percent in 240-360 lactation days. ILSTS72 has significant effect on fat and protein percent in 120-240 days of lactation. BM5004 must have effectsand BMS1979 might have more significant effect on protein and fat percent in 240-360 lactation days.

ILSTS72 has significant effect on fat and protein percent in 120-240 days of lactation. BM5004 must have effects on fat and protein percent traits in 1-120 and 240-360 days of lactation. ILSTS97 might have significant effect on both fat and protein percent on 0-120 days of lactation. Two markers BM143 and BM1824 might have significant effect on both fat and protein percent on all days of lactation. And at last BM4307 might have effects on fat and protein percent in 120-360 days of lactation.

## Conclusion

Based on this study, it was reviewed that markers associated with QTL of milk fat and protein have significant effects on the Iranian Holstein cattle population. Although, Iranian crossbreed Holstein cattle are unknown to the scientific society, markers with peak of QTL effects on milk fat and protein found out by other researchers have shown the same result in our sample population. We need more exploration in our future researches based on QTL analysis to check out if QTLs are significant. Anyway, due to Iran's major policies for dairy production which is quantity and milk fat, we can use these markers to identify potential individuals for breeding.

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