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EXPRESSION PROFILE OF IMPORTANT MILK PRODUCING GENES IN CATTLE BREEDS FROM INDIA

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ABSTRACT

Milk production, a polygenic trait, involves several genes, which may be selected to act as markers for improved milk quality and quantity. Present study aims at identification of genes which are involved in the lactation pathway and later can be used for finding Quantitative Trait Markers (QTM), through real-time based gene expression for assessing milk production trait in four of our Indian cattle breeds. Milk samples were collected from different cattle breeds from major milk producing regions of the country. A total of 104 samples belonging to the five major cattle breeds (Sahiwal, Tharparkar, Jersey, Holstein Friesian and Hariana) were collected. Somatic cells were extracted from milk and RNA extraction was carried out followed by conversion to cDNA. 12 genes were sorted based on bioinformatics analysis and previously published literature. Expression of the 12 genes was studied using Taq Man chemistry in the different cattle breeds, and distance-based clustering of genes was carried out using heat map. We observed a ~67 fold CEL gene expression in Holeisten Fries. MFGE-8 was expressed ~3 fold in Jersey. TGF-β showed a ~1.5 fold increase in Holeisten Fries. Holeisten Fries exhibited a ~2 fold lactoferrin gene expression. Sahiwal exhibited a ~67 fold expression of CSN2. CSN3 was expressed ~20 fold in Jersey. LALBA showed highest expression (8 fold) in Sahiwal. FDFT1 and LPL were expressed ~99 fold and ~1.8 fold respectively in Holeisten Fries. XDH showed a ~3 fold expression in Jersey. Holeisten Fries, exhibited highest PRL gene expression. Markers analyzed in the study are of significance in milk yield and milk quality especially CSN, LALBA, XDH, PRL, MFGE8, LTF, LPL and FDFT1. These are essential markers when deciding about the milk quality and quantity, and therefore should be considered while deciding on the quality of any breed. Additionally, CEL, MFGE8, TGF-β and Lactoferrin genes do not directly affect the milk productivity, but are indispensible for their significant roles.

Keywords: Cattle, Milk, Real Time PCR, Gene, Heat Map, Quantitative Trait Markers

INTRODUCTION

Cattle form the most common ungulate raised worldwide for their milk. This can be attributed to their milk production capacity which is much more than that required by its offspring and a high nutritive value of the milk such that it may be used to cater our daily nutritional requirement. Through the passage of time we have learnt that strategies such as hybridization, artificial insemination, etc. could be devised to get an improved breed, a phenomenon called heterosis. With the advent of molecular techniques, it has now become possible to study genes responsible for superior traits in animals.

Milk production is a quantitative trait as it involves several genes. Individual quantitative trait loci (QTL) or selection of linked genetic markers may be used to increase the rate of genetic gain through the use of DNA-based genetic markers or Quantitative trait markers (QTM). Such a strategy has been used by several researchers to determine the markers that may be useful in increasing milk production, but at the

same time not compromising with the milk quality.

Previous studies have proposed several genes as potential candidates for dairy traits, and *prolactin* gene (PRL) seems to be the most promising since it plays a crucial role in mammary gland development, initiation and maintenance of lactation, and expression of milk protein genes. Prolactin stimulates mammary development and promotes the formation and action of the corpus luteum during the female reproductive cycle in mammals (1, 2). Allelic variation in the structural or regulatory sequences of PRL and variations in genes upstream and downstream to PRL in lactation pathway would be of interest because of its possible direct or indirect effect on milk production (3,4,5,6).

Such genes along with many others influence the chemical composition of milk and can serve as effective DNA markers in dairy cattle selection (7,8,9). There have been several studies which focussed on Single nucleotide polymorphisms (SNPs) in lactation pathway genes with respect to milk production and quality, none of them was successful in providing significant results since they lacked a holistic approach and the real functional variants remain encrypted. Additionally, there have been reports on expression of several milk producing genes as well, especially from India.

The present study aimed at identification of Quantitative Trait Markers (QTM). The study employs real-time based gene expression as the strategy to ascertain the plausible markers for assessing milk production trait in four of our Indian cattle breeds namely Sahiwal, Tharparkar, Jersy, Holstein Friesian and Hariana, which form the major breeds of cattle in India.

MATERIALS & METHODS

Study Samples

Milk samples were collected from several centres in Haryana, Delhi and Rajasthan, the major milk producing regions of the country. These included Local Dairy Farms / Veterinary clinics in Delhi, Rajasthan and Haryana. A total of 104 samples were collected which belonged to the five major cattle breeds in the country namely Sahiwal (24), Tharparkar (15), Jersy (20), Holstein Friesian (25) and Hariana (20). From each animal, 100 ml of milk was collected.

Isolation of somatic cells from milk

50 ml of the milk was placed in crushed ice for 2-4hrs. Later, it was centrifuged at 2200 g at room temperature for 10 minutes. Supernatant containing the hard fat layer was aspirated and discarded, leaving a 5ml residual fluid at the bottom of the tube. PBS (5ml) was added to the fluid and was re-suspended and centrifuged again at 2200g for 5min. Supernatant was discarded and 1ml of the residual fluid at the bottom was transferred by the pipette into 1.5ml eppendorf and centrifuged at 2200g for 5min. The pellet obtained was washed with PBS three times.

RNA Extraction

RNA was extracted from epithelial cells (extracted from milk) using the TRIzol reagent (*Ambion*), as per the manufacturer's protocol.

Quality Check of RNA using Bioanalyzer 1200

QC was carried out on 2100 bioanalyzer (Agilent) using Eukaryotic Total RNA Nano Series II, passing criteria was RIN value of 6 or more.

Conversion to cDNA

The total RNA extracted was converted to cDNA using the capacity cDNA Reverse Transcription kit according to manufacturer's protocol (*Applied Biosystems*).

Selection of Genes

An extensive review of literature was carried out to determine the significant genes that could be used as markers of milk production in cattle.

Real Time PCR (Lactation gene's optimization and validation for gene expression)

Gene expression was carried out using primers and probes for the shortlisted genes. Primers and probes were designed using primer express 3.0 software. The sequence of primers and probes is mentioned in Table 1. The 18S housekeeping gene was used as our reference gene.

Gene	***Forward Primer***	***Reverse Primer***	***Probe***
CEL	*ACCCTCTGCGGGTGACTCT*	*GCCAAGGACATTAGAAACCAATG*	*AGGTGGCTCAGATGC*
CSN2	*GCTCTGGCCCTTGCAAGA*	*TTTCCACAATCTCACCAGGTACA*	*AGCTGGAAGAACTCA*
CSN3	*GATGAAGAGTTTTTTCCTAGTTGTGACTAT*	*CTCCTGGGCACCCAAAAAT*	*CTGGCATTAACCCTGC*
FDFT1	*TCGCAGTTTCGCAGCTGTTA*	*CATACTGCATGGCGCATTTC*	*CAGGCGCTGGATGG*
LALBA	*AGCCCTGGGCCCTGTAGT*	*TTCATGCACCCCTGGAGATT*	*CAATGGACATGTAAGGAC*

LPL	*CAAGTCGCCTTTCTCCTGATG*	*TGACCCCCTGGTGAATGTGT*	*CGGATTTTGTAGACGTTTTA*
LTF	*CAGCTGTGTTCCCTGCATTG*	*CCCCCTTGCACAGTTGACA*	*AGACAAGCATACCCCAAC*
MFGE8	*CTGCAAGTGCCCTCTTGGAT*	*GTGAGGTGCAGGTGGTCTCA*	*CGTGGGCATCCACT*
MVD	*CCCCCTAAGTGCCTCACCTT*	*CAGGTCCCCATCTCAGCAA*	*CCCTGACCTGCACCCA*
PRL	*CCTGGTGAAGTGTGTTTCTTGAAA*	*TTCTGCGACGAACCTTTGC*	*CATCACCACCATGGAC*
TGFB	*CTCCCTGAAGGCCTCAACTCT*	*GGGTCTCCCGAGGAAAAGG*	*CCGCAAACAGACCC*
XDH	*CCCCCGGCATCGTCAT*	*GTGGGCTCGGGCTGATT*	*AGCATGTACACACTGCT*

Table 1: Table showing primer and probe sequences of the different genes included in our study.

Amplification was carried out at the following thermal profile: 50°C for 2 mins, 95°C for 10 mins, and 95°C for 15s and 60°C for 1 min for 40 cycles. The samples were run on Applied Biosystems, 7900HT machine.

Heat Map

Heat Map was constructed using the DataAssist™ software version 3.0 (Applied Biosystems). The software clusters groups based on distance calculated through ΔC_T values, using Pearson's Correlation as the measure of distance.

RESULTS

The relative quantitation plot (RQ plot) obtained after running our gene expression analysis is shown in Figure 1, and the values are given in Table 2. The values have been normalized with reference to the Hariana breed expression levels.

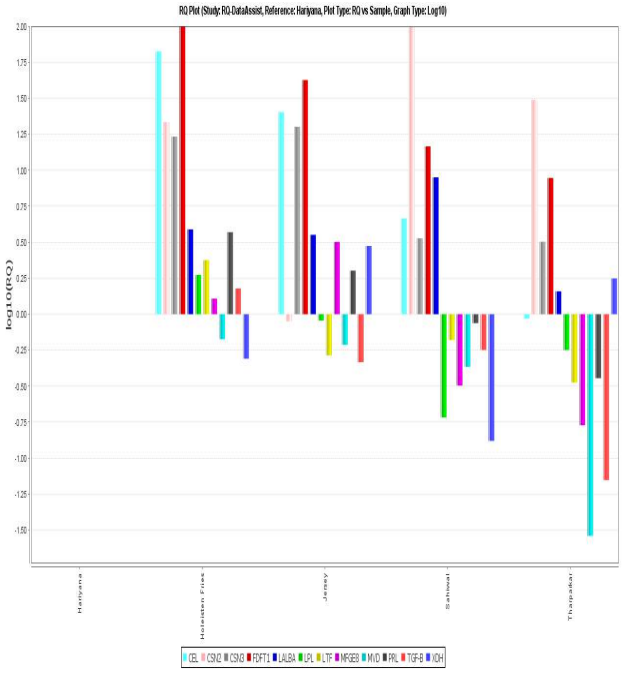

Fig 1: Gene Expression profile in different breeds

Assay	*Type*	*Hariana*	*Holeisten Fries*	*Jersey*	*Sahiwal*	*Tharparkar*
18S	Control	1	1	1	1	1
CEL	Target	1	67.1284	25.3157	4.6274	0.9325
CSN2	Target	1	21.5713	0.8908	67.3136	30.8193
CSN3	Target	1	17.121	20.0326	3.3648	3.1868
FDFT1	Target	1	99.8448	42.2945	14.6391	8.8453
LALBA	Target	1	3.8848	3.5559	8.9175	1.4416
LPL	Target	1	1.8761	0.9035	0.1912	0.5638
LTF	Target	1	2.3738	0.5169	0.6604	0.3345
MFGE8	Target	1	1.2821	3.1791	0.3189	0.169
MVD	Target	1	0.6695	0.6126	0.4304	0.0288
PRL	Target	1	3.7099	2.0049	0.8667	0.3586
TGF-B	Target	1	1.5086	0.4632	0.5639	0.0703
XDH	Target	1	0.4898	2.9754	0.1315	1.7709

Table 2: Table showing expression profile of the different genes involved in milk production in the five cattle breeds (Hariana Holeisten Fries, Jersey, Sahiwal, Tharparkar). The values have been normalized with reference to the Hariana breed expression levels.

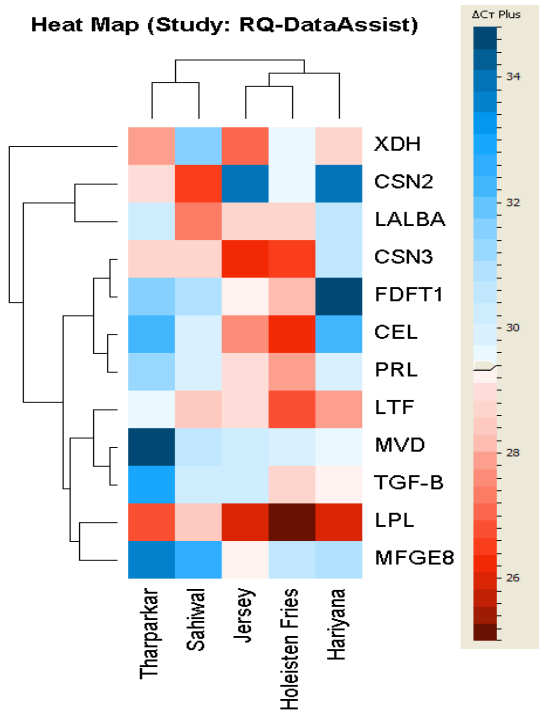

Fig 2: Heat Map (Correlation between B/W Different breeds and different gene)

DISCUSSION

The cattle breeds Sahiwal, Tharparkar, Jersey, Holstein Friesian and Hariyana, form the major and most significant milk producing breeds in India, especially in the states of Delhi, Haryana and Punjab.

The genes CEL, MFGE8, TGF-β and Lactoferrin do not directly affect the milk productivity, but are indispensible for their significant roles. CEL (Carboxy ester lipase) is an enzyme that is

involved in digestion of triglycerides. A study by Hui DY et al. reported that administration of CEL inhibitor in mice lead to improper milk fat and lipid digestion, thereby causing damage to the ileum villi (10). Expression of CEL was determined in the different cattle breeds. We observed a ~67 fold, the highest expression among all the breeds in Holeisten Fries. The protein helps in an efficient absorption in neonates. Significance of the gene is also evident from the fact that it is expressed in abundance in human milk.

Milk fat globule–EGF factor 8 (MFGE8), a milk mucin associated protein provides protection against enteric pathogens. Its expression was ~3 fold in Jersey as compared to Hariana, and was the highest MFGE-8 gene expression observed among all the breeds. It forms an important component of Milk fat globule. This was followed by ~1.2 fold in Holeisten Fries.

TGF-β (Transforming Growth Factor-β) is involved in development of an immature GI tract as in newborns, and is also involved in development of the immune system. A ~1.5 fold increase in TGF-β gene expression was observed in Holeisten Fries.

Lactoferrin, another immune defensive protein, is found in cow milk and human breast milk. Lactoferrin levels are seven times higher in the colostrum as compared to the regular breast milk. Lactoferrin can provide protection against several bacterial and viral infections. Holeisten Fries exhibited a ~2 fold higher lactoferrin gene expression, the highest among all the breeds.

Breed Performance	***Sahiwal***	***Tharparkar***	***Jersey***	***Holestein Fries***	***Hariana***
Total lactation yield (kg)	2266	2334	2727	4295	1500
Fat %	5.1	5.3	5.3 %	3.4	low
SNF %	9.2	9.1	15%	low	low

Table 2: Table showing milk performance in terms of the total yield, and Fat% and SNF % in the five cattle breeds

Source:http://www.indg.in/agriculture/animalhusbandary/breeds-of-cattle-and-their-selection
http://www.scribd.com/doc/37910717/Know-About-Important-Breeds-of-Cattle-and-Buffalos-in-India

In terms of the total lactation yield, Holstein Friesan is reported to exhibit the highest yield of 4295 kg, which is followed by 2727 kg by Jersey, 2334 kg by Tarparkar, 2266 kg by Sahiwal and the least is given by Hariana, 1500 kg.

CSN (Casein) and LALBA (Lactalbumin) refer to milk producing genes exclusive to the mammary gland, and also form the two highest expressing genes of human mammary epithelium during lactation. Significance of CSN is evident from mice experiments which report abnormal lactation and milk composition in knockout experiments. Human milk CSN is low, but mice studies reveal a high CSN. CSN2 (Casein beta), and CSN-3 (Casein-kappa) are majorly involved in formation of casein, that forms 80% of milk. Casein is important as its increased expression increase the nutritional value as well as processing properties. Thus both form important markers to decide on milk quality. CSN-2 expression has been related to milk calcium levels, whereas CSN-3 is linked with properties such as heat stability and cheese making properties. Sahiwal exhibited a ~67 fold increased expression of CSN2, followed by ~30 fold in Tharparkar, and ~17 fold in Holeisten Fries. This probably is the cause for a higher SNF% in Sahiwal (9.2%). CSN3 is expressed about ~20 fold in Jersey, and ~17 fold in Holeisten Fries. This may be involved in increasing the SNF% in Jersey (15%). LALBA is involved in milk protein and lactose synthesis, and is highest (8 fold) in Sahiwal, causing an increased total lactation yield, as well as SNF%.

FDFT1 (farnesyl-diphosphate farnesyltransferase1), a membrane linked enzyme involved in biosynthesis of cholesterol, and Lipoprotein lipase (LPL is involved in fat synthesis, and regulation of triglycerides. These are expressed ~99 fold and ~1.8 fold in H. Fries, exhibiting the highest expression among the breeds.

XDH (xanthine dehydrogenase) is involved in milk fat synthesis and lipid droplet formation. Our study reports a ~3 fold higher expression in Jersey, followed by 1.7 fold expression in Tharparkar, and ~0.13 fold expression. The highest fat% is reported from Jersey (5.3%), Tharparkar (5.3%) and Sahiwal (5.1%).

Prolactin (PRL) is involved in synthesis of milk proteins, lactose, lipids and all major milk components. It plays significant role in development, different and regulation of mammary gland. Holeisten Fries, exhibits highest expression of this gene, and is known for its high milk yield, and an average Fat% though the SNF% is low.

As is evident from the heat map (Fig 2), Holeisten Fries and Jersy, are observed to cluster closely, along with Hariana, and the breeds Sahiwal and Tharpakar cluster together. This clustering is on the basis of the expression levels of the 12 marker genes identified as significant.

In conclusion, Holeisten Fries forms the breed with the highest milk production yield, and contains an average fat percentage of 3%. But, in terms of SNF %, the Jersey is the leading breed with 15%. The markers analyzed are of significance in milk yield and milk quality especially CSN, LALBA, XDH, PRL, MFGE8, LTF, LPL and FDFT1. These give a good idea about the milk quality and quantity, and should be considered while deciding on the quality of any breed. Additionally, CEL, MFGE8, TGF-β and Lactoferrin genes do not directly affect the milk productivity, but are indispensible for their significant roles, as discussed above.

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REFERENCES

1. S. Sharifi, H. Roshanfekr, S.R. Khatami and K.H. Mirzadeh. Prolactin Genotyping of Najdi Cattle Breed Using PCR-RFLP. *Journal of Animal and Veterinary Advances*. 2010; 9 (2):281-283.
2. Le Provost, F., C. Leroux, P. Martin, P. Gaye, J.Dijane. Prolactin gene expression in ovine and caprine mammary gland. *Neuroendocrinology*. 1994; 60: 305-313.
3. Ghasemi N, Zadehrahmani M, Rahimi G, Hafezian SH. Associations between prolactin gene polymorphism and milk production in montebeliard cows. *International Journal of Genetics and Molecular Biology*.2009; 1(3):048-051.
4. Alipanah M, Kalashnikova L, Rodionov G. Association of prolactin gene variants with milk production traits in Russian Red Pied Cattle. *Iranian Journal of Biotechnology*. 2007; 5 (3):158-161.
5. He F, Sun D, Yu Y, Wang Y, Zhang Y, Association between SNPs within prolactin gene and milk performance traits in Holstein dairy cattle. *Asian-Australian Journal of Animal Science*. 2006; 19(10):1384-1389.
6. Brym P, Kaminski S, Wojcik E. Nucleotide sequence polymorphism within exon 4 of the bovine prolactin gene and its associations with milk performance traits. *Journal of Applied Genetics*. 2005; 46 (2): 179-185.
7. Bionaz M, Loor JJ. Gene networks driving milk fat synthesis during the lactation cycle. *BMC Genomics*. 2008; 9:1-21.
8. Ogorevc J, Kunej T, Razpet A, Dovc P. Database of cattle candidate genes and genetic markers for milk production and mastitis. *Animal Genetics*.2009; 40 (6): 832-51.
9. Bionaz M, Loor JJ. Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics*. 2008; 9:366.
10. Hui DY, Howles PN. Carboxyl ester lipase structure-function relationship and physiological role in lipoprotein metabolism and atherosclerosis.