Analysis of sequence variability and expression pattern of lactoferrin gene in Sahiwal cows (*Bos indicus*) and Murrah buffaloes (*Bubalus bubalis*)

Ankita Sharma[1, 2], S K Swami[1], Manoj Kumar [1], Monika Sodhi[1], R S Kataria[1], Pranay Jain[2], Avnish Kumar Bhatia[1], A K Mohanty[3], S K Niranjan[1], U K Shandilya[1], Parvesh Kumari[1] and Manishi Mukesh[1]*
[1]*ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana, India*
[2]*University Institute of Engineering and Technology, Kurukshetra University, Kurukshetra, Haryana, India*
[3]*ICAR-National Dairy Research Institute, Karnal, Haryana , India*

ABSTRACT

Lactoferrin (LF), a bioactive glycoprotein is member of transferrin family and plays an important role in immune defense, iron homeostasis, antioxidant and regulation of cell growth. The present investigation was undertaken to analyze the sequence variations and expression pattern of LF gene between cattle and buffaloes. Blood was collected from Sahiwal cows (SAC), Karan Fries cows (KFC) and Murrah Buffaloes (MUB) to isolate peripheral blood mononuclear cells (PBMCs). Total RNA was extracted from PBMCs to synthesize cDNA. Sequence analysis has shown an ORF of 2127 bp with 780 amino acids in all cattle types and buffaloes. The analysis revealed no difference between LF cDNA sequences of SAC and KFC. Whereas, on comparing with exotic cattle, a total of 6 amino acid changes viz., I145V, S538T, T546N, T596S, K627E, and H632R were identified. The comparison between SAC and MUB revealed a total of 22 amino acid changes. The comparison of MUB LF cDNA sequences revealed homology of 96.23%, 96.75%, 91.67%, 91.95%, 73.16%, 70.20%, 64.21%, 73.83% with Indian native cattle, cross bred cattle, taurine cattle, sheep, goat, horse, human, mouse and pig, respectively. Additionally, expression pattern of LF mRNA in somatic cells from different lactation stages (colostrum early, mid and late lactation) of SAC and MUB was successfully evaluated. The analysis revealed highest transcript abundance of LF mRNA in colostrum samples indicating its role in enhancing immune system of new born calves. The study has led to sequence characterization LF cDNA in native, cross bred and riverine buffaloes and identified several variations that could prove important resource for future genotype:phenotype association studies. Further, the expression data has indicated that milk derived somatic cells could be utilized as valuable source to understand mammary gland functioning of Indian cows and buffaloes.

Keywords: Lactoferrin, Sahiwal cattle, Murrah buffaloes, SNP, somatic cells, expression pattern
***Corresponding author:** mmukesh_26@hotmail.com

INTRODUCTION

Lactoferrin (LF) an iron binding glycoprotein from the transferrin family, is synthesized by glandular epithelial cells and neutrophils (Plaffl et al. 2003). It is present in most of the biological fluids including milk, mucosal uterine fluid, saliva, tears, bile and pancreatic juice with most abundance in colostrum. It constitutes an important component of innate immune system and functions as the first line of defense against infectious micro-organisms. The main physiological function of LF is to bind iron that is basic requirement for microorganism's metabolism. The binding action impounds the necessary nutritional requirement for most bacterial pathogens (Gram-negative as well as Gram-positive bacteria), thereby inhibiting their growth. It is also involved in intracellular destruction of bacteria by inducing hydroxyl radical formation, which is catalyzed by iron (Fang and Oliver 1999). It modulates the inflammatory process by early recognition of invading microorganisms and enhance immunity by activating the transcription of important immune-related genes (Plaffl et al. 2003; Yamauchi et al. 2006).

Besides immunomodulatory, anti-inflammatory, antibacterial activity, LF is also known for its anti-viral (both naked and enveloped), anti-fungal, anti-parasitic, osteogenic and anti-cancerous potential (Gibbons et al. 2015, Duarte et al. 2011; Małaczewska and Rotkiewicz 2007, Gonzalez-Chavez et al. 2009). It also inhibits enteric absorption of iron in neonates and serves as a natural antioxidant (Detilleux, 2002). LF is also involved in modulation and regulation of macrophages, lymphocytes and neutrophil function (Smith and Oliver 1981; Sordillo et al. 1987) and hence help in prevention and control of mastitis in

cows (Seyfert and Kuhn 1994; Hirvonen et al. 1999; Teng, 2002). Thus LF, a multifunctional protein plays important role in health of mammary gland and could be a potential candidate gene against disease resistance. In bovines, LF gene spans 34.5 Kb of genomic region with 17 exons and 16 introns. It is single polypeptide chain of 708 amino acids with a molecular weight of 80 KDa (Seyfert and Kuhn, 1994). The polypeptide chain is folded into two symmetrical globular lobes N (amino) and C (carboxyl) terminals, containing one iron and two bicarbonate binding sites each (O'Halloran et al. 2009). The N lobe residue comprises of 1–333 amino acids while C-lobe comprises of 345–708 amino acids. The two lobes are connected by a peptide of residues 334–344 to forms a 3-turn α-helix (Berlutti et al. 2011).

In past, several studies have shown association of certain variants of LF gene with mastitis, somatic cell counts, uterine infections and reproduction trait in dairy cattle (Cao et al. 2011; Li et al. 2004; Hajibemani et al. 2012; Valadan et al. 2011; Wojdak-Makysmiec et al. 2006; Zupin et al. 2015).The association of different LF variants with health status might be attributed to the altered surface properties and iron chelating ability of lactoferrin (Mohammed et al, 2007). Several studies related to characterization of LF gene and its biological role have also been published in mouse, human (Teng et al, 1987; Teng and Gladwell 2006; Fine et al. 2013), pig (Wang 1998), exotic cattle (O'Halloran et al. 2009; Daly et al. 2006; Zhou et al. 2006) and several other species. However, similar systematic efforts are lacking for Indian cattle and buffaloes. Although, few isolated efforts (Raja et al. 2014; Kumari et al. 2014; Kathivaran et al. 2009) have been made in Indian cattle and buffaloes but these studies mainly focused on few exons or genotyping of certain alleles of LF gene. Further, data on comparative sequence variations of LF gene across native, cross-bred and exotic cattle and its expression pattern is also lacking. Hence, this particular study was planned to characterize the complete coding region of LF gene in two cattle types (native and cross-bred cattle) and compare its sequences with riverine buffaloes. Further, efforts were made to evaluate the expression pattern of LF mRNA in somatic cells across lactation stages of native cows and riverine buffaloes.

MATERIALS AND METHODS

Selection of animals, RNA isolation and cDNA synthesis

For sequence characterization of lactoferrin gene, blood samples were collected from 9 adult animals,3 each of Sahiwal cows (SAC), Karan Fries cows (KFC) and Murrah buffaloes (MUB)maintained at cattle farm of National Dairy Research Institute, Karnal. The blood samples were transported to laboratory for isolation of peripheral blood mononuclear cells (PBMCs). The PBMCs were isolated using density gradient centrifugation method by employing HiSep reagent (Himedia). Briefly, blood samples were diluted (1:1) with 1X PBS, and gently over laid on HiSep reagent. The mixture was centrifuged at 400g for 30min at room temperature. After removing the buffy coat, cells were treated with 2 ml chilled RBC lysis buffer and mixed gently with pasture pipette at room temperature for 10 minutes. The reaction was stopped by adding 8.0 ml of 1X PBS to remove the traces of HiSep and RBC lysis buffer followed by centrifugation at 260g for 10 min at room temperature. The supernatant was discarded to obtain a white pellet of PBMCs. The isolated PBMCs were washed twice with 1X PBS. Total RNA was extracted from 9 PBMC samples using Trizol reagent (Invitrogen Corp., CA). To remove the traces of genomic DNA, RNeasy Mini kit columns (Qiagen, Germany) along with on column digestion by RNAse free DNase enzyme (Qiagen, Germany) was used. The RNA quantity and quality was assessed using Nanovue (GE healthcare). RNA integrity was confirmed by denaturing agarose gel electrophoresis. First strand cDNA was synthesized using 1.5µg of purified RNA with Revert Aid First Strand cDNA Synthesis Kit (Fermentas, Thermo Scientific) following manufacturer's instructions. Briefly, the mixture containing RNA, 1 µl Oligo $dT_{(12–18)}$, 1 µl 10mM dNTP mix and 1 µl random primers, was incubated at 65°C for 5min and kept on ice for 3min. Further, a total of 6.0 µl of enzyme mix composed of 5X enzyme buffer, 1.0 µl M-MuLV RT (Fermentas, Thermo Scientific) and 1.0 µl of RNase inhibitor was

Table 1. Checking of contaminations in the RNA isolated from spermatozoa

Primer	Sequence	Purpose
LF1F	GTCCCATGGCCCCGAGGAAAAACGTTCGATGGTGTA	Amplification
LF1R	ACGTGCACCCCTCGTCAGGAAGGCGCAG	
LF2In-1	GGAATCCTTCGCCCGTACTT	Sequencing
LF3In-2	AGGCGCAGGAGAAATTTGGA	Sequencing
LF4In-3	CCTGGCAGAGAACCGGAAAT	Sequencing

added in the reaction. The reaction was performed in an Eppendorf Gradient cycler using the program as 25°C for 5 min, 50°C for 60 min and 70°C for 15 min.

PCR amplification, sequencing and analyzing sequence data

To amplify 2.1 Kb coding region of lactoferrin gene, specific primers were designed using Primer3 NCBI tool. The LF cDNA was amplified using primers designed based on *Bos taurus* sequence (Acc No.L08604.1). The description about the primers used for amplification and sequencing are given in Table 1. The amplification of LF cDNA was performed in 25 µl reaction containing cDNA as template, 10 pmol of forward and reverse primers, 10mM of dNTPs (Invitrogen Corp., CA), 1.0 unit of Taq DNA polymerase (Fermentas, Thermo Scientific) and 5.0µl of 5X reaction buffer. The thermal cycle conditions used were as follows: initial denaturation at 95°C for 2 min 30 sec, 32 cycles at 94°C for 45 sec, 64°C for 45 sec and extension at 72°C for 1.0 min, followed by final extension at 72°C for 10 min. PCR amplified product was visualized on 1.5% agarose gel. After purification, the PCR products were sequenced using BigDye Terminator Cycle Sequencing kit (Applied Biosystems). The chromatogram of each sequence obtained was checked manually. Sequences base calling was performed with Phred available in the suite Codon code Aligner v. 3.5.1. (Codon Code Corp., Dedham, USA). Contigs produced by the overlapping primers were aligned in consensus with *Bos taurus* reference sequence (Acc.No.L08604.1) to generate complete sequence of LF gene using Codon-code aligner and MEGA 6.0 tools. To identify variations/SNPs across coding region of LF gene, the sequences were subjected for multiple sequence alignment using CLC genomic workbench 8.5 software. Protparam tool (http://ca.expasy.org) was utilized to analyze the physiochemical properties such as molecular weight, pI, instability index and grand average of hydropathy (GRAVY) of LF. MEGA 6.0 (Tamura et al. 2011) was used for the phylogenetic analyses Distances were estimated by the p-distance model (Kimura and Crow, 1964) and the standard errors of the estimates were obtained through 1000bootstrap replicates.

Expression analysis of LF gene

To understand the expression pattern of LF mRNA across lactation stages in Sahiwal cows and Murrah buffaloes, a total of 40 milk samples, 20 each from both the dairy species were collected. Five milk samples representing each specific stage of lactation: colostrum (0-2 days), early (10-30 days), mid (90-120 days) and late (>240 days) were utilized to

Table 2. Primer details, annealing temperature, amplification efficiency and slope for target (LF) and reference (GAPDH, ACTB, UBC) genes in qPCR based expression analysis

Gene	Primer sequences	Tm	Efficiency	Slope
LF	GAACATCCCCATGGGCCT	60°C	102.21	-3.19
	CAGCCAGGCACCTGAAAG			
GAPDH	TGGAAAGGCCATCACCATCT	60°C	101.11	-3.28
	CCCACTTGATGTTGGCAG			
ACTB	GCGTGGCTACAGCTTCACC	60°C	104.80	-3.10
	TTGATGTCACGGACGATTTC			
UBC	TCCCTACCTGCATCATGTGC	60°C	102.45	-3.17
	GGAATTTGGGCCAGTGCTC			

isolate somatic cells. Post collection, milk samples were immediately defatted by centrifugation at 4000 rpm at 4°C for 20 min. After removing the fat layer, somatic cells were washed twice with 1X PBS at 3000 rpm for 10 min at 4°C. The cells were trizolated and processed for RNA isolation using Trizol reagent (Invitrogen Corp., CA). The quality and quantity of extracted RNA was assessed using nanovue (GE Healthcare). The purified RNA was used to synthesize first strand cDNA using Revert Aid First Strand cDNA Synthesis Kit (Fermentas, Thermo Scientific) following manufacturer instructions. The reaction was performed in an Eppendorf Gradient cycler using the program: 65°C for 5 min, 42°C for 60 min and 70°C for 5 min. The amplification was performed in 10 µl volume using StepOne Plus instrument (Applied Biosystems) using 96-well optical plate (Applied Biosystem). The reaction mixture consisted of 4 µl diluted cDNA, 6 µl mix of 5µl 2X Syber Green with ROX master mix (Fermentas, Thermo Scientific), 0.4 µl each of 10µM forward and reverse primers, and 0.2µl DNase/RNase-free water. Each sample was run in duplicate along with the reference gene. The amplification reaction conditions followed were: 10 min at 95°C, 40 cycles of 15 sec at 95°C (denaturation) and 1 min at 60°C (annealing + extension). To assess the sensitivity and specificity of the assay, a dissociation protocol with an incremental temperature of 95°C for 15 sec plus 65°C for 15 sec was performed at the end of each run. The qPCR data was normalized using panel of reference genes (*ACTB, GAPDH* and *UBC*) identified in a previous study (Varshney et al. 2012). The description of target gene and reference genes in terms of gene symbols with primer sequences, annealing temperature and their respective reaction efficiency and slope are presented in Table 2.

RESULTS AND DISCUSSION

Sequence analysis of Lactoferrin gene

This study was planned to characterize Lactoferrin, one of the important multifunctional gene in Indian native cattle (*Bos indicus*) and compared with crossbred, exotic cattle (*Bos taurus*) and riverine buffaloes (*Bubalus bubalis*). For characterization of LF coding region of about 2.1 Kb length, 9 cDNA samples of SAC, KFC and MUB, were successfully amplified using specific primer pairs (Figure 1). The manual inspection of chromatograms indicated good quality of nucleotide sequences in each of the sample. The initial sequences analysis of LF cDNA revealed the presence of complete CDS region in all the samples. The gene was found to have an ORF of 2127 bp with 780 amino acids and predicted molecular mass of 78.08 KDa. The comparison of sequence data indicated the existence of highly conserved structural organization of LF gene across the native, crossbred, exotic cattle and buffaloes. The molecular weight, pI and instability index of LF as deduced from Protparam in SAC and KFC cows showed similar

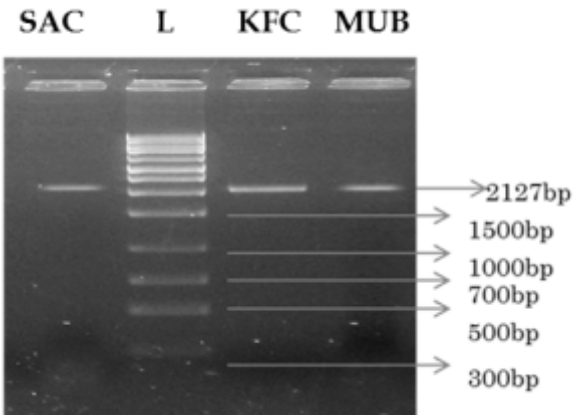


Figure 1. *Full length PCR product of lactoferrin cDNA. Lane 1: Sahiwal cattle (SAC); Lane 2: Molecular weight marker (L); Lane 3 Karan Fries cattle (KFC) and Lane 4: Murrah buffalo (MUB)*

Table 3. Physiochemical properties of LF in SAC, KFC, exotic cattle and MUB

	Physiochemical properties				
Lactoferrin	Length	Mol. Weight	Theoretical PI	Instability index	GRAVY
Exotic cattle	708	78.05	8.69	40.99	-0.289
SAC	708	78.08	8.65	41.79	-0.301
KFC	708	78.06	8.65	41.97	-0.285
MUB	708	77.69	8.36	40.90	-0.253

values as78.08 KDa, 8.65, 41.79, respectively. However, the values were slightly different from deduced *Bos taurus* values as 78.05KDa, 8.69, 40.99; whereas the values in Murrah were 77.69 KDa, 8.36 and 40.90, respectively (Table 3). The GRAVY index, an indicator of the solubility of proteins revealed hydrophilic nature of LF with values of -0.289, -0.301, -0.285, -0.253 for exotic cattle, SAC, KFC and MUB, respectively. The physiochemical parameters observed for LF in present study were somewhat similar to that reported in other cattle breeds (Pierce et al. 1991; Shashidharan et al. 2011).

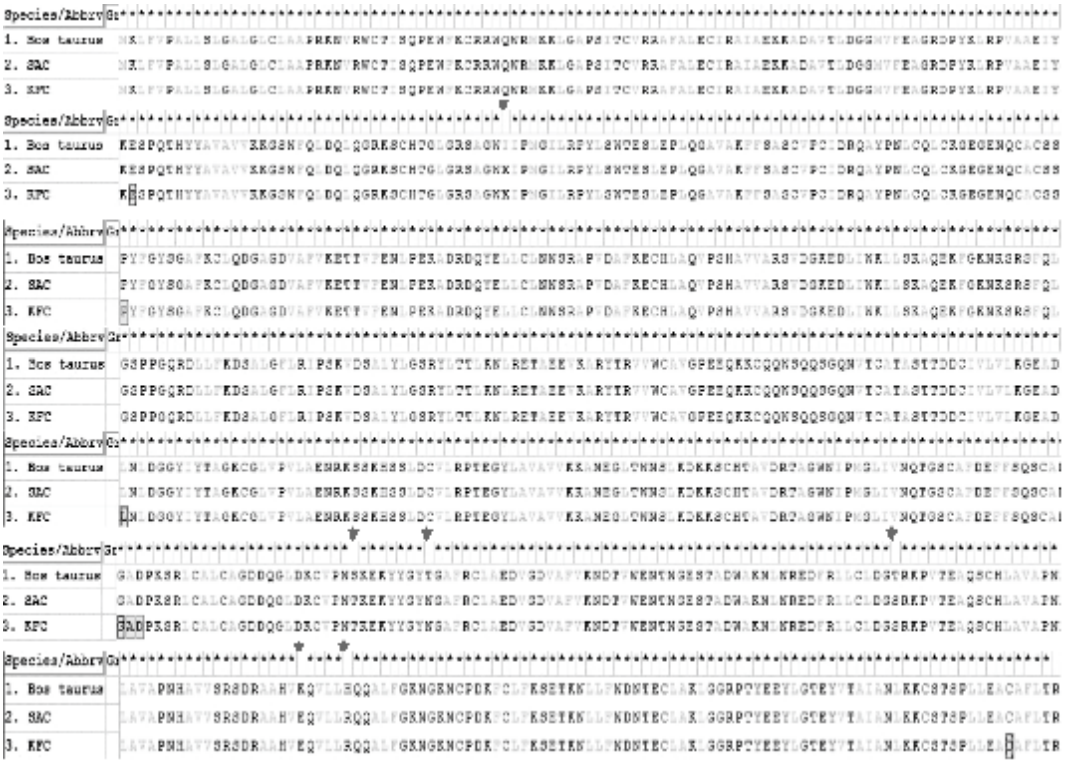
Comparison of Lactoferrin cDNA sequences across native, crossbred and exotic cattle

To identify the sequence variations in LF gene across different cattle types (native, crossbred and exotic), the sequence data for SAC (native) and KFC(cross bred) cows were compared with *Bos taurus* (exotic) reference sequence (acc. no. L08604.1). Interestingly, no variation was observed between SAC and KFC, either at nucleotide or amino acid level. However, when the sequences were aligned with *Bos taurus* sequences, a total of 6variations covering exons 4, 13 and 15 were identified (Figure 2). The 6SNPs found in the present data set were non-synonymous in nature as these could bring changes in amino acid composition in the transformed products. Amongst the 6 amino acid variations, 1 variation was observed in exon 4, 2 in exon 13, and 3 in exon 15. Remaining 14 exons were found to be monomorphic across the three cattle types.

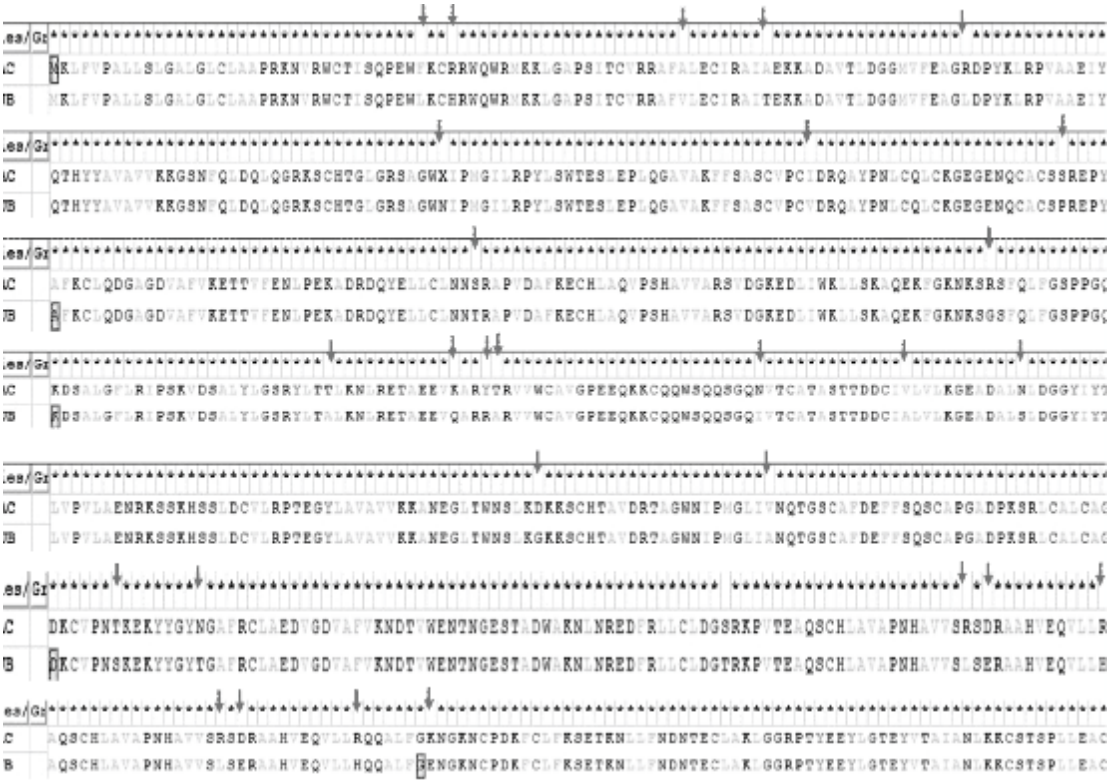
The SNP (A433G) in exon 4 resulted in substitution of isoleucine to valine at amino acid position 145. This particular SNP has also been reported in few other studies in exotic cattle (Li et al. 2004; O'Halloran et al. 2009). The nucleotide changes in exon 13 (T1612A and C1637A) also led to change in amino acid between sequences of SAC/KFC and exotic cattle. The SNP at locus T1612A resulted in substitution of threonine from serine at amino acid position 538. The second locus in exon 13 (C1637A), also led to substitution of a new amino acid. The nucleotide variation of C to A resulted in substitution of asparagine from threonine at 546 amino acid position. Both these SNPs, were found to be located at C lobe of the protein. These changes have also been reported earlier in different exotic cattle types in dbSNP data base (rs379782196 and rs208566369). Similarly, the three nucleotide changes in exon 15 also resulted in amino acid changes. The T1786A resulted in threonine to serine substitution, A1879G resulted in lysine to glutamic acid substitution, and A1895G resulted in histidine to arginine substitution at amino acid positions 596, 627 and 632, respectively.

Sequence analysis of Lactoferrin gene in SAC and MUB

The comparison of LF sequences of SAC and MUB revealed a total of 22 amino acid changes (Fig. 2b and Table 4). Out of 17 exons, 6 were conserved (monomorphic) between SAC and MUB. Exon 9 was most diverse with 5changes followed by 4 changes in exon 2;3 changes in exon 15;2 changes in exon 5,8 & 12; and one change in exon 3,4,7,10 and 16. Other than variations observed at position 145 and 632 amino acids, all other polymorphic sites were unique to buffaloes. At position 145, SAC and KFC had valine while MUB had asparagine. At position 632, both MUB had histidine while it was substituted with arginine in SAC and KFC. These changes depicted the species specific differences between cattle and buffaloes. Although large number of variations were identified in MUB, two variations *viz*., leucine to phenylalanine at amino acid (aa) 172 and threonine to isoleucine at aa 286 in MUB as reported by (Kathiravan et al. 2010) were not observed in the present study. Further, on comparison of MUB LF sequences with that of *Bubalus Arnee* (AJ005203.1), three amino acid changes *viz*., asparagine to lysine at 132, phenylalanine to leucine at 164 and cysteine to serine at 322 amino acid positions were identified. Similar to our findings, Kang et al. (2008) also observed higher variability among species than within species while analyzing 60 LF sequences from 11 species. O'Halloran et al. (2009) identified 47 variations within exonic region in six different Irish cattle populations. Amongst these, two changes were observed to affect the iron chelating ability of protein and hence overall immune function. Other studies have also shown changes in lactoferrin amino acids and altered functional and structural properties (Velliyagounder et al. 2003; Lee et al. 1997). Wojdak-



(a)



(b)

Figure 2. a) Alignment of amino acid sequences of lactoferrin across SAC, KFC and Bos taurus cattle b) Alignment of amino acid sequences of lactoferrin between SAC and MUB

Table 4. Data highlighting changes in LF gene in SAC vs MUB and exotic vs SAC/ KFC

S. No			SAC vs. MUB*		Exotic vs. SAC/ KFC**	
	Exonic Location	Location in Protein	Changes in Nucleotide	Changes in amino acids	Changes in Nucleotide	Changes in amino acids
1.	2	N lobe	T106C	Phe36Leu		
2.	2	N lobe	G116A	Arg39His		
3.	2	N lobe	C182T	Ala61Val		
4.	2	N lobe	G205A	Ala69Thr		
5.	3	N lobe	G263T	Arg88Leu		
6.	4	N lobe	G434A	Val145Asn	A433G	Ile145Val
7.	5	N lobe	A538G	Ile180Val		
8.	5	N lobe	T610C	Ser204Pro		
9.	7	N lobe	G761C	Ser254Thr		
10.	8	N lobe	C907G	Arg303Gly		
11.	8	C lobe	A1036G	Thr346Aal		
12.	9	C lobe	A1072C	Lys358Gln		
13.	9	C lobe	T1081C	Tyr361Arg		
14.	9	C lobe	A1084G		C1086G	Thr362Ala
15.	9	C lobe	A1160T	Asn387Ile		
16.	9	C lobe	T1202C	Val401Ala		
17.	10	C lobe	A1235G	Asn412Ser		
18.	12	C lobe	A1415G	Asp472Gly		
19.	12	C lobe	T1481C	Val494Aal		
20.	13	C lobe			T1612A	Ser538Thr
21.	13	C lobe			C1637A	Thr546Asn
22.	15	C lobe			T1786A	Thr596Ser
23.	15	C lobe	1856	Arg619Leu		
24.	15	C lobe			A1879G	Lys627Glu
25.	15	C lobe	A189G	Arg632His	A1895G	His632Arg
26.	16	C lobe	1915	Lys639Glu		

*The first nucleotide/amino acid is for SAC and second is for MUB. ** The first nucleotide/amino acid is for exotic cattle and second is for SAC/KFC.

Maksymiec et al. (2006) showed significant association between the somatic cell counts and LF variants in Holstein Friesian cows. Huang et al. (2010) successfully tried to associate LF haplotypes with mastitis in Chinese Holstein cattle. Association of polymorphism in LF with occurrence of mastitis has also been observed in crossbred cattle by Rahmani et al. (2012). The present data provides the basic information of genetic structure and polymorphism in lactoferrin, a potent candidate gene for disease resistance in different cattle types and buffalo. The SNPs identified in present study can be explored further to examine their impact on functional properties of this major protein.

Comparative sequence analysis for SAC and KFC revealed hundred percent homology at nucleotide level while homology percent with taurine (exotic) cattle was 96.07% and 96%, respectively. The MUB LF sequences revealed homology of 96.23%, 96.75%, 91.67%, 91.95%, 73.16%, 70.20%, 64.21%, 73.83% with Indian native cattle, cross bred cattle, taurine cattle, sheep, goat, horse, human, mouse and pig, respectively at amino acid level, indicating the high similarity of this gene among the mammalian species. Phylogenetic analysis of SAC, KFC, and MUB LF gene with different species revealed close clustering of cattle types followed by buffaloes and small ruminants (sheep and goat) (Figure 3). The

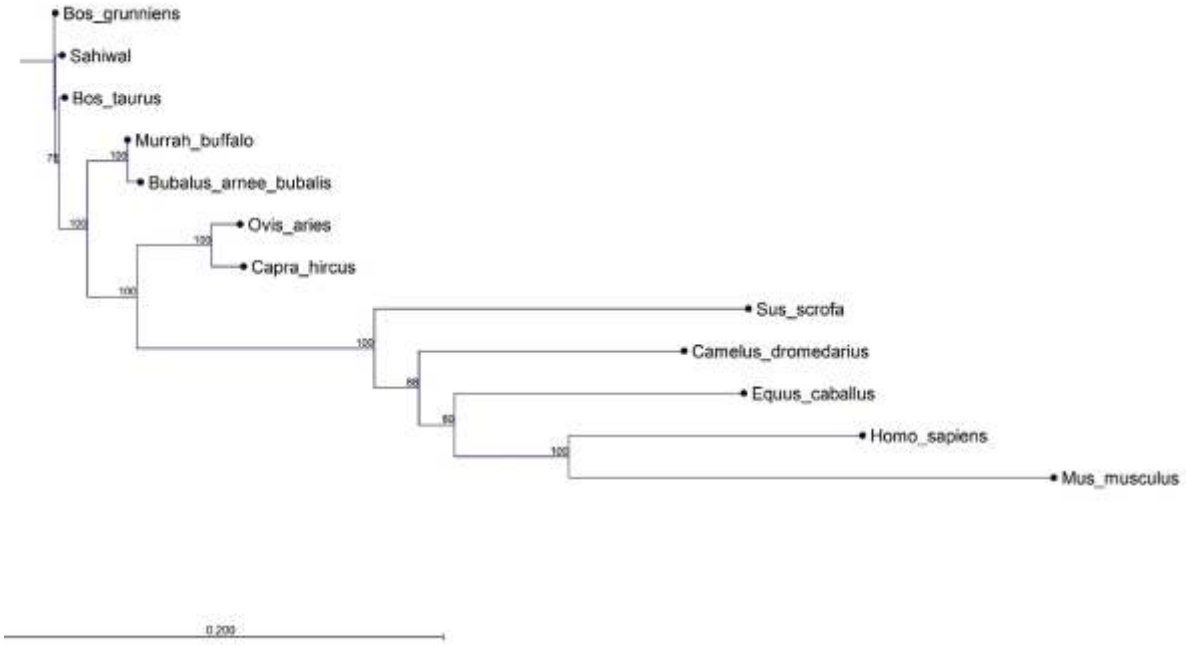


Figure 3. Evolutionary relationship across different species based on LF cDNA sequence data

high sequences similarity of LF gene across livestock species was also reported by Shashidharan et al. (2011) and Teng et al. (2012).

Relative expression pattern of LF mRNA in somatic cells of SAC and MUB

The study has also evaluated the expression pattern of LF gene in milk derived somatic cells harvested across different stages of lactation in MUB and SAC. The somatic cells were used as a source for LF mRNA as this gene is known to be abundantly present in milk secretions. All the 40 somatic cells representing different lactation stages *viz*., colostrum, early, mid and late lactation showed good quality RNA with $A_{260/280}$ ranging from 1.8-2.0. The normalized qPCR data of LF mRNA when compared across different lactation stages, it showed maximum abundance ($p<0.01$) in colostrum samples in both the dairy species (Fig. 4). The presence of LF mRNA at maximal level in colostrum samples could be attributed to its role in providing immunity to the calves. Subsequently as the lactation progressed, its mRNA level decreased continuously from colostrum to early-, and mid- lactation stages before increasing significantly again during late lactation stage. Overall, the pattern of expression of LF transcript was more or less similar in the two species and its expression was higher in colostrum and during late lactation stages. Higher abundance of LF gene in colostrum may be due to its contribution in transferring iron into the colostrum milk, and act as an important immune proteins specialized to combat various infections in new born calves. Whereas, the higher expression in late lactation stage could be due to its role in developmental process and help mammary gland to be prepared for involution stage. The present study also provides a strong clue that milk derived somatic cells could be used as an alternative non-invasive resource to study mammary

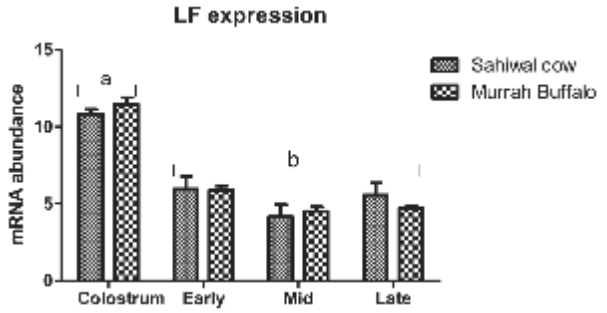


Figure 4. *mRNA abundance of LF across different lactation stages in SAC and MUB. Different letter showed significant changes in expression level ($p<0.01$)*

gland functioning of mammary gland in dairy animals.

In conclusion, the present study has characterized the LF cDNA in native, crossbred and riverine buffaloes. The comparison of coding sequences of LF gene between native cattle (SAC) and exotic cattle resulted in identification of 6 variations. Similarly, the analysis of LF cDNA between native cattle (SAC) and riverine buffaloes (MUB) has identified a total of 22 variations. All the SNPs identified between native and exotic cattle were non-synonymous in nature as these led to substitution of amino acids. Further, the study has successfully delineated the expression pattern of LF mRNA in milk derived somatic cells of SAC and MUB across different lactation stages. The analysis has shown maximum abundance of LF mRNA in colostrum samples of both SAC and MUB attributing to its role in providing immunity to the calves. Such type of studies will provide information on new variations/SNPs in LF gene in native cattle and riverine buffaloes. In future, these could be utilized as genomic resource for *in-silico* as well as genotype : phenotype association studies especially for disease resistance traits in the two major dairy species of our country.

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