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# Bovine lactoferrin and its functions in animals - A review

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**ABSTRACT**

Lactoferrin (Lf) was discovered in 1939 as "red protein from milk whey". Bovine lactoferrin (bLf) gene is located on *Bos taurus* autosome, long arm of chromosome no.22 (BTA 22q24) in cattle. Its size varies from 23-35kbp among different species. The lactoferrin gene consists of 17 exons and 16 introns ranging from 82bp (exon-1) to 225bp (exon-17). The presence of multiple regulatory elements within lactoferrin promoter contributes differential gene expression and variable content of lactoferrin in milk. The concentration of lactoferrin in normal bovine milk is about 0.02-0.2 mg/ml. The primary function of Lf lies in its role in iron metabolism including iron transport, storage and chelation. Lf exhibits strong antimicrobial activity against a broad spectrum of bacteria (gram-positive & negative), fungi, yeasts, viruses and parasites. Lf exerts bacteriostatic and bactericidal activity. Its main contribution to antiviral defence consists in its binding to the cell membrane glycosaminoglycan, thus lactoferrin prevents viruses from entering cells and infection is stopped at an early age. More than 140 SNPs in this gene have been identiûed. Such a high variability in Lactoferrin gene implies that it may be used as candidate gene for screening animals also a marker of milk yield.

**Key words:** Antibacterial, Antiviral, Lactoferrin, Milk and Polymorphism.

Lactoferrin (Lf) was discovered in 1939 as "red protein from milk whey" (Sorenson and Sorenson, 1939). It was initially identified in bovine milk and later found in milk from other species (Masson and Heremans, 1971). It is a non-hemachrome ferric ($Fe^{3+}$) ion-binding glycoprotein of the serum transferrin (Tf) family, and is synthesized mainly by glandular epithelial cells and neutrophils (Plaff *et al.*, 2003). It was first purified from human and bovine milk in 1960 and defined as transferrin like glycoprotein (Wakabayashi *et al*., 2006). It is abundant in milk and in most biological fluids (saliva, tears, nasal secretions etc.) and brings about adaptive and innate immune response.

**Structure of lactoferrin protein:** Bovine Lactoferrin (bLf) is an 80kDa glcosylated, monomeric metal binding protein of 708 amino acids with 19 amino acid signal peptides (Mead and Tweedie, 1990; Pierce *et al*., 1991). The single polypeptide chain is folded into two symmetrical globular lobes representing N (Amino) and C (Carboxy) terminal halves of the polypeptide connected with an alpha-helix (Moore *et al.*, 1997). Lactoferrin are glycosylated and position of potential glycosylation sites may vary among proteins. Asparagine (Asn) residues in the N and C terminal lobes provide several potential N-glycosylation sites. The amino acid sequence of lactoferrin has N-glycosylation sites as Asn-233, Asn-281, Asn-368, Asn-476 and Asn-545 (Pierce *et al.*, 1991). Lf is capable of binding $Fe^{2+}$ or $Fe^{3+}$ ions and can also bind with $Cu^{2+}$, $Zn^{2+}$ and $Mn^{2+}$ ions. Within the iron binding sites of each lobe, amino acids Asp-60, Tyr-92, and His-253 are directly involved in iron binding while Arg-121 is involved in binding the anion ($CO_3^{2-}$ ions) (Crichton, 1990).

**Bovine lactoferrin gene:** Bovine lactoferrin (bLf) gene is located on *Bos taurus* autosome, long arm of chromosome no.22 (BTA 22q24) in cattle (Schwerin *et al*., 1994). Its size varies from 23-35kbp among different species (Teng, 2002). In bovines, it spans about 34.5kbp in which the promoter region consists of 1122bp with GC rich region and has a non-canonical TATA box, multiple stimulating protein (sp-1) and other putative binding sites for transcription factors including nuclear factor-κB (NF-κB), activator protein 1 (AP1), signal transducer and activator of transcriptions 3 and 5 (STAT 3 and STAT 5) and steroid hormone receptors (Zheng *et al.*, 2005).

The lactoferrin gene consists of 17 exons and 16 introns ranging from 82bp (exon-1) to 225bp (exon-17). It is having two lobes: N lobe and C lobe. N lobe is having two regions N-I and N-II and C lobe also consists of C-I and C-II. N-I lobe is comprised of exon-2, exon-3 and exon-4, whereas N-II lobe is comprised of exon-9, exon-10, exon-11 and exon-12. C-I lobe consists of exon-4, exon-5, exon-6,

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exon-7 and C-II lobe consists of exon-12, exon-13, exon-14, and exon-15. In between N lobe and the C lobe is the hinge region which comprises of exon-9. The mRNA (2351 bases) codes for a 708 amino acids protein with a 19 amino acids signal peptide immediately preceding a sequence identical to N-terminal, 40 amino acids have been reported for bovine lactoferrin gene (Goodman and Schanbacher, 1991). The presence of multiple regulatory elements within lactoferrin promoter contributes differential gene expression and variable content of lactoferrin in milk.

**Lactoferrin concentration in milk in cows at different stages of lactation:** Lactoferrin is present in variable amounts in humans, mouse, mare and sow milk but rat, rabbit and dog milk appears to be devoid of lactoferrin. The concentration of lactoferrin in normal bovine milk is about 0.02-0.2 mg/ml (Neville *et al*., 1998). Increase in concentration of lactoferrin occurs in mammary gland during involution, colostrum and udder inflammation.

**Biological functions of lactoferrin:** The primary function of Lf lies in its role in iron metabolism including iron transport, storage and chelation but it is also thought to play a role in innate defence and exhibits a diverse range of biological activities, including antimicrobial activities, antiviral activities, antioxidant activities, immunomodulation, modulation of cell growth, and binding and inhibition of several bioactive compounds, such as lipopolysaccharide and glycosaminoglycan (Chierici, 2001). The *in vitro* activity of Lf also includes transcriptional activation of several genes (Oh *et al.*, 2004). It is one of the important proteins that have anti-infective properties & can prevent or control mastitis in dairy cattle (Teng, 2002). Lactoferrin stimulates the immune system and serves as a natural antioxidant (Detilleux, 2002).

**Antibacterial function:** Lf exhibits strong antimicrobial activity against a broad spectrum of bacteria (gram-positive & negative), fungi, yeasts, viruses and parasites (Kirkpatrick *et al*., 1971) but has beneficial effects on *lactobacillus* and *bifidobacterium* (Garcia *et al.*, 2011). Lf exerts bacteriostatic and bactericidal activity. The bacteriostatic activity is by binding to iron and bactericidal is by interaction of lactoferrin and bacterium. Lactoferrin has ability to bind free iron, one of the elements responsible for growth of bacteria, is responsible for the bacteriostatic effect of lactoferrin (Arnold *et al.*, 1980). Lactoferrin binds to lipopolysaccharide of bacterial cell wall and via peroxide formation oxidizes bacteria, thus, affecting membrane permeability and resulting in cell lysis (Farnaud and Evans, 2003). Antimicrobial activity of lactoferrin affecting the bacterial cell wall occurs due to antimicrobial peptides of an N-terminal part amino acids chain of this protein, lactoferricin and lactoferrampin. These proteins are released from native proteins by pepsin-mediated digestion. Lactoferrin potentiates the activity of beta-lactam antibiotics like penicillin (Diarra *et al*., 2002).

**Antiviral function:** Lactoferrin is capable of binding certain DNA and RNA viruses (Yi *et al.*, 1997). Its main contribution to antiviral defence consists in its binding to the cell membrane glycosaminoglycan, thus lactoferrin prevents viruses from entering cells and infection is stopped at an early age (Ward *et al.*, 2005). Lactoferrin takes part in specific immune reactions, but in an indirect manner (Legrand *et al.*, 2005). It is a potent activator of immunological functions such as granulopoiesis, cytokine production, antibody synthesis, natural killer cell toxicity, lymphocyte proliferation and complement activation and production of interleukins (IL-1), (IL-2) and TNF (Kimber *et al.*, 2002).

**Antiparasitic function:** Lactoferrin acts against parasites like the infectivity of *Toxoplasma gondi* and *Eimeria stiedai*. Sporozoites are reduced after their incubation with lactoferricin-B. It breaches parasitic membrane integrity and causes subsequent interactions between host and parasite (Omata *et al.*, 2001). The lactoferrin dependent cytokine mediated stimulation of activity of NK cells and lymphocytes stimulation $CD\text{-}4^{+}$ and $CD\text{-}8^{+}$ plays important role in defence against tumor cells. Lactoferrin has potent antifungal action (Kirkpatrick *et al*., 1971).

**Others:** Lactoferrin is also an anabolic factor affecting osteocytes. Lactoferrin stimulates osteoblast proliferation and incorporates thymidine thus reducing apoptosis of osteoblasts (Cornish *et al*., 2004). It is demonstrated that lactoferrin is involved not only in the transport of iron, zinc and copper, but also in the regulation of their intake (Shongwe *et al*., 1992). Presence of loose ions of zinc and copper does not affect the iron binding ability of lactoferrin, and might even increase it.

**Concentration of lactoferrin in secretions of the bovine mammary gland**

| Lactation stage | Lf conc. mg/ml | Reference |
|---|---|---|
| Immediately after parturition | 2.4 | Nonnecke and Smith (1984) |
| Lactating cows | 0.02-0.35 | Welty *et al.* (1976) |
| 2-4 days of involution | 2.4 | Welty *et al.* (1976) |
| 5-7 days of involution | 2.6-17.8 | Welty *et al.* (1976) |
| Sub clinical mastitis | 0.2-1.2 | Hagiwara *et al.* (2003) |
| Clinical mastitis | 0.3-2.3 | Kawai *et al.* (1999) |

**Polymorphism of lactoferrin gene:** Lactoferrin gene polymorphism occurs in coding and regulatory regions as well as in introns. A total of 29 SNP mutations were found in the promoter region of the lactoferrin gene and 47 in exons (O'Halloran *et al*., 2009). More than 140 SNPs in this gene have been identiûed (Huang *et al*., 2010). Such a high variability implies that a mastitis-resistance marker is very likely to exist within this gene, or possibly also a marker of milk yield.

Seyfert *et al*. (1994) reported polymorphism in lactoferrin gene promoter region and a polymorphic *Eco*RI restriction site in intron-6 during isolation and characterization of complete bovine Lf gene. The deletion of C and T in 5' at 838 and 810 were reported, while, substitution of A and T to G and C at 156 and 151 were also reported.

Wang *et al*. (1998) characterized the elements responsible for the expression of porcine lactoferrin gene. 5' region was cloned from a porcine liver genomic library. They analyzed 5' region consists approximately of 4kbp, two exons and an intron.

Zheng *et al*. (2005) characterized the infective responsive bovine lactoferrin promoter and an 8.2kbp of bovine lactoferrin gene containing 4.4kbp of 5' flanking region, exon 1, intron 1 and exon 2 was isolated and sequence analysis revealed that lactoferrin gene promoter contain high G/C content.

Daly *et al*. (2006) analyzed the sequence of the bovine lactoferrin gene promoter in five different cattle breeds (Holstein Friesian, New Zealand Holstein, Montebéliard, Normande, and Norwegian Red) to determine the extent of polymorphic variation, which exists in this region both within and across cattle breeds. Fifteen different single nucleotide polymorphisms (SNPs) were identified throughout this region. Numerous polymorphisms were found throughout the Holstein Friesian, New Zealand Holstein, Montebéliard, and Normande populations.

Sender *et al*. (2006) reported the association between lactoferrin gene polymorphism occurring in intron-6 using PCR-RFLP and SCC in Polish Black and White Holstein cows. Genotype BB was found to have low SCC.

Wojdak-Maksymiec *et al*. (2006) associated bovine lactoferrin gene polymorphism with SCC in milk using PCR-RFLP method using *Eco*RI enzyme in 124 Polish Black and White dairy cows of Holstein Friesian breed. Two alleles of LTF, A and B were found in the population having frequencies 67.74% and 32.56% respectively. Statistically significant associations were established between SCC, Lactoferrin genotype, lactation month, parity and HF gene share. No significant association was found between SCC and season. The highest somatic cell count was found in the milk of AB genotype, whereas the lowest was found in cows of AA genotype.

Zhou *et al*. (2006) characterized PCR-SSCP markers of bovine lactoferrin gene for clinical mastitis. PCR-SSCP analysis on four fragments with in 5' region and two fragments of exon 4, 15 of bovine lactoferrin revealed that four fragments P-1, P-4, E-4 and E-15 had polymorphism of six base mutations and two of them had significant differences in allele frequencies between resistant and susceptible groups.

Cheng *et al*. (2008) performed sequencing to detect the polymorphisms and found 6 SNPs in a region of 602bp promoter from 128 dairy cows. In the -241bp and -190bp, there were a T to C mutation and a G to A mutation, and those mutations were first discovered. The others mutations were in -28bp (A/C), +33bp (C/G) -131bp (T/C) and -156bp (A/G), respectively.

O'Halloran *et al*. (2009) characterized SNPs in bovine lactoferrin gene sequences across a range of dairy cow breed. They identified 29 polymorphisms with in 2.2kb regulatory region. 19 novel polymorphisms were also identified and some of them found within transcription factor binding sites including GATA-1 and SP-1, 47 polymorphisms were identified within exon sequences with unique polymorphism which were associated with amino acid substitutions. These included T/A SNP in Holstein Friesian animal which resulted in valine to aspartic acid substitution (Val89Asp) in the mature lactoferrin protein.

Huang *et al*. (2010) explored SNP, haplotypes and combined genotypes of lactoferrin and its association with mastitis in Chinese Holstein cattle. Three previously reported SNPs in the 5' flanking region and one novel SNP in exon-1 of lactoferrin gene were identified. Twenty two and nineteen combinations of three SNP were observed. The result of haplotype analysis of four SNPs showed that fourteen different haplotypes were observed.

O'Halloran *et al*. (2010) reported the polymorphism at the position -586, -190, and -28 of the bovine lactoferrin promoter in Holstein Friesian. The C to T polymorphism was at -586, which distorts a putative activating protein 2 (AP-2) and was associated with shorter calving interval and higher somatic cell score. The G to A polymorphism at -190 was associated with longer calving interval and decreased functional survival. Third polymorphism A to C at position -28 was associated with functional survival.

Bahar *et al*. (2011) reported that polymorphism in the bovine lactoferrin (bLf) gene promoter has the potential

to affect milk lactoferrin concentrations. In silico analysis of the 2.2kb promoter revealed two major haplotypes (BtLTF_H1a and BtLTF_H2a) that differed at 10 SNP loci that affect transcription factors of both a constitutive (at -28, -1702) and an inducible (at -131, -270, -586, -2047, -2077, -2122, -2140 and -2151) nature.

Huang *et al*. (2011) identified novel splice variants of the bLf gene in mastitis-infected and healthy cows. Sequencing analysis showed that the new splice variant was 251 bp in length, including exon 1, part of exon 2, part of exon 16, and exon 17.

Jemmali *et al*. (2011) genotyped 52 imported Tunisian Holstein cows for polymorphism of lactoferrin gene by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Amplification of the lactoferrin gene fragment revealed an 1143bp long product by electrophoresis following PCR. After restriction enzyme digestion with *HinfI*, two alleles were characterized by only one restriction fragment. Only the A/A genotype was detected.

Shashidharan *et al*. (2011) cloned and sequenced the full coding region of the Lf gene of Vechur breed of *Bos indicus* .Structural differences were located due to 11 SNPs that lead to functional variations. Out of 11 SNPs, 5 amino acid variations were under alpha helix and beta sheet regions, this might be of functional significance.

Sharifzadeh and Doosti (2011) analyzed the lactoferrin gene polymorphism in semen samples of 160 Iranian Holstein bulls with PCR-RFLP method using *EcoR*I enzyme. Two alleles, A and B with frequencies 67.74% and 32.26%, respectively and three genotypes AA, BB and AB with frequencies 32.50%, 10% and 57.50%, respectively were found.

Nanaei *et al*. (2012) also reported the lactoferrin gene polymorphism in 404 Isfahan Holstein cows by using PCR-RFLP/*EcoR*I technique. Two alleles, A and B were found in the examined population.

Rahmani *et al*. (2012) investigated the association of genotypes at promoter region of lactoferrin gene with mastitis in crossbred cattle. The study revealed the polymorphism at the 1049bp long fragment corresponding to the promoter lactoferrin locus by using PCR-RFLP/*Hinf* I technique. Genotype AA was higher in mastitis free group while BB was higher in mastitis group.

Sharma (2013) reported genotypic frequency of AA, AB and BB as 0.50, 0.10 and 0.40, respectively for the Jersey crossbred cattle in India.

## CONCLUSION

Lactoferrin is an important component of milk and its concentration in milk also an indicator of health status of the animals. It has wide range of activities including defence mechanism. Lactoferrin gene is highly polymorphic and has been associated with different diseases and production traits. So, it may be used as candidate gene for screening animals and also be incorporated for selection criteria for animal health improvement as well as production improvement.

## REFERENCES

Arnold, R. R., Brewer, M. and Gautheir, J. J. (1980). Bactericidal activity of human lactoferrin: sensitivity of a variety of microorganisms. *Infection and immunity*, **28**:893-898.

Bahar, B., Halloran, F., Callanan, M. J., McParland, S., Giblin, L. and Sweeny,T. (2011). Bovine Lactoferrin gene haplotypes have different basal transcriptional activities. *Animal Genetics*, **42** (3): 270-279.

Chierici, R. (2001). Antimicrobial actions of lactoferrin. *Advances in Nutritional Research*, **10**: 247–269.

Cornish, J., Callon, K. E., Naot, D., Palmano, K. P., Banovic, T., Bava, U., Watson, M., Lin, J. M., Tong, P. C., Chen, Q., Chan, V. A., Reid, H. E., Fazzalari, N., Baker, H. M., Baker, E. N., Haggararty, N. W., Grey, A. B. and Reid, I. R. (2004). Lactoferrin is a potent regulator of bone cell activity and increases bone formation *in vivo*. *Endocrinology*, **145**: 4366–4374.

Crichton, R. R., (1990). Proteins of iron storage and transport. *Advances in Protein Chemistry*, **40**: 281-363.

Daly, M., Ross, P. and Giblin, L. (2006). Polymorphism with the Lactoferrin gene promotion in various cattle breeds, *Animal Biotechnology*, **17:** 33-42.

Detilleux, J. C. (2002). Genetic factors affecting susceptibility of dairy cows to udder pathogens. *Veterinary Immunology and Immunopathology*, **88**: 103-110.

Diarra, M. S., Petitclerc, D. and Lacasse, P. (2002). Effects of lactoferrin in combination with penicillin on the morphology and the physiology of *Staphylococcus aureus* isolated from bovine mastitis. *Journal of Dairy Science*, **85**: 1141-1149.

Farnaud, S. and Evans, R. W. (2003). Lactoferrin a multifunctional protein with antimicrobial proteins. *Molecular Immunology*, **40**: 395-405.

Garcia, A. M., Cendon, T. S., Gallegos, S. A. and Cruz, Q. R. (2011). Lactoferrin a multiple bioactive protein. *Biochimica et Biophysica* Acta, 11-14.

Goodman, R. E. and Schanbacher, F. L. (1991). Bovine lactoferrin mRNA: sequence, analysis and expression in the mammary gland. Biochemical *and Biophysical* Research Communications, **180** (1): 75-84.

Hagiwara, S., Kawai, K., Anri, A. and Nagahata, H. (2003). Lactoferrin concentrations in milk from normal and subclinical mastitis cows. *Journal of Veterinary Medical Science*, **65**:319-323.

Huang, J., Wang, C., Jianbig, L., Quiling L., Hou, M. and Zhoung, L. (2010). Single Nucleotide polymorphism, haplotypes and combined genotypes of Lactoferrin gene and their association with mastitis in Chinese Holstein cattle. *Molecular Biology Reports,* **37**: 447-483.

Huang, J. M., Wang, Z. Y., Ju, Z. H., Wang, C. F., Li, Q. L., Sun, T., Hou, Q. L., Hang, S. Q., Hou, M. H. and Zhong, J. F. (2011). Two splice variants of the bovine lactoferrin gene identified in *Staphylococcus aureus* isolated from mastitis in dairy cattle. *Genetics and Molecular Research*, **10**: 3199-3203.

Jemmali, B., Kamoun, M., Ksouri, M., Rekik, B. and Mhirsi, S. (2011). PCR-RFLP Analysis of Genetic Polymorphism of the Lactoferrin gene in Tunisian Imported Holsteins. *Wayamba Journal of Animal Science*, ISSN: 2012-578X.

Kawai, K., Hagiwara, S., Anri, A. and Nagahata, H. (1999). Lactoferrin concentration in milk of bovine clinical mastitis. *Veterinary Resource Community,* **23**: 391-398.

Kimber, I., Cumberbatch, M., Dearman, D. R., Bhusman, M. and Griffiths, C. E. M. (2002). Lactoferrin influences on Langerhans cells, epidermal cytokines and cutaneous inflammation. *Biochemistry and Cell Biology*, **80**: 103-107.

Kirkpatrick, C. H., Green, I., Rich, R. R. and Schade, A. L. (1971). Inhibition of growth of candida albicans by iron–unsaturated lactoferrin: relation to hot defense mechanism in chronic muco-cutaneous candidiasis. *The Journal of Infectious Diseases*, **124**: 539-544.

Legrand, D., Elass, E., Carpentier, M. and Mazurier, J. (2005). Lactoferrin: a modulator of immune and inflammatory responses. *Cellular and Molecular Life Sciences*, **62**: 2549-2559.

Masson, P. L. and Hermans, J. F. (1971). Lactoferrin in milk from different species. *Compendium of Biochemical physiology*, **39**B: 119- 129.

Mead, P. E. and Tweedie, J. W. (1990). cDNA and protein sequence of bovine Lactoferrin. *Nucleic Acids Research*, **18:** 7167.

Moore, S. A., Anderson, B. F., Groom, C. R., Haridas, M. and Baker, E. N. (1997). Three dimentional structure of bovine lactoferrin at 2.8 L resolution. *Journal of Molecular Biology*, **274**: 222-236.

Nanaei, H. A., Edriss, M. A., Mahyari, S. A., Rahmani, H. R. and Tabatabaei, B. E. S. (2012). Lactoferrin gene polymorphism of Holstein cows in Isfahan province. *Annals of Biological Research*, **3**: 2365-2367.

Neville, M. C., Chatfield, K., Henson, L., Lewis, A., Monks, J., Nuijens, J., Oliver-Bousquet, M., Schanbacher, F., Sawicki, V. and Zhang, P. (1998). Lactoferrin secretion into mouse milk, development of secretory activity, the localization of lactoferrin in the secretory pathway and interactions of lactoferrin with milk iron. *Advances in Experimental Medicinal Biology*, **443**: 141-153.

Nonnecke, B. J. and Smith, K. L. (1984). Biochemical and antibacterial properties of bovine mammary secretions during involution and at parturition. *Journal of Dairy Science*, **67**: 2863-2872.

Oh, S. M., Pyo, C. W., Kim, Y. and Choi, S. Y. (2004). Neutrophil lactoferrin upregulates the human p53 gene through induction of NF-kB activation cascade. *Oncogene*, **23**: 8282–8291.

O'Halloran, Berry, D. P., Bahar, B., Howard, D. J., Sweeny, T. and Giblin, L. (2010). Polymorphism in the bovine Lactoferrin promoter are associated with reproductive performance and somatic cell count. *Journal of Dairy Science*, **93**: 1253-1259.

O' Halloran, F. B., Bahar, B., Buckley, F., O'Sullivan, O., Sweeney, T. and Giblin, L. (2009). Characterization of single nucleotide polymorphism identified in the bovine lactoferrin gene sequences across a range of dairy cow breeds. *Biochimie,* **91:** 68-75.

Omata,Y., Satake, M., Maeda, R., Saito, A., Shimazaki ,K., Yamauchi, K.,Uzuka,Y., Tanabe, S., Sarashina, T. and Milkami, T. (2001). Reduction of the infectivity of *Toxoplasma* and *Eimeria stiedai* sporozoites by treatment with bovine lactoferricins. *Journal of Veterinary Medical Science*, **63**: 187-190.

Pierce, A., Colavizza, D., Bennaisa, M., Maes, P., Tartar, A., Montreuil, J. and Spik, G. (1991). Molecular cloning and sequence analysis of bovine lactotransferrin. *European Journal of Biochemistry*, **196**: 177-184.

Plaff, M. W.,Whittmann, S. L., Meyer, H. H. D. and Bruckmaier, R. M. (2003). Gene expression of immunologically important factors in blood cells, milk samples and mammary tissue of cows. *Journal of Dairy Science*, **86**: 538-545.

Rahmani, S. F., Jayashankar, M. R., Nagaraja, C, S., Bhaskaran, R., Byregowda, S. M. and Satyanarayan, K. (2012). Association of genotypes at promoter region of lactoferrin gene with mastitis in crossbred cattle. *Indian Journal of Animal Sciences*, **82** (7): 718-720.

Schwerin, M., Solinas-Toldo, S., Eggen, A., Brunner, R., Seyfert, H. M. and Fries, R. (1994). The bovine lactoferrin gene (LTF) maps to chromosome 22 and synthetic group $U_{12}$. *Mammalian genome*. **5**: 486-489.

Sender, G., Korwin-Kossakowska, A., Hameed, K.G.A. and Prusak, B. (2006). Medycyna *Weterynaryjna*, **62**: 563-565.

Seyfert, H., Turkoricz, A., Interthal, H., Koczan, D and Gerd, H. (1994). Structure of the bovine lactoferrin encoding gene and its promoter. *Gene*, **143**: 265-269.

Sharma, R. (2013). DNA polymorphism of lactoferrin gene in Jersey crossbred cattle. Submitted to SKUAST-Jammu for the fulfilment of MVSc degree.

Sharifzadeh, A. and Doosti, A. 2011. Study of lactoferrin gene polymorphism in Iranian Holstein cattle using PCR-RFLP technique. *Global Veterinaria*, **6** (6): 530-536.

Shashidharan, A., Singh R., Bhasker S. and. Mohankumar C. (2011). Physicochemical characterization and functional site analysis of lactoferrin gene of Vechur cow. *Bioinformation*, **6** (7): 275-278.

Shongwe, M. S., Smith, C. A., Ainscough, E. W., Baker, H. M., Brodie, A. M. and Baker, E. N. (1992). Anion binding by human lactoferrin: results from crystallographic and physicochemical studies. *Biochemistry*, **31** (18): 4451–8.

Sorenson, M. and Sorenson, S. P. L. (1939). The proteins in whey. *Comptes-rendus des Travaux du* Laboratoire**.** Carlsberg, **23**: 55-99.

Teng, C. T. (2002). Lactoferrin gene expression and regulation: An overview. *Biochemistry and cell-Biology*, **80**: 7-16.

Wakabayashi, H., Yamauchi, K. and Takase, M. (2006). Lactoferrin research, technology and applications. *International Dairy Journal*, **16**: 1241-1251.

Wang, S. R., Jyhshiun, L., Cheng, I. C. and Yun, T. L. (1998). Characterization and functional analysis of porcine Lactoferrin gene. *Gene*, **215**: 203- 212.

Ward, P. P., Paz, E. and Conneely, O. M. (2005). Multifunctional roles of lactoferrin: A overview. *Cellular and Molecular Life Sciences*, **62**: 2540-2548.

Welty, F. K., Smith, K. H and Schanbacher. (1976). Lactoferrin concentrations during involution of the bovine mammary gland. *Journal of Dairy Science*, **59:** 224-231.

Wojdak-Maksymiec, K., Kmiec, K. and Ziemak, J. (2006). Association between bovine lactoferrin gene polymorphism and somatic cell count. *Veterinary Medicine*, **51:** 14-20.

Yi, M., Kaneko S., Yu, D.Y. and Murakami, S. (1997). Hepatitis virus envelope proteins bind lactoferrin. *Journal of Virology*, **71:** 5997-6002.

Zheng, J., Ather, J. L., Sonstegard, T. S. and Kerr, D. E. (2005). Characterization of the infection responsive bovine lactoferrin promoter. *Gene*, **353**: 107-117.

Zhou, L., Yang, Y., Zhong, H., Li, Kong, Li, Xin, G. D., Di, H. S. and Wang, G. L. (2006). Detection and Characterization of PCR–SSCP Markers of the Bovine Lactoferrin Gene for clinical mastitis. *Journal of Animal Science*, **19**: 1399-1403.