DOI: 10.22055/ivj.2024.455006.2713

Expression profiles of pro-inflammatory cytokine genes in milk somatic cells at different stages of the first lactation in Holstein dairy cattle

Elnaz Heidari Arjlo[1], Hajar Al-sadat Hoseini-Dolatabady[1] and Mustafa Muhaghegh-Dolatabady[2*]

1 MSc Graduated of Animal Genetics and Breeding, Faculty of Agriculture, University of Yasouj, Yasouj, Iran
2 Associate Professor, Department of Animal Science, Faculty of Agriculture Science, University of Yasouj, Yasouj, Iran

Received: 30.04.2024 Accepted: 24.09.2024

Abstract

Milk somatic cells produce numerous soluble proteins like cytokines that play important roles in the immunity of the mammary gland. This study aimed to investigate the expression profiles of bovine pro-inflammatory cytokine genes including IL-2, IL-6, IL-8, TNF-α, IFN-γ, and GM-CSF in the somatic cells of milk in healthy Holstein cows at different lactation stages in their first lactation cycle. For this purpose, milk samples were collected from eighteen dairy cows at the early, middle, and late lactation stages. Total RNA was extracted from the somatic cells of milk and then the first strand of cDNA was synthesized. Real-time PCR was performed for the bovine pro-inflammatory cytokine genes. As reference genes, the β-actin and GAPDH genes were used to normalize the data. The real-time PCR data were analyzed with the REST and SAS programs. According to the results, the six-cytokine genes were expressed in the milk somatic cells of healthy cows in different lactation stages. The results showed that the expressions of almost all cytokine genes (except for the TNF-α gene) were significantly higher in animals at the middle compare to the early lactation stage. However, the expression of cytokine genes also showed a trend to be higher at the late lactation stage compared to early lactation. Still, these differences were only significant for mRNA levels of TNF-α and GM-CFS genes. Furthermore, the expression differences of cytokine genes were not significant in cows at the late relative to animals at the middle lactation stage. In the entire lactation cycle, the mRNA transcription levels of IL-6 and IL-2 were observed at high and low concentrations compare to other cytokine genes, respectively. The highest stability was shown for IL-6 throughout the three lactation stages, while the lowest stability was found for the expression of TNF-α. The correlation between the gene expression levels was almost not significant for most of the studied genes in different stages of lactation, however, a significant correlation was found between IL-8 and GM-CSF in the entire, early and late stages of lactation.

Key words: Gene expression, Cytokine, Cattle, Lactation stage

* **Corresponding Author**: Mustafa Muhaghegh-Dolatabady, Associate Professor, Department of Animal Science, Faculty of Agriculture Science, University of Yasouj, Yasouj, Iran
E-mail: mmuhaghegh@yu.ac.ir

Refrences

Abdel-Azim, G.A., Freeman, A.E., Kehrli, M.E., Jr Kelm, S.C., Burton, J.L., Kuck, A.L., & Schnell, S. (2005) Genetic basis and risk factors for infectious and noninfectious diseases in US Holsteins. I. Estimation of genetic parameters for single diseases and general health. *Journal of Dairy Science,* 88, 1199-1207.

Alluwaimi, A.M. (2000). Detection of IL-2 and IFN-gamma mRNA expression in bovine milk cells at the late stage of the lactation period with RT-PCR. *Research in Veterinary Science,* 69, 185-187.

Alluwaimi, A.M., & Cullor, J.S. (2002). Cytokines gene expression patterns of bovine milk during mid and late stages of lactation. *Journal of Veterinary Medicine, Series B,* 49,105-100.

Belardelli**,** F., & Ferrantini**,** M. (2002). Cytokines as a link between innate and adaptive antitumor immunity. *Trends in immunology,* 23, 201-208.

Bennedsgaard, T.W., Enevoldsen, C., Thamsborg, S.M. and Vaarst, M. (2003). Effect of mastitis treatment and somatic cell counts on milk yield in Danish organic dairy cows. *Journal of Dairy Science*, 86(10), 3174-3183.

Beutler, B., & Cerami, A. (1988). Tumor necrosis, cachexia, shock, and inflammation: A common mediator. *Annual review of biochemistry,* 57, 505-518.

Bhatt, V.D., Khade, P., Tarate, S., Tripathi, S.B., Nauriyal, A.K., Dh Rank, D.S., Kunjadia, N.A.P., & Joshi, C.G. (2012) Cytokine expression pattern in milk somatic cells of subclinical mastitis-affected cattle analyzed by real time PCR. *Korean Journal of Veterinary Research*, 52, 231-238.

Bonnefont, C.M., Toufeer, M., Caubet, C., Foulon, E., Tasca, C., Aurel, M.R., Bergonier, D., Boullier, S., Robert-Granié, C., Foucras, G., & Rupp, R. (2011). Transcriptomic analysis of milk somatic cells in mastitis resistant and susceptible sheep upon challenge with Staphylococcus epidermidis and Staphylococcus aureus. *BMC genomics*, 12, 208.

Brenmoehl, J., Ohde, D., Wirthgen, E., & Hoeflich, A. (2018). Cytokines in milk and the role of TGF-beta. *Best Practice & Research Clinical Endocrinology & Metabolism*, 32(1),47-56.

Connelly, L., Barham, W., Pigg, R., Saint-Jean, L., Sherrill, T., Cheng, D.S., Chodosh, L.A., Blackwell, T.S., & Yull, F.E. (2010). Activation of nuclear factor kappa B in mammary epithelium promotes milk loss during mammary development and infection. *Journal of cellular physiology*, 222, 73–81.

Curone, G., Filipe, J., Cremonesi, P., Trevisi, E., Amadori, M., Pollera, C., Castiglioni, B., Turin, L., Tedde, V., Vigo, D., & Moroni, P. (2018). What we have lost: Mastitis resistance in Holstein Friesians and in a local cattle breed. *Research in veterinary science*, 116, 88-98.

Daley, M., Williams, T., Coyle, P., Furda, G., Dougherty, R., & Hayes, P. (1993). Prevention and treatment of Staphylococcus aureus infections with recombinant cytokines. *Cytokine*, 5, 276-284.

Ferens, W.A., Davis, W.C., Hamilton, M.J., Park, Y.H., Deobald, C.F., Fox L., & Bohach, G. (1998). Activation of bovine lymphocyte subpopulations by staphylococcal enterotoxin c. *Infection and immunity*, 66, 573-80.

Fonseca, I., Silva, P.V., Lange, C.C., Guimarães, M.F., Weller, M.M., Sousa, K.R.S., Lopes, P.S., Guimarães, J.D., & Guimarães, S.E. (2009). Expression profile of genes associated with mastitis in dairy cattle. Genetics and Molecular Biology, 32, 776-781

Godson D.L., Baca-Estrada M.E., & Babiuk L.A. (1997). Chapter 1. Applications of bovine cytokines. In Schijns, V.E.C.J., & Horzinek, M.C. (Ed.). Cytokines in Veterinary Medicine (1nd ed., pp 3-13). Wallingford, UK. CAB Int.

Griesbeck-Zilch B., Osman M., Kuhn C., Schwerin M., Bruckmaier R.H., Pfaffl M.W., Hammerle-Fickinger A., Meyer H.H.D., & Wellnitz O. (2009). Analysis of key molecules of the innate immune system in mammary epithelial cells isolated from marker-assisted and conventionally selected cattle. Journal of *Dairy Science,* 92, 4621-4633.

Guo, W., Liu, B., Hu, G., Kan, X., Li, Y., Gong, Q., Xu, D., Ma, H., Cao, Y., Huang, B., & Fu, S. (2019). Vanillin protects the blood–mill barrier and inhibits the inflammatory response in LPS-induced mastitis in mice. *Toxicology and applied pharmacology*, 365, 9-18.

Hagnestam-Nielsen, C., Emanuelson, U., Berglund, B., & Strandberg, E. (2009). Relationship between somatic cell count and milk yield in different stages of lactation. *Journal of Dairy Science*, 92(7), 3124-3133.

Harati, H., Narenji Sani, R., Jebelli Javan, A., Ahmadi-hamedani, M. and Naeimi, S. (2022). Efficacy of Zataria multiflora essential oil for treatment of Staphylococcus aureus detected by polymerase chain reaction in lactating dairy cows with subclinical mastitis. *Iranian Veterinary Journal*, 18(1), 34-45.

Heiser, A., McCarthy, A., Wedlock, N., Meier, S., Kay, J., Walker, C., Crookenden, M.A., Mitchell, M.D., Morgan, S., Watkins, K., & Loor, J.J. (2015). Grazing dairy cows had decreased interferon-γ, tumor necrosis factor, and interleukin-17, and increased expression of interleukin-10 during the first week after calving. *Journal of Dairy Science*, 98(2), 937-946.

Ito T., & Kodama M. (1996). Demonstration by reverse transcription-polymerase chain reaction of multiple cytokine mRNA expression in bovine alveolar macrophages and peripheral blood mononuclear cells. *Research in veterinary science*, 60, 94-96.

Jonsson N.N., Fortes M.R.S, Piper E.K., Vankan D.M., Prada J. de Cisneros J., & Wittek T. (2013). Comparison of metabolic, hematological, and peripheral blood leukocyte cytokine profiles of dairy cows and heifers during the periparturient period. *Journal of Dairy Science*, 96, 2283-2292.

Karcher E.L., Beitz D.C., & Stabel J.R. (2008). Modulation of cytokine gene expression and secretion during the periparturient period in dairy cows naturally infected with *Mycobacterium* avium subsp. paratuberculosis. *Veterinary immunology and immunopathology*, 123, 277-288.

Khan, M.Z., Khan, A., Xiao, J., Ma, J., Ma, Y., Chen, T., Shao, D., & Cao, Z. (2020). Overview of research development on the role of NF-κB signaling in mastitis. *Animals*, 10(9), 1625.

Khan, M.Z., Wang, J., Ma, Y., Chen, T., Ma, M., Ullah, Q., Khan, I.M., Khan, A., Cao, Z., & Liu, S. (2023). Genetic polymorphisms in immune-and inflammation-associated genes and their association with bovine mastitis resistance/susceptibility. *Frontiers in Immunology*, 14, 1082144.

Koeck A., Miglior F., Kelton D.F., & Schenkel F.S. (2012). Health recording in Canadian Holsteins: data and genetic parameters. *Journal of Dairy Science*, 95, 4099-4108.

Kulms, D., & Schwarz, T. (2006). NF-κB and Cytokines. *Vitamins & Hormones*, 74, 283-300.

Lee J., Banermann D., Paape M.J., Huang M., & Zhao X. (2006). Characterization of cytokine expression in milk somatic cells during intramammary infections with *Escherichia coli* or *Staphylococcus aureus* by real-time PCR. *Veterinary Research*, 37, 219-229.

Leutenegger C.M., Alluwaimi A.M., Smith W., Perani L., & Cullor J.S. (2000). Quantitation of bovine cytokine mRNA in milk cells of healthy cattle by realtime Taq Man polymerase chain reaction. Veterinary Immunology and Immunopathology, 77, 275-287.

Mallard B.A., Atalla H., Cartwright S., Hine B.C., Hussey B., Paibomesai M., Thompson-Crispi K.A., & Wagter-Lesperance L. (2011). Genetic and Epigenetic Regulation of the Bovine Immune System: Practical Implications of the High Immune Response Technology. Proc National Mastitis Council 50th Annual Meeting; pp. 53-63.

Nakajima Y., Mikami O., Yoshioka M., Motoi Y., Ito T., Ishikawa Y., Fuse M., Nakano K., & Yasukawa K. (1997). Elevated levels of tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) activities in the sera and milk of cows with naturally occurring coliform mastitis. Research in veterinary science, 62, 297-298.

Naugler, W.E., & Karin, M. (2008). NF-κB and cancer—identifying targets and mechanisms. *Current Opinion in Genetics & Development*, 18(1), pp.19-26.

Ogorevc J., Kunej T., Razpet A., & Dovc P. (2009). Database of cattle candidate genes and genetic markers for milk production and mastitis. *Anim Genet*. 40, 832-851

Okada H., Ito T., Ohtsuka H., Kirisawa R., Iwal H., Yamashita K., Yoshino T., & Rosol J. (1997). Detection of interleukin-1 and interleukin-6 on cryopreserved bovine mammary epithelial cells in vitro. *Journal of veterinary medical science,* 59, 503-507.

Orsi, N.M., Gopichandran, N., Ekbote, U.V., & Walker, J.J. (2006). Murine serum cytokines throughout the estrous cycle, pregnancy and postpartum period. *Animal Reproduction Science*, 96:54-65.

Oviedo-Boyso J., Valdez-Alarcon J.J., Cajero-Juárez M., Ochoa-Zarzosa A., López-Meza J.E., Bravo-Patiño A., & Baizabal-Aguirre V.M. (2007). Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. *Journal of Infection,* 54, 399-409.

Parker Gaddis K.L., Cole J.B., Clay J.S., & Maltecca C. (2014). Genomic selection for producer-recorded health event data in US dairy cattle. *Journal of Dairy Science,* 97, 3190-3199.

Pfaffl M.W., Horgan G.W., & Dempfle L. (2002). Relative Expression Software Tool (REST©) for group wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research,* 30, e36

Pfaffl M.W., Wittmann S.L., Meyer H.H.D., & Bruckmaier R.M. (2003). Gene Expression of Immunologically Important Factors in Blood Cells, Milk Cells, and Mammary Tissue of Cows. *Journal of Dairy Science,* 86, 538-545.

Pighetti, G.M., & Sordillo L.M. (1995). Enhanced mammary gland immunity following primary immunization with interferon-g. *Journal of Dairy Science,*78, 528.

Rewiniski M.J., & Yang T.J. (1994). Lactation stage-dependent changes in levels of tumor necrosis factor/cachectin in milk. *American Journal of Reproductive Immunology,* 31, 170-176.

Riollet C., Rainard P., & Poutrel B. (2001). Cell subpopulations and cytokine expression in cow milk in response to chronic Staphylococcus aureus infection. Journal of Dairy Science, 84, 1077-1084.

Rouveix B. (1997). Clinical pharmacology of cytokines. In: Proceeding of the Euro conference, Cytokines: Tools and Targets for Tomorrow's Therapies, Institut Pasteur, France, 15-17 October

Sarikaya H., Schlamberger G., Meyer H.H.D., & Bruckmaier R.M. (2006). Leukocyte populations and mRNA expression of inflammatory factors in quarter milk fractions at different somatic cell score levels in dairy cows. *Journal of Dairy Science,* 89, 2479-2486.

Smith, C.W., & Goldman, A.S., 1968. The cells of human colostrum. I. In vitro studies of morphology and functions. *Pediatric Research,* 2(2), 103-109.

Smith, K.A. (1988). Interleukin-2: inception, impact, and implications. *Science*, 240(4856),1169-1176.

Sordillo L.M., & Peel J.E. (1992). Effect of interferon–γ on the production of tumor necrosis factor-α during acute *E.coli* mastitis. *Journal of Dairy Science,* 75, 2119-2125

Sordillo L.M., Pighetti G.M., & Davis M.R. (1995). Enhanced production of bovine tumor necrosis factor-α during the periparturient period. *Veterinary Immunology and Immunopathology,* 49, 263-270.

Sordillo L.M., Shafer-Weaver K., & De Rosa D. (1997). Immunobiology of the mammary gland. *Journal of Dairy Science,* 80, 1851-1865.

Taylor, B.C., Keefe, R.G., Dellinger, J.D., Nakamura, Y., Cullor, J.S., & Stott, J.L. (1997). T cell populations and cytokine expression in milk derived from normal and bacteria-infected bovine mammary glands. *Cellular Immunology,* 182(1), 68-76.

Tiezzi F., Parker-Gaddis K .L., Cole J.B., Clay J.S., & Maltecca C. A. (2015). Genome-wide association study for clinical mastitis in first parity US Holstein cows using single-step approach and genomic matrix re-weighting procedure. *POLS One*, 6, 10(2):e0114919.

Trevisi, E., Jahan, N., Bertoni, G., Ferrari, A., & Minuti, A. (2015). Pro-inflammatory cytokine profile in dairy cows: consequences for new lactation. *Italian Journal of Animal Science*, 14(3), 3862.

Usman, T., Wang, Y., Liu, C., Wang, X., Zhang, Y., & Yu, Y. (2015). Association study of single nucleotide polymorphisms in JAK 2 and STAT 5B genes and their differential mRNA expression with mastitis susceptibility in Chinese Holstein cattle. *Animal Genetics*, 46(4), 371-380.

Watanabe A., Yang Y., Shiono H., & Yokomizo Y. (2000). Effect of intramammary infusion of tumour necrosis factor-α on milk protein composition and induction of acute-phase protein in the lactating cow. *ournal of Veterinary Medicine, Series B*, 47, 653-662.

Wood P.R., & Rothel J.S. (1997). The IFN-c assay is a diagnostic test for bovine tuberculosis. In: Schijns, V.E.J., Horzinek, M.C. (Eds.), Cytokines in Veterinary Medicine. Cab International, New York, USA, pp. 35-40.

Young H.A., & Hardy K.J. (1995). Role of interferon-gamma in immune cell regulation. *Journal of leukocyte biology*, 58, 373-381