Vol. 21, No. 1, Spring, 2025, 85-96 *Original Article*
DOI: 10.22055/ivj.2024.455006.2713
Journal Homepage: http://www.ivj.ir/

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

Introduction

The breeding strategy continues to increase its focus on health traits in dairy cattle (Koeck et al, 2012; Parker et al, 2014). It has been recommended to include immune response traits in breeding indices to improve the inheritance of disease resistance in dairy cattle (Abdel-Azim et al, 2005; Mallard et al, 2011). For example, it is suggested to use somatic cell score (SCS) as an indicator trait to obtain genetic improvement in mastitis resistance, and the somatic cell count (SCC) is widely used as

[*] **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

a selection criterion for the genetic improvement of mastitis resistance in dairy cattle (Odegard et al, 2003). Since variations in milk SCC depend mainly on the leukocyte recruitment in response to an inflammatory reaction, SCC is usually used to distinguish between healthy and infected mammary glands and, thus, to monitor udder health (Schukken et al, 2003). The somatic cells are also normally present in milk at low levels. They consist of different cell types such as lymphocytes, polymorphonuclear leukocytes (PMN), macrophages, and a smaller number of epithelial cells. The number and composition of milk somatic cells depend on many factors such as animal health, milk productivity, genetics, and environmental factors (Riollet et al, 2002; Alhussein, 2018). In addition, variations are observed in the composition of milk somatic cells including the lymphocyte population in the lactating healthy gland during lactation stages and cycles (Alluwami, 2002; Park et al, 1992). Furthermore, an increase in SCC is associated with a decrease in daily milk production, which depends on the lactation stages and parity, and it was very extensive in late lactation stage regardless of parity (Hagnestam-Nielsen et al, 2009). Decreased milk production due to increased SCC in multiparous cows was more than primiparous cows (Bennedsgaard et al, 2003; Hagnestam-Nielsen et al, 2009). Generally, total milk SCC is less than 10^5/ml in the healthy lactating mammary gland (Leutenegger et al, 2000). However, the high milk production of the Holstein breed creates a constant state of stress in lactating animals, and therefore a small amount of tissue damage and possibly the presence of low-grade bacteria are common. This assumption is important to understand the expression of cytokines that indicate a certain level of inflammation in the healthy state of lactating cows (Leutenegger et al, 2000). Bacteria in infected mammary glands release their metabolites and cell walls, chemoattractants for leukocytes (Kehrli et al, 1994). Then, larger amounts of various soluble factors are secreted by somatic cells in the mammary gland, which leads to the massive recruitment of additional leukocytes into the gland. These factors involve cytokines, complement components, leukotrienes, prostaglandins, serotonin, and histamine (Giri et al, 1984; Harati et al, 2022; Rose et al, 1989; Shuster et al, 1993; Zia et al, 1987). Cytokines are small proteins that transmit intercellular signals for inflammation, immunity, hematopoiesis, stress, mammary gland development, and tissue repair (Belardelli and Ferrantini, 2002; Brenmoehl et al, 2018; Rouveix, 1997; Wood and Rothel, 1997). The use of bovine cytokines in mastitis immunotherapy also indicates their important role in the defense regulation of the mammary glands (Godson et al, 1997). Accordingly, the cytokine genes have considered as strong candidate markers for mastitis resistance selection in dairy cattle (Sarikaya et al, 2006; Oviedo-Boyso et al, 2007; Curone et al, 2018). Understanding the cytokines activity at different stages of the lactation cycle is important to monitor the well-being of the mammary gland. Therefore, the objective of the present study was to investigate the mRNA levels of normal transcription of cytokines, IL-2, IL-6, IL-8, IFN-γ, TNF-α, and GM-CSF in the milk cells of the bovine mammary gland at the early, mid, and late stages of lactation period in the first lactation of Holstein dairy cows.

Materials and methods

Eighteen healthy dairy Holstein cows in their first lactation were grouped according to their lactation stages (6 at 7-10, 6 at 140-150, and 6 at 290-295 days after parturition). The criterion for selecting animals at the early stage was the SCC< 350,000, while this criterion was SCC<100,000 for the two middle and late lactation stages. One liter of milk sample representing all four quarters was collected

in sterile tubes. Then the milk sample was centrifuged for 20 min at 1500 g at 4 °C. The obtained cell pellet was washed in PBS pH 7.4 twice and centrifuged for 20 min at 4 °C and 220g according to Liebe (1996). The pellets were lysed with 500 µl PBS-EDTA and, then, total RNA was isolated using a Denazist kit according to the manufacturer's protocol. The extracted RNA samples were treated with *DNase* I to remove DNA contamination. RNA quality and quantity were assessed by agarose gel electrophoresis and spectrophotometric readings. Synthesis of first strand cDNA was performed with *AccuPower® RocketScriptTM RT PreMix* kit (Bioneer) and random hexamer primers (Takapozist) according to the manufacturer's instructions. The final volume was adjusted to 50 µl with RNase-free water. The amplified cDNA samples were then stored at -20 °C until use in real-time PCR. The primers for the gene expression evaluation were used as described by Lee et al, (2006) and the β-actin and GAPDH genes were used as endogenous references for the calculation of dCP (Table 1). Real-time PCR was performed using CFX96 (Bio-Rad, USA) and *Hot Taq Eva Green qPCR* kit (Cinnagen) according to the manufacturer's instructions. All reactions were carried out in duplicate. Amplification conditions were an initial step of 95 °C for 15 min, followed by 50 cycles of 94 °C for 15 s, 60 °C for 30 s, and 72 °C (depending on the product length, 5 s per 100 bp) in a 10-µl reaction volume. After amplification, all samples were submitted to analysis of the dissociation curve to confirm the absence of nonspecific products and primer dimmers (melting curve by 95 °C for 5 s, 65 °C for 15 s, and 95 °C for 0 s). In each reaction of real-time PCR, the cycle number at which the fluorescence rises appreciably above the background fluorescence is determined as the crossing point (CP). Descriptive statistics are calculated using the derived CPs for each cytokine gene by the SAS program. The real-time PCR results were analyzed with the REST program to compare differences in gene expression across groups (Pfaffl et al, 2002).

Table 1: Primers sequences of bovine cytokines and references genes in real-time PCR.

Gene	Primer	Sequence (5'-3')	Length	Accession
Il-2	IL-2.107f IL-2.271r	5'-GGATTTACAGTTGCTTTTGGAGAAA-3' 5'-GCACTTCCTCTAGAAGTTTGAGTTCTT-3'	165	M12791
IL-6	IL-6.f209 IL-6.r313	5'-TCATTAAGCGCATGGTCGACAAA-3' 5'-TCAGCTTATTTTCTGCCAGTGTCT-3'	105	NM173923
IL-8	IL-8.f251 IL-8.r355	5'-CACTGTGAAAATTCAGAAATCATTGTTA-3' 5'-CTTCACAAATACCTGCACAACCTTC-3'	105	NM173925
IFN-γ	IFN-γ.f296 IFN-γ.f480	5'-TCATTAAGCGCATGGTCGACAAA-3' 5'-TCAGCTTATTTTCTGCCAGTGTCT-3'	185	M29867
TNF-α	TNF-α.f2377 TNF-α.r2794	5'-TCTTCTCAAGCCTCAAGTAACAAGC-3' 5'-CCATGAGGGCATTGGCATAC-3'	418	AF011926
GM-CFS	GM-CFS.f170 GM-CFS.r250	5'-AGTAATGACACAGAAGTCGTCTCTG-3' 5'-GCCGTTCTTGTACAGCTTCAGG-3'	87	U22385
β-actin	β-actin.f38 β-actin.r428	5'-CCTTTTACAACGAGCTGCGTGTG-3' 5'-ACGTAGCAGAGCTTCTCCTTGATG-3'	391	AH00130
GAPDH	GADPH.463f GADPH582r	5'-GGCGTGAACCACGAGAAGTATAA-3' 5'-CCCTCCACGATGCCAAAGT-3'	120	AF022183

Results

The form of data observations is the raw CP or threshold cycles (Ct) that are generated by a real-time PCR. Usually, the raw CPs or Ct are normally distributed and seem to be the best estimators of the gene expression levels, thus, a parametric test can be performed. In real-time PCR, the amount of amplicon is inversely related to the numerical value of the Ct (i.e., the greater the amount of amplicon, the lower the value of the Ct). Six cytokine genes were expressed in somatic cells of all milk samples at the early, middle, and late stages of lactation (Fig 1). Although the Ct average in all cytokines, except for IL-6 with an average of 25.8, was higher than 30, which indicates the low expression of studied genes in the milk somatic cells of healthy cows. The lowest expression level was detected for IL-2 (average Ct= 34.1) in the entire lactation cycle (Figure 1a). As shown in Fig 1b, the mRNA level of IL-6 was also higher in each lactation stage compared to the other cytokines. Among all cytokine genes, the expression of IL-2 was lower in the early (mean Ct= 35.1) and late (mean Ct= 34.3) lactation stages, while a lower expression level was observed for TNF-α (mean Ct=36.3) in the middle lactation stage (Figure 1b).

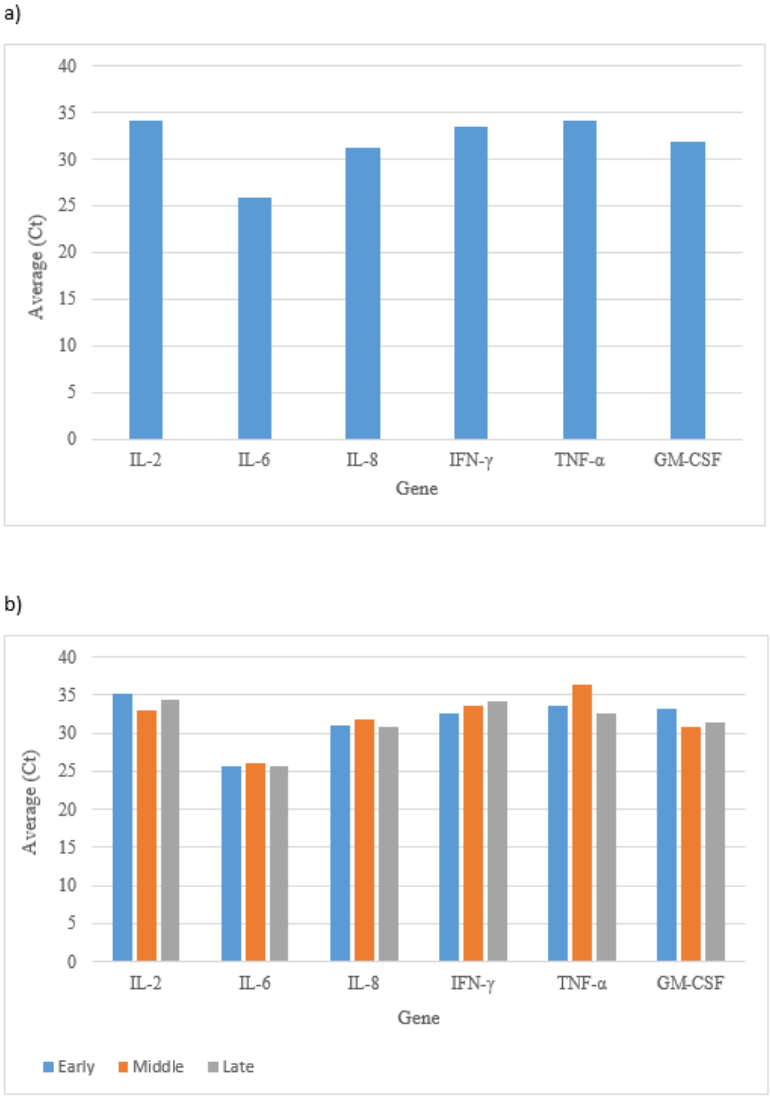

Figure 1: Average of Ct values for cytokine genes in the entire lactation (a) and different lactation stages (b)

Based on the descriptive results of Ct values for cytokine genes, the IL-6 and TNF-α, with standard deviations of 0.54 and 3.21, showed the highest and lowest expression stability during the entire lactation cycle, respectively (Figure 2a). In the early stage of lactation, as in the entire lactation period, the IL-6 and TNF-α revealed the highest and lowest expression stability, with a standard deviation of 0.44 and 3.94, respectively (Figure 2b). The IL-6 gene showed maximum expression stability in the middle (SD Ct=0.70) and late (SD Ct=0.43) lactation stages (Figure 2b). The highest expression variations were observed for IL-8 (SD Ct= 2.26) and IFN-γ (SD Ct=3.29) genes in the middle and late lactation stages, respectively (Figure 2b).

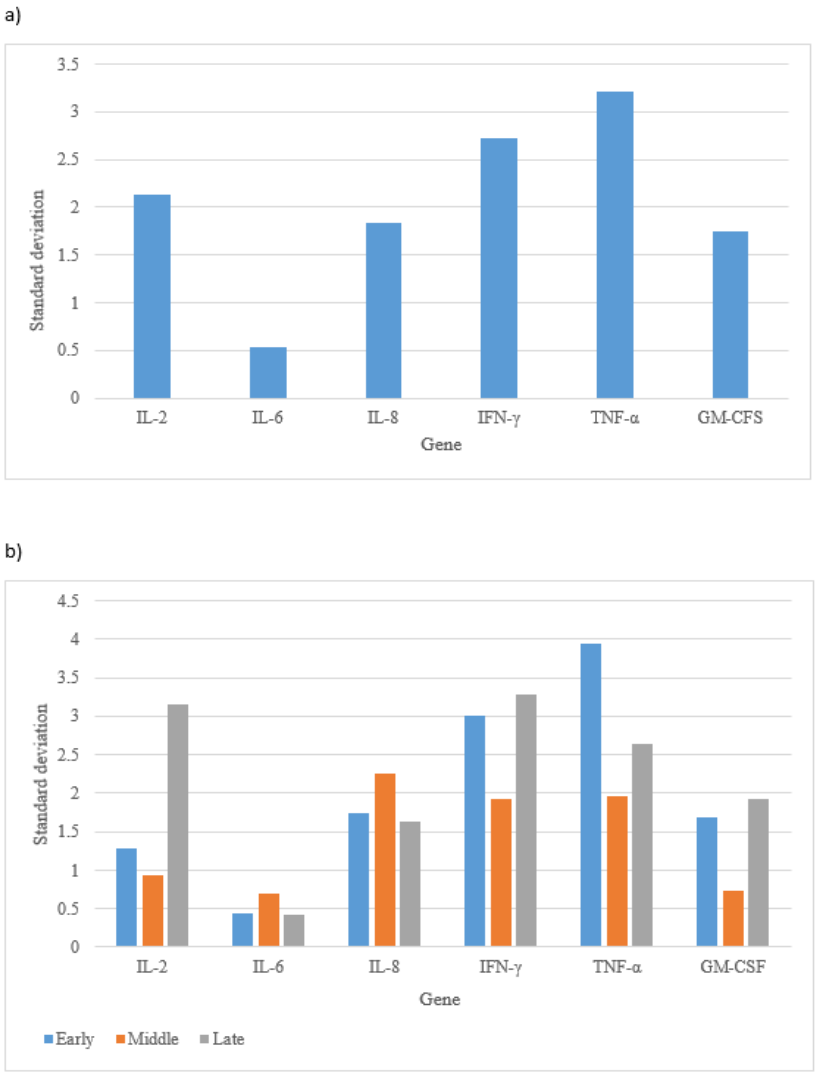

Figure 2: The standard deviations of Ct values for cytokine genes in the entire lactation (a) and different lactation stages (b)

The comparison of the relative expression of cytokine genes at different lactation stages is given in Table 2. The comparison of mRNA levels in cytokine genes was represented as folds' induction, and large variations were shown among the studied genes in terms of magnitude (Table 2). The results showed no significant difference in the expressions of the TNF-α gene in cows at the middle lactation stage

compared to animals at the early lactation stage. However, in cows at middle lactation, the expression of the TNF-α was 3.05 times higher than in cows at early lactation, but this difference was not significant (p=0.24). The expressions of other studied cytokine genes were significantly higher in cows at middle lactation compared to the early lactation stage (P<0.05).

Table 2: Relative gene expression in animals at different lactation stages (E: Early lactation stage, M: middle lactation stage, and L: late lactation stage).

Gene	Expression rate			Results		
	M vs E	L vs E	L vs M	M vs E	L vs E	L vs M
IL-2 *p*-value	92.46** 0.002	9.34 0.19	0.10 0.12	Up	–	–
IL-6 *p*-value	15.43* 0.01	5.44 0.19	0.35 0.31	Up	–	–
IL-8 *p*-value	15.52* 0.04	6.23 0.25	0.30 0.36	Up	–	–
–γIFN *p*-value	12.23* 0.03	1.81 0.71	0.148 0.19	Up	–	–
TNF-α *p*-value	3.05 0.24	11.70* 0.03	3.830 0.24	–	Up	–
GM-CSF *p*-value	100.45** 0.001	16.97* 0.02	0.169 0.10	Up	Up	–

* Statistically significant (p < 0.05)
** Statistically significant (p < 0.01)

The striking differences were found in the mRNA levels of GM-CSF and IL-2 between cows in the early and middle lactation stages. The cows in the middle lactation stage showed 100.45 and 92.46-fold increases in GM-CSF and IL-2 transcripts compared with animals in the stage of early lactation, respectively. In addition, significant differences were observed in the expression of TNF-α and GM-CFS genes at late relative to early lactation stage (p<0.05); however, the expression of other cytokine genes also showed a trend to be higher at the late lactation stage relative to early lactation, but these differences were not significant. No significant differences were observed in the expression of cytokine genes in cows at the late lactation stage relative to cows at the middle lactation stage (Table 2).

Significant correlations were found between the expressions of cytokine genes at different lactation stages (Table 3). In the early stage of lactation, the correlation between the expression of IL-8 and GM-CSF genes was significant (p<0.05). Whereas, only the correlation between expression of IL-6 and IFN-γ genes was significant in the middle stage of lactation (p<0.05). In the late lactation stage, a significant correlation was observed in the expression of IL-2 and IL-6 (p<0.01). In addition, the correlation between IL-8 and GM-CSF was significant in the late lactation stage (p<0.05). In the entire lactation cycle, correlations between IL-8 and GM-CSF, and also, IL-8 and TNF-α were significant (p<0.05).

Table 3: Correlations between the transcriptional activity of cytokine genes in the different lactation stages and the entire lactation cycle

		IL-2	IL-6	IL-8	IFN-γ	TNF-α
IL-6	Early	0.39				
	Middle	-0.65				
	Late	0.98**				
	Entire	0.14				
IL-8	Early	-0.10	-0.05			
	Middle	0.48	-0.19			
	Late	0.25	0.28			
	Entire	0.11	0.03			
IFN-γ	Early	0.66	0.39	-0.77		
	Middle	0.56	-0.89*	0.05		
	Late	0.31	0.34	-0.34		
	Entire	0.31	-0.01	-0.41		
TNF-α	Early	0.48	0.64	-0.28	0.63	
	Middle	-0.68	0.25	-0.55	-0.09	
	Late	0.57	0.50	0.25	0.64	
	Entire	0.05	0.52	-0.07	0.40	
GM-CSF	Early	0.13	0.17	-0.89*	0.78	0.54
	Middle	-0.36	-0.04	0.47	-0.13	0.27
	Late	0.001	0.001	-0.82*	0.35	-0.23
	Entire	0.23	-0.12	-0.52*	0.28	0.01

* Statistically significant ($p < 0.05$)
** Statistically significant ($p < 0.01$)

Discussion

The somatic cells of milk play an important role in the innate immune defense of the bovine mammary gland. Macrophages are the most abundant cell type (54–83%) in milk from healthy mammary glands, whereas neutrophils become the predominant cell type (>95%) in milk during mastitis. Milk somatic cells rapidly increase to reach 10^6 cells/ml or higher in the acute phase of mastitis (Lee et al, 1980; Sordillo et al, 1997). Gene expression of milk somatic cells mainly depends on mammary gland conditions (healthy or infected), lactation stages and cycle, and pathogens types (Alluwami, 2002; Boulanger et al, 2003; Lee et al, 2006; Pisoni et al, 2010). Expression of immunity-related genes represents only 1% of the total expressed genes in the milk somatic cells of the caprine mammary gland under normal conditions, while a significant transcriptomic disruption occurred in milk somatic cells after 24 h in experimentally infected mammary glands by *S. aureus* (Pisoni et al, 2010). The most abundant genes expressed in somatic cells of infected goat mammary glands included pro-inflammatory cytokines, chemokines, and their receptors (Pisoni et al, 2010). The cytokines are produced in various types of milk somatic cells such as leukocytes, especially T-cells, macrophages, and mammary epithelial cells (Smith and Goldman, 1968). They are known as not only pro- and/or anti-inflammatory agents to neutralize harmful pathogens but also act as growth stimulants by affecting the immune system maturation and development (Brenmoehl et al, 2018). Furthermore, the cytokines are sensitive tools to study the immune response of the bovine mammary gland and serve as a suitable marker to monitor udder health. The regulatory role of cytokines in mobilizing innate and adaptive immunity of bovine mammary glands is well-documented (Sordillo et al, 1997). In this study, transcriptions of pro-inflammatory cytokines (IL-2, IL-6, IL-8, IFN-γ, TNF-α, and GM-CSF genes) were detected in milk somatic cells of all healthy cows at the early, middle, and late stages of lactation.

Leutenegger et al, (2000) also reported high levels of transcription for TNF-a, GM-CSF, IL-12 p40, and IFN-γ in milk somatic cells of healthy Holstein cattle at middle lactation. However, mRNA expressions of IL-12 p40 and IL-8 genes were significantly lower than the TNF-a, GM-CSF, and IFN-γ (Leutenegger et al, 2000). IFN-γ and IL-6 mRNAs were also identified in the milk somatic cells of all healthy cows, but no transcribed mRNA was detected for TNF-α in any samples (Taylor et al, 1997). Bovine GM-CSF was found in normal bovine mammary glands at middle and late lactation with a significant elevation in its transcriptional activity in late lactation (Alluwaimi and Cullor, 2002). The high transcriptional level of TNF-α was also observed at the late lactation stage (Rewinski and Yang, 1994; Sordillo et al, 1995; Allowiami and Cullor, 2002). A comparison of healthy animals in two cattle breeds (Gyr with Black and White Holstein cattle) showed a significantly lower expression of IL-2 and IFN-γ genes in Gyr cows, whereas no significant difference was observed in mRNA levels between Gyr and BW Holstein cows for IL-4, IL-6, IL-8, IL-10, and TNF-α genes (Fonseca et al, 2009). In dairy cows and heifers, the expressions of IFN-γ and TNF-α displayed in two groups; however, the mRNA level of IFN-γ was significantly higher in cows 2 weeks after parturition (Jonsoon et al, 2013). The highest expressions of TNF-α and COX-2 were also found in the milk somatic cells of Holstein and Brown Swiss dairy cows (Pfaffl et al, 2003). The serum concentrations of pro-inflammatory cytokines (i.e. TNF-α, IL-1β, and IL-6) increased during middle and late gestation in the murine model (Orsi et al, 2006). In our study, among cytokine genes, higher expression was observed for IL-6 in the entire and three lactation stages, while IL-2 showed lower mRNA transcription levels in the entire, early, and late stages of lactation. However, higher expression was found for the TNF-α gene in the middle lactation stage. IL-2 plays an important role in stimulating the immunological memory of T cells and is necessary for the proliferation and activation of specific cytotoxic T cells (Smith, 1988). In this study, the lower levels of IL-2 expression with low somatic cells may indicate a lower probability of mammary glands' exposure to foreign antigens.

Based on the observed variability, the studied cytokine genes can be ordered from the most stably expressed, exhibiting the lowest variation, to the least stable one, exhibiting the highest variation. In this study, the expression profile of IL-6 showed the highest stability in the entire stages of lactation. This means that there is low variation in mRNA levels of IL-6 among samples compared to other genes. In contrast, high variability was observed for TNF-α, IL-8, and IFN-γ for the early, middle, and late lactation stages, respectively. Johnson et al, (2013) found higher variability in the expression of cytokine genes between heifers and dairy cows. A relationship was found between the expression of IL-2 and the lactation numbers, in which the variability in heifers was lower than in cows (Jonsoon et al, 2013). The mRNA expression of blood cells for IFN-γ, TNF-α, IL-17, and IL-10 greatly varied between individual healthy dairy cows during the first week after calving (Heiser et al, 2015). Genetic variations in immunity-related genes can cause variations in the inflammatory response to pathogens in different inflammatory conditions. Additionally, single nucleotide polymorphisms (SNPs) in promoter regions of cytokine genes may alter the transcription rate. Numerous studies have confirmed the association of genetic variations in bovine cytokine genes with susceptibility or resistance to mastitis disease in dairy cattle (Khan et al, 2023; Usman et al, 2015). Therefore, genetic polymorphisms in bovine cytokine genes could play an important role in differences between animals in their cytokine

expressions and immune responses or resistance to mastitis pathogens.

Also, no significant correlations were found between transcriptional activities of the most studied cytokine genes in the entire and different lactation stages in animals at first lactation cycle. Expression of IL-2 positively correlated with the IL-6 in the late lactation. In the middle of lactation, mRNA levels of IL-6 negatively correlated with the IFN-γ. In addition, positive significant correlations were observed between the expression of IL-8 and GM-CSF in the entire, early and late stages of lactation. In an earlier study, a significant correlation was reported between GM-CSF and TNF-α in the late and middle lactation period in the second-and third-lactating Holstein cows (Alluwaimi and Cullor, 2002). Furthermore, the correlation analysis indicated that the transcriptional activity of GM-CSF and IFN-γ significantly correlated to TNF-α at the middle lactation stage (Alluwaimi and Cullor, 2002). Bhatt et al, (2012) reported a significant and positive correlation among IFN-γ, GM-CSF, and TNF-α in crossbred cows but no significant correlation was found among these cytokine genes in Gir and Kankrej cattle breeds. IL-1β concentration was significantly correlated with IL-6 during the first month after calving in multiparous Holstein dairy cows (Trevisi et al, 2015). In general, the production of pro-inflammatory cytokines occurs due to the stimulation of NF-kB and MAPK cell signaling pathways by Pathogen Recognition Receptors (PRRs), namely Toll-like receptors (TLRs) (Guo et al, 2017). NF-kB is a key transcription factor that plays an important role in various physiological and pathological processes such as cell growth, metastatic functions, and inflammation (Kulms et al, 2006). The NF-κB activation has been demonstrated in regulated patterns during the various stages of mammary gland development (Connelly et al, 2010). In addition, the effect of NF-κB could be observed in the obvious symptoms of many infectious diseases, including inflammation of the mammary glands, and mastitis (Naugler and Karin, 2008). The bacterial lipopolysaccharides (LPS) start their pathogenesis using NF-κB cell signaling to cause mastitis after binding with relevant TLRs on epithelial cells of mammary glands (Khan et al, 2020). The IL-8 and GM-CSF are NF-κB-dependent cytokines that are involved in initiating and perpetuating neutrophilic inflammation. The level of NF-κB activity was extremely correlated with the expression levels of IL-8 and GM-CSF in milk cells of mastitis-affected cows (Boulanger et al, 2003).

Acknowledgment

The Yasouj University of Iran supported this study. The author's thanks from the company of agriculture and animal husbandry at Yasouj that provided samples for this study.

Conflict of Interest

There was not any conflict of interest in this research.

Funding

This study was financially supported by Yasouj University, Yasouj, Iran

References

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

Received: 30.04.2024
Accepted: 24.09.2024

DOI: 10.22055/ivj.2024.455006.2713

پروفایل بیان ژن‌های سیتوکین گاوی در سلول‌های سوماتیک شیر در مراحل مختلف اولین دوره شیردهی گاوهای شیری هلشتاین

الناز حیدری ارجلو[۱]، هاجرالسادات حسینی‌دولت‌آبادی[۱] و مصطفی محقق‌دولت‌آبادی[۲*]

[۱] دانش‌آموخته کارشناسی ارشد ژنتیک و اصلاح نژاد دام، دانشکده کشاورزی، دانشگاه یاسوج، یاسوج، ایران

[۲] دانشیار گروه علوم دامی، دانشکده کشاورزی، دانشگاه یاسوج، یاسوج، ایران

تاریخ دریافت: ۱۴۰۳/۲/۱۱ تاریخ پذیرش: ۱۴۰۳/۷/۳

چکیده

سلول‌های سوماتیک شیر پروتئین‌های محلول متعددی مانند سیتوکین‌ها را تولید می‌کنند که نقش مهمی در ایمنی غدد پستانی ایفا می‌کنند. این مطالعه با هدف بررسی پروفایل‌های بیان ژن‌های سیتوکین پیش التهابی گاو شامل IL-2، IL-6، IL-8، TNF-α، IFN-γ و GM-CSF در سلول‌های سوماتیک شیر در مراحل مختلف شیردهی در گاوهای هلشتاین سالم در اولین دوره شیردهی خود انجام شد. برای این منظور، RNA کل از سلول‌های سوماتیک شیر تعداد ۱۸ رأس گاو شیری سالم در مراحل اولیه، میانی و اواخر دوره شیردهی استخراج شد و واکنش real-time PCR جهت بررسی بیان ژن‌های سیتوکین برای تمام نمونه‌ها انجام شد. نتایج نشان داد که بیان تقریباً تمام ژن‌های سیتوکین به جز ژن TNF-α در حیوانات در میانه نسبت به مرحله اولیه شیردهی به طور معنی‌داری بیش‌تر بود. با این حال، بیان ژن‌های سیتوکین نیز در مرحله اواخر شیردهی در مقایسه با اوایل شیردهی روند بالاتری را نشان داد، اما این تفاوت‌ها تنها برای سطوح mRNA ژن‌های TNF-α و GM-CFSمعنی‌دار بود. علاوه بر این، تفاوت بیان ژن‌های سیتوکین در گاوها در مرحله انتهای شیردهی نسبت به حیوانات در مرحله میانی شیردهی معنی‌دار نبود. در کل دوره شیردهی، سطوح رونویسی mRNA برای IL-6 و IL-2 به ترتیب در غلظت‌های بالا و پایین نسبت به سایر ژن‌های سیتوکین مشاهده شد. همبستگی بین سطوح بیان ژن برای اکثر ژن‌های مورد مطالعه در مراحل مختلف شیردهی تقریباً معنی‌دار نبود. با این حال، ارتباط معنی‌داری بین IL-8 و GM-CSF در کل، مراحل اولیه و اواخر دوره شیردهی یافت شد.

کلمات کلیدی: بیان ژن، سیتوکین، دوره شیردهی، گاو

* نویسنده مسئول: مصطفی محقق‌دولت‌آبادی، دانشیار گروه علوم دامی، دانشکده کشاورزی، دانشگاه یاسوج، یاسوج، ایران

E-mail: mmuhaghegh@yu.ac.ir