

The inflammation of the cow mammary gland causes changes in the chemical composition of milk and contributes to economic losses, both in the livestock sector and in dairy industry. Moreover, the presence of pathogen bacteria in milk producing thermostable toxins, which are not destroyed during pasteurization process, poses a risk to the health of consumers. The losses due to mastitis reach billion dollars and euro in America and Europe every year.

The udder is not defenseless against pathogens. Protection against pathogens of the mammary gland is possible thanks to immune mechanisms, which are supervised by the different populations of cells and mediators secreted by them. Characteristics of the immune profile of genes in bovine tissues from both healthy animals and characterized by the presence of different types of bacteria in milk show alterations in expression of many genes responsible for the changes of immunological system.

The term epigenetics refers to the study of traits whose expression is not dependent on the DNA sequence. These changes are heritable but reversible. The term "DNA methylation" means the binding of methyl group (-CH₃) mainly to cytosines which are present in so called CpG islands (dinucleotides) in the gene promoters. They have big regulatory importance for gene expression. The methylation of gene causes the lowering of gene expression. In turn, microRNA is the short (ca. 20 nucleotides), one strand, non-coding RNA particles which regulate (negative regulation) the expression of genes. The epigenetic modifications connecting with methylation of DNA are heritable, but reversible. They cause changes of gene expression or its function which do not result from changes in the DNA sequence. The most frequent effect of methylation of DNA is inhibition effect of transcription processes, thus the lowering of particular gene expression. In turn, the short (ca. 20 nucleotides) a single-stranded, non-coding particles of RNA called microRNA have negative regulation influence on expression of other genes.

The first findings on methylation processes were related to aging of organisms – the older animal the lower gene methylation level. However, the idea that DNA methylation pattern is not only species- and age-specific but also tissue-, cell- and even organelle-specific was also proved. Because most tissues are characterized by their own methylation pattern this phenomenon could be used to the diagnosis of some diseases and to estimate age (biological clock - especially human).

The study on the influence of pathogens on epigenetics regulation mechanisms in the host were conducted on some husbandry animal species such horse and cattle. The exposure of neonates to environment with high level of pathogens such as bacteria and fungi causes temporal increase of expression of interferon-gamma. The importance of bacterial microflora in development of adaptive immunity horse foals (2 - 16 old) throughout epigenetic mechanisms was also documented. The presence of bacteria pathogens in the foals' rearing environment is one of the main effect which causes the decreasing of the methylation level of the interferon-gamma promoter region and its demethylation is connected with increased gene expression.

The methylation level of alphaS1-casein gene is increasing as the mammary gland becomes more involuted, but also that hypomethylated CpG dinucleotides become methylated during lactation as the result of *Escherichia coli* infection of the gland. Moreover, the methylation level of CpG dinucleotides of pubertal, non-lactating and mastitic mammary tissue were similar to each other (between 36 and 47%).

It is a common knowledge that the total protein content in milk during (sub)mastitis is elevated by 10-20% as compare to its average content. However, in (sub)mastitic milk the ratio between various protein is also changed. Usually, the content of caseins decreases in favor of whey protein. Moreover, the ratio between casein proteins also is changed. The content of alpha- and kappa-caseins is lowered, therefore the technological parameter of milk are worse, causing economic losses in manufacturing.

Till now, there is limited information on the influence of bacterial infection on DNA methylation pattern. Therefore, the aim of study is to investigate the effects of the infection of mammary gland secretory tissue (parenchyma) of dairy cows with coagulase-negative and coagulase-positive *Syaphylococci*, which cause the permanent changes in the gene expression level, on the methylation pattern of selected genes and microRNA transcriptome. This study will be a continuation and complement to the earlier carried out analysis of transcriptomic profile, using the parenchyma samples coming from the same quarters of the udders. It is assumed, that bacterial infections induce changes in mRNA transcriptome by methylation-specific regulatory regions of genes which are involved in immune response to infection and have an impact on non-coding RNA expression profile having regulatory significance for both the mRNA transcriptome and methylation of DNA. The analysis of methylation status of selected genes and microRNA transcriptome will allow to identify the impact of the infections on epigenetic regulations and on the changes in expression of genes which are involved in response to infection.

The project consists of four closely complementary analysis:

- 1) Methylation level of genes: The sequencing-based methylation analysis will be done. Methylation level of CpG islands from the selected genes will be evaluated.
- 2) Characteristics of microRNA profile: The level of mRNA and the level of DNA methylation is closely associated with the activity of specific non-coding RNAs, thus the microRNA profile will be analyzed.
- 3) The protein level of selected genes will be established using ELISA kits.
- 4) The association between methylation level and expression of genes will be established.