

Expression of Neurofilament Medium Chain (*NEFM*) and DEAD-Box Helicase 5 (*DDX5*) genes in ovine mammary epithelial cells after stimulation with *Staphylococcus aureus* in vitro

ABSTRACT

Being a Gram-positive bacterium, *Staphylococcus aureus* is causing mastitis disease in livestock. Mastitis is a condition where the udder of lactating animals gets inflamed due to various pathogens, mainly bacteria. This ailment affects the health of dairy animals and reduces milk production, significantly negatively impacting the dairy industry's economy. In this study, the mammary tissue from a local Turkish breed (Akkaraman) was collected from the slaughterhouse and was isolated according to the used protocol. First the mammary cells were taken from the mammary epithelial cells, then the cells were cultured, stimulated with *S. aureus*, then total RNA was extracted, and cDNA was synthesized. Finally, by using the quantitative real-time PCR, we investigated the mRNA expression levels of *Neurofilament Medium Chain (NEFM)* and *DEAD-Box Helicase 5 (DDX5)* as they are involved in the pathways related to the innate immunity, and *GAPDH* gene as the reference gene. Our results showed that the differential expression of *NEFM* mRNA and *DDX5* mRNA is higher in stimulated group as compared to control which is involved in the resistance mechanism of ovine mastitis. In conclusion our study revealed for the first time that the exposure to *S. aureus* stimulates the immune response in the sheep mammary gland.

MATERIALS and METHODS

- Physical examination of the sheep was done, and mammary tissues were collected after slaughtering of the Akkaraman sheep.
- Mammary cells were cultured by using Dulbecco's modified Eagle's medium (DMEM, 500 mL, high glucose, Sigma-Aldrich, USA) and stimulated with *Staphylococcus aureus*.
- cDNA and total RNA was extracted from the stimulated and unstimulated samples.
- qRT-PCR was used for the expression of genes.
- Statistical analysis was performed by using R Studio.

RESULTS

- To examine differential expression of immune-related gene in the ovine mammary epithelial cells qRT-PCR array analysis was performed to detect the expression of immune related genes. In this expression 96 well qRT-PCR array plate was used to study the regulation of immune related genes in ovine mammary epithelial cell and two genes were checked *NEFM*, *DDX5* and *GAPDH* as a reference gene to see the expression level against the *S. aureus*.
- For the statistical analysis and the graph preparation, R Studio was used, and compared the control samples with treated samples. When the p-value was ≤ 0.05 , it was considered significant. The relative gene expression was calculated $2^{-\Delta Ct}$, and the fold change expression between the stimulated and unstimulated mammary epithelial cells was calculated as $2^{-\Delta Ct \text{ stimulated}} / 2^{-\Delta Ct \text{ control}}$.

RESULTS

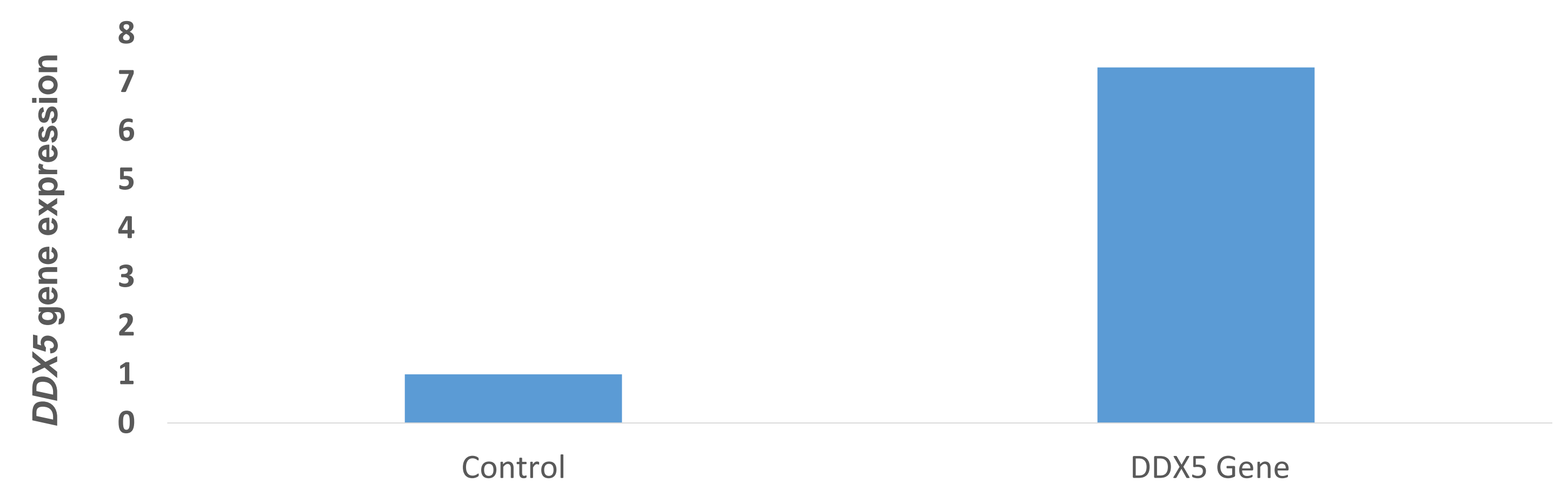


Fig.1 The expression of *DDX5* mRNA is lower in control as compared to stimulating group. *DDX5* showed more resistance against mastitis in stimulating group. Quantitative real time reverse transcription PCR (qRT-PCR) was used to determine the *DDX5* expression

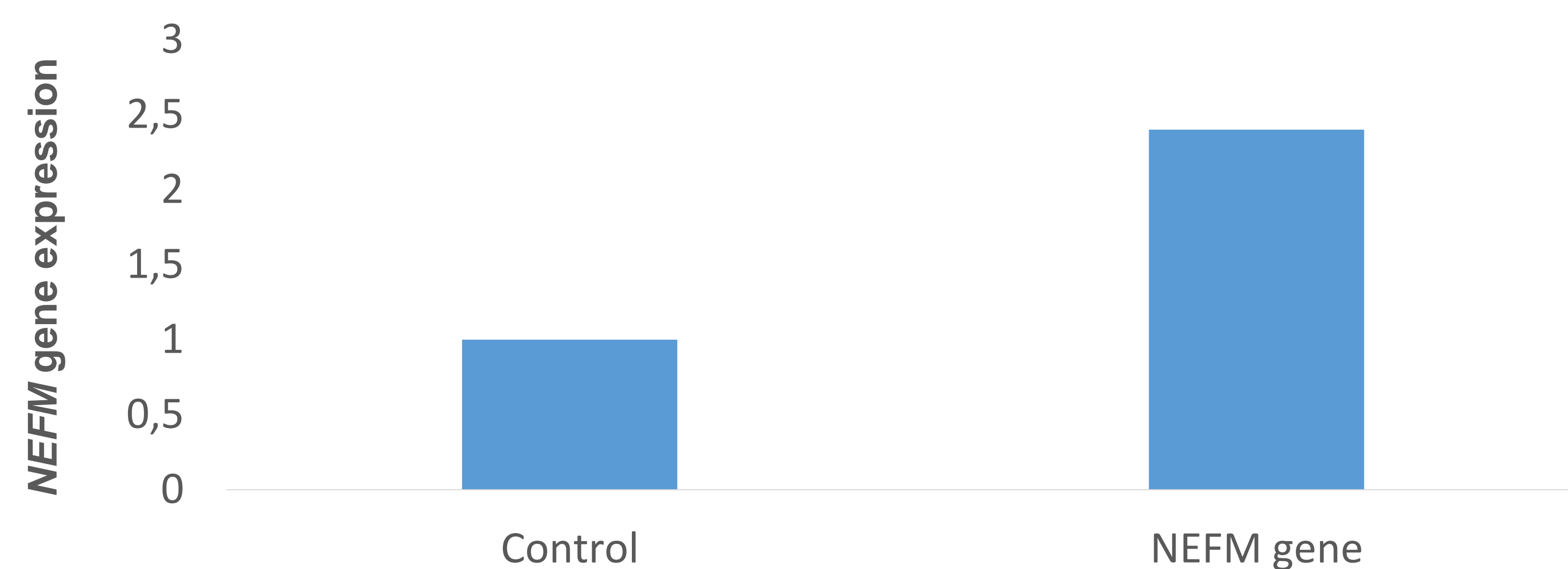


Fig.2 The expression level of *NEFM* mRNA is lower in control as compared to stimulating group. *NEFM* showed more resistance in stimulating group than control.

CONCLUSION

- According to the previous *DDX5* is used as a candidate gene for targeted breast cancer therapy of breast tumor in human (Cancer Discov; 2(9); 812–25. ©2012 AACR)
- According to (Xinyu Zhang et al, 2021) Differential transcriptional expression of *NEFM* was described in tumor and multiple cancer types using Oncomine database.
- Our results highlight novel potential functions of *NEFM* and *DDX5* in regulation of ovine mastitis immune microenvironment in sheep.

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