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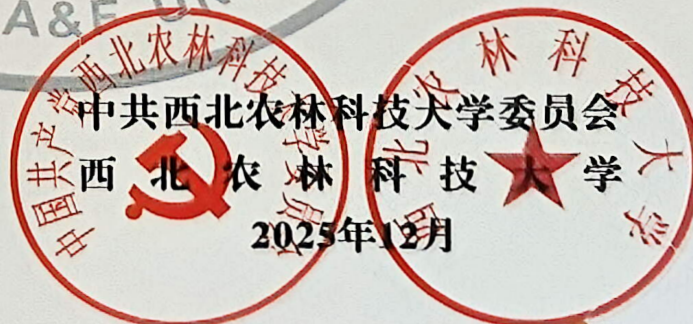


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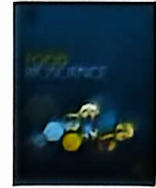
证书编号: YJYX20250336

誠 樸 勇 毅



Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Integrative transcriptomics and QTL analysis identify lactoperoxidase and deubiquitinase as key targets for mastitis control in dairy animals

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ARTICLE INFO

Keywords:
Deubiquitinase
QTL
Mastitis

ABSTRACT

Mastitis is a prevalent disease in dairy livestock, posing a significant threat to milk quality and the economic efficiency of the dairy industry. Transcriptomic analysis of mammary gland samples from healthy and mastitis-affected animals revealed that inflammatory responses and immune regulatory processes play central roles in the progression of the disease. Differentially expressed genes were significantly enriched in Gene Ontology (GO) categories related to cytokine-cytokine receptor interactions, cell adhesion molecules, and the JAK-STAT signaling pathway. Quantitative trait locus (QTL) association analysis indicated the importance of *CXCR1/CXCR2*. Literature evidence further suggests that lactoperoxidase (LPO) protein can mitigate inflammation by reducing CXCL2, a ligand of CXCR1/CXCR2. In this study, siRNA-mediated knockdown of the *LPO* gene demonstrated that LPO silencing under bacterial lysate stimulation markedly increased the expression of pro-inflammatory cytokines (*IL8*, *CXCL2*, *IL1β*; $p < 0.01$). Moreover, weighted gene co-expression network analysis (WGCNA) highlighted a key role for ubiquitination in mastitis responses. Experimental results showed that inflammatory stimulation significantly upregulated both inflammatory mediators (NF- κ B, *IL1β*, Caspase1) and deubiquitinating enzymes (*CYLD*, *A20*; $p < 0.01$). Based on these findings, this study proposes that future mastitis intervention strategies may benefit from enhancing endogenous antimicrobial proteins while modulating ubiquitination processes to attenuate excessive inflammatory responses. In summary, this study systematically elucidates the key molecular mechanisms underlying mastitis and proposes therapeutic and breeding approaches, providing a foundation for more effective clinical interventions and the development of mastitis-resistant breeding lines, with potential implications for enhancing animal health, milk quality, and the sustainable development of the dairy industry.

1. Introduction

Milk, with its distinctive taste and high quantity of proteins (whey and casein), lipids, and vitamins, has become a vital beverage in people's lives. It offers great potential in alleviating malnutrition and increasing farmers' income (Headey et al., 2024). Research indicates that milk oligosaccharides can promote brain development and shape the immune system in infants (Dinleyici et al., 2023; Yao et al., 2024). Globally, approximately 83 % of the milk and dairy products consumed by humans are produced by cows, 13 % by buffaloes, 2 % by dairy goats, 1 % by sheep, and 0.4 % by camels (Bittante et al., 2022; FAO, 2019). In recent years, with the improvement of living standards, the quality of raw milk and dairy products has received increasing attention. However, mastitis is a major cause of the decline in dairy product quality, leading

to global economic losses of at least \$37 billion (Lelito de Campos et al., 2023). Therefore, mastitis in dairy cattle has become a pressing global issue that needs to be addressed.

To date, various treatment strategies have been attempted to mitigate the impact of mastitis. Antibiotic therapy is a commonly used treatment both domestically and internationally (Sharun et al., 2021), however, prolonged use of antibiotics can lead to resistance and drug residues in dairy animals, posing a threat to human health (Wilm et al., 2021). With the advancement of bioinformatics, proteomics, transcriptomics, and metabolomics, analysis methods have been improved, becoming scientific approaches to investigate the potential reasons for differences in milk yield (Chen et al., 2024), flavor (Ren et al., 2023) and nutrition (Zou et al., 2022) between healthy and diseased dairy animals.

Lactoperoxidase (LPO) and Deubiquitinases (DUBs) play pivotal

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<https://doi.org/10.1016/j.fbio.2025.107965>

Received 3 July 2025; Received in revised form 30 October 2025; Accepted 17 November 2025

Available online 25 November 2025

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编号: SCIE-J-20260409-09

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标题: Integrative transcriptomics and QTL analysis identify lactoperoxidase and deubiquitinase as key targets for mastitis control in dairy animals

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来源出版物: FOOD BIOSCIENCE 卷: 74 文献号: 107965

DOI: 10.1016/j.fbio.2025.107965

出版时间: DEC 2025 在线发表: NOV 2025 已索引: 2025-12-15

入藏号: WOS:001633475500001 语言: English 文献类型: Article

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出版商: ELSEVIER

出版商地址: RADARWEG 29, 1043 NX AMSTERDAM, NETHERLANDS

Web of Science Index: Science Citation Index Expanded (SCI-EXPANDED)

Web of Science 类别: Food Science & Technology

研究方向: Food Science & Technology

IDS 号: P3304 ISSN: 2212-4292 eISSN: 2212-4306

Web of Science 核心合集中的 "被引频次": 0

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2025 年公布的影响因子: 5.9, JCR 分区情况:

JCR 学科类别	类别排序	类别分区
FOOD SCIENCE & TECHNOLOGY 其中 SCIE 版本	32/182	Q1

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2026 年 4 月 9 日