Effect of Staphylococcus aureus-induced mastitis on milk fatty acid composition in Chinese Holstein dairy cows: Postprint

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Abstract

This study aimed to investigate the effects of Staphylococcus aureus-induced mastitis on fatty acid composition and absolute contents in milk from Chinese Holstein dairy cows. Based on the mammary gland structure, the experimental group established an induced Staphylococcus aureus mastitis model by injecting Staphylococcus aureus bacterial suspension into the mammary gland via the teat canal using a milk catheter, while the control group was infused with phosphate-buffered saline. Gas chromatography was employed to determine the absolute fatty acid contents in milk. The results showed that a total of 34 fatty acids were detected in milk, and Staphylococcus aureus induction directly affected fatty acid synthesis in infected mammary quarters. Among the six major categories of fatty acids classified by degree of unsaturation and carbon chain length, the absolute contents of short-chain fatty acids, medium-chain fatty acids, and saturated fatty acids in the experimental group were significantly decreased at 11 h post-infection compared with 24 h before infection ($P < 0.05$); the control group exhibited the same trend, but none reached statistical significance except for short-chain fatty acids ($P > 0.05$). At 23 h post-infection, the absolute contents of all six categories of fatty acids in the experimental group were lower than those in the control group. These results suggest that Staphylococcus aureus-induced mastitis affected the fatty acid synthesis process in dairy cows and reduced the absolute contents of all fatty acid components.

Full Text

Effects of Staphylococcus aureus-Induced Mastitis on Milk Fatty Acid Composition of Chinese Holstein Cows

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Abstract

This study investigated the effects of Staphylococcus aureus-induced mastitis on milk fatty acid composition and absolute content in Chinese Holstein cows. Based on udder anatomy, the experimental group received intramammary infusion of S. aureus bacterial suspension via teat canal using a milk catheter to establish an induced S. aureus mastitis model, while the control group received phosphate-buffered saline. Gas chromatography was employed to determine fatty acid absolute contents in milk. The results demonstrated that 34 fatty acids were detected in milk, with S. aureus induction exerting direct effects on fatty acid synthesis in infected quarters. When classified into six categories by saturation degree and carbon chain length, the experimental group exhibited significantly reduced absolute contents of short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), and saturated fatty acids (SFA) at 11 hours post-infection compared to 24 hours pre-infection ($P<0.05$). The control group displayed similar trends, though no significant differences were observed ($P>0.05$) except for SCFA. At 23 hours post-infection, all six fatty acid categories in the experimental group showed lower absolute contents than the control group. These findings indicate that S. aureus-induced mastitis impairs fatty acid synthesis processes in dairy cows, reducing the absolute content of each component.

Keywords: Chinese Holstein cows; fatty acids; Staphylococcus aureus; mastitis

Introduction

Bovine mastitis is an inflammatory response of the mammary gland triggered by microorganisms, physical, and chemical factors, representing one of the most significant diseases affecting global dairy industry development due to its high incidence and substantial economic impact. While mastitis etiology is complex, pathogenic microbial infection constitutes the primary cause, with Staphylococcus aureus predominating, followed by Escherichia coli and Streptococcus

species, which together account for over 90% of mastitis pathogens. Consequently, research on defense mechanisms against S. aureus mastitis and its effects on mammary gland metabolism has become a focal point in mastitis prevention and control.

With advancing research on S. aureus, numerous investigators have utilized artificially induced S. aureus mastitis models to explore pathogenesis. Chandler first successfully established an experimental S. aureus mastitis mouse model in 1970 and subsequently employed it for penicillin efficacy trials in 1971. Bramley et al. found that intramammary infusion of lysostaphin could prevent and treat S. aureus mastitis in mice. Hu et al. demonstrated that the ligand-binding domain of lactoferrin-binding protein (lFnBP) conferred immune resistance against S. aureus in a mouse mastitis model. Guo et al. established an S. aureus mouse mastitis model and discovered that baicalin could downregulate p38 and nuclear factor- B (NF- B) phosphorylation, thereby reducing pro-inflammatory cytokine expression. Domestic researchers have conducted multifaceted investigations on artificially induced S. aureus mastitis mouse models. Yang et al. constructed an induced S. aureus mouse model using isolates from milk to study oxidative damage in visceral organs (liver, kidney, and spleen) and antimicrobial resistance.

Although mouse models facilitate S. aureus mastitis research and enable precise investigation of bacterial surface proteins and virulence factors, substantial differences in mammary duct structure between mice and cows limit complete simulation of the pathogenic process. Therefore, Yuan established a bovine mastitis model using S. aureus induction, while Liu et al. developed an artificially induced S. aureus bovine mastitis model to investigate DNA methylation status and mRNA expression of the bovine αs1-casein (CSN1S1) gene promoter region. Cui successfully induced apoptosis in bovine mammary epithelial cells using S. aureus and studied its signaling pathways. These studies collectively demonstrate that S. aureus induction has become an effective approach for investigating bovine mastitis etiology, pathogenesis, and therapeutic efficacy.

However, no reports have examined the effects of artificially induced S. aureus mastitis models on the synthesis of major milk components, particularly fatty acid metabolism. This study aimed to establish an S. aureus bovine mastitis model and investigate the impact of S. aureus infection on milk fatty acid synthesis and metabolism, providing theoretical reference for improving milk quality.

1.1.1 Experimental Animals

Three healthy, primiparous Chinese Holstein cows with no history of disease were selected. The cows exhibited similar body condition, well-developed udders, and negative bacterial culture results [somatic cell count (SCC) <100,000/mL]. All animals were in mid-lactation [(175$±$31) days] with an average daily milk yield of 19.20 kg and underwent 7 days of isolation observation before the experiment.

1.1.2 Feeding Management

Selected Chinese Holstein cows were fed total mixed ration (TMR) with the following formula: concentrate 19%, corn silage 50%, hay 10%, and dried distillers grains with solubles 21%. Concentrate composition included: corn 52%, wheat bran 8%, wheat middlings 10%, soybean meal 13%, cottonseed meal 5%, rapeseed meal 5%, bone meal 1%, salt 1%, and premix 5% (containing trace elements, vitamins, yeast culture, etc.). The TMR concentrate-to-forage ratio was approximately 40:60 on a dry matter basis, with 35 kg TMR provided per cow daily, ensuring feed access for at least 20 hours per day.

1.1.3 Bacterial Strain

Staphylococcus aureus (strain ATCC2913) was isolated from mastitic bovine mammary glands and provided by the College of Veterinary Medicine, Yangzhou University.

1.2 Establishment of S. aureus-Induced Mastitis Model

Based on udder anatomy, the experimental group received intramammary infusion of 5 mL S. aureus bacterial suspension (1$×10^{7}$ CFU/mL) via teat canal using a milk catheter, while the control group received an equal volume of sterile phosphate-buffered saline (PBS). The distribution of infused quarters is shown in Table 1 .

Table 1 The distribution of injected breast area of cow

Note: A1 and A2 represent left front and right front quarters of experimental cow 1, respectively; B1 and B3 represent left front and right rear quarters of experimental cow 2, respectively; C1 and C4 represent left front and left rear quarters of experimental cow 3. The same as below.

1.3 Milk Sample Collection

Fresh milk samples (50 mL) were collected at 24 hours pre-inoculation and 11 and 23 hours post-inoculation, transported to the laboratory in insulated containers, and immediately subjected to mastitis detection and SCC measurement. Remaining samples were stored at -20°C and analyzed for fatty acid (FA) composition and absolute content within 10 days.

1.4 Milk Fatty Acid Detection

Fatty acid detection followed the protocol of GB/T 21676-2008 “Determination of fatty acids in milk and milk products—Gas chromatography method”. Samples were filtered using organic-phase syringe filters before instrumental analysis, and gas chromatography was performed using programmed temperature detection.

Standard and sample fatty acids were analyzed under identical conditions for qualitative and quantitative determination based on retention time and peak

area. The quantification formula was:

$$C_{\text{sample}}(\text{mg/mL}) = C_{\text{standard}} \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$$

where C_{sample} and A_{sample} represent the concentration and peak area of a specific fatty acid in the sample, respectively, and C_{standard} and A_{standard} represent the corresponding values in the standard.

A 37-component fatty acid methyl ester mixture standard from Sigma-Aldrich (USA) was selected. Under established GC conditions, the standard was analyzed using an autosampler, with the chromatogram shown in Figure 1 [Figure 1: see original paper].

Figure 1 The chromatogram of 37 fatty acids mixture

1.5 Bovine Mastitis Detection

Mastitis detection employed the BMT (Beijing mastitis test) method. Two milliliters of milk sample were placed in a test plate, mixed with 2 mL of diagnostic reagent using a quantitative dispenser or graduated pipette, and gently rotated. Results were evaluated during mixing (within 10-25 seconds) and recorded.

1.6 Milk SCC Detection

On-site SCC measurement utilized a portable SCC-100 milk somatic cell rapid detector (Chemometec, Denmark). The procedure involved adding 0.5 mL Reagent C to a 2 mL centrifuge tube, followed by 0.5 mL milk sample, mixing thoroughly (10-20 s), aspirating the dissolved mixture with a sampler, immediately placing it in the detector slot, closing the lid, pressing "RUN" , and recording the results.

1.3 Statistical Methods

Data were organized using Excel software and subjected to one-way ANOVA using SPSS 17.0 statistical software.

Results

2.1 Clinical Manifestations Post-Infection

Following intramammary inoculation with S. aureus, cows gradually developed clinical symptoms. Inoculated quarters exhibited mild redness and swelling, with slight pain upon palpation. As time progressed, symptoms intensified, characterized by hardened udders, severe redness, swelling, and pain, along with abnormal milk containing clots, flocculent material, and watery appearance.

Affected cows displayed depression and anorexia, confirming successful induction of clinical mastitis.

2.2 BMT Test Results Pre- and Post-Infection

As shown in Table 2 , experimental group quarters (A2, B3, C4) exhibited weak positive reactions at 11 hours post-inoculation, while the control group remained negative. At 23 hours post-inoculation, the experimental group showed strong positive reactions, whereas only quarter C1 in the control group displayed weak positivity, with the remaining two quarters negative.

Table 2 The BMT result of cows

2.3 Milk SCC Measurement

Milk SCC results are presented in Table 3 . Following artificial S. aureus infection, SCC in infected quarters increased dramatically. All six quarters from three experimental cows had SCC <10$×10^{4}$ cells/mL before infection. Post-infection, SCC in three experimental quarters rose substantially, exceeding 100$×10^{4}$ cells/mL at 23 hours post-infection, with individual quarters reaching 200$×10^{4}$ cells/mL, indicating clinical mastitis. Although three control quarters showed slight SCC increases, only quarter C1 exceeded 50$×10^{4}$ cells/mL at 23 hours post-infection, while others remained below this threshold.

Table 3 Milk SCC test results of cows (10^4 cells/mL)

2.4 Milk Fatty Acid Chromatography Results

Under conditions identical to the standard, milk samples were analyzed for fatty acids based on retention time and peak area percentage for qualitative and quantitative determination. Figure 2 [Figure 2: see original paper] illustrates a sample milk chromatogram, identifying 34 fatty acids comprising 17 saturated fatty acids (SFA), 7 monounsaturated fatty acids (MUFA), and 10 polyunsaturated fatty acids (PUFA). These included 1 short-chain fatty acid (SCFA), 9 medium-chain fatty acids (MCFA), and 24 long-chain fatty acids (LCFA).

Figure 2 The chromatogram of milk fatty acids of cows

2.5 Changes in Individual Fatty Acid Absolute Content Pre- and Post-Infection

As shown in Table 4 , most fatty acids in the experimental group (except C4:0, C13:0, C20:3n6, C20:4, C22:6, C24:1) exhibited a trend of initial decrease followed by increase across the three time points, with highest absolute contents generally observed at 24 hours pre-infection. Short-chain fatty acid C4:0 was not detected at 24 hours pre-infection but showed significantly higher content

at 11 hours post-infection (P<0.05). Among medium-chain fatty acids (C6-C15), all except C6:0, C11:0, C13:0, and C15:0 displayed significantly lower absolute contents at 11 hours post-infection compared to 24 hours pre-infection. In long-chain fatty acids (C16-C24), C16:0 and C18:3n3 showed significant differences between 24 hours pre-infection and 11 hours post-infection (P<0.05), while C20:1 differed significantly between 24 hours pre-infection and both post-infection time points (P<0.05). Other fatty acids showed no significant differences (P>0.05). C24:1 increased then decreased, while C20:3n3 was undetectable at 11 hours post-infection and C22:6 at both 11 and 23 hours post-infection.

The control group exhibited similar decreasing-then-increasing trends for most fatty acids (except C4:0, C21:0, C22:6, C24:0), with highest absolute contents generally at 23 hours post-infection. Minimally abundant C20:3n3 and C22:6 were difficult to detect throughout the study, and no significant differences were observed among the three time points (P>0.05). Overall, experimental group fatty acid absolute contents were lower than control group values at all corresponding time points.

Table 4 The changes of the absolute contents of milk fatty acids of cows before and after infection (mg/mL)

Note: Values in the same row with different small letter superscripts indicate significant difference (P<0.05), and different capital letter superscripts indicate extremely significant difference (P<0.01). The same as above.

2.5 Changes in Different Fatty Acid Categories Pre- and Post-Infection

The 34 detected fatty acids were categorized into six groups based on carbon chain length and saturation degree: short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Statistical analysis and one-way ANOVA results are presented in Table 5 . Control group values for all six categories were lower at 11 hours post-infection compared to the other two time points, with significant or extremely significant differences compared to 23 hours post-infection (P<0.05 or P<0.01). In the experimental group, SCFA, MCFA, and SFA absolute contents were significantly reduced at 11 hours post-infection compared to 24 hours pre-infection (P<0.05), while the other three categories also decreased but without significant differences (P>0.05). At 23 hours post-infection, all six fatty acid categories in the experimental group remained lower than control group values.

Table 5 The changes of the absolute contents of different kinds of milk fatty acids of cows before and after infection

Discussion

3.1 Establishment and Application of Artificially Induced S. aureus Mastitis Model

Bovine mastitis poses a complex, chronic, and difficult-to-eradicate challenge, representing the greatest obstacle to economic efficiency in dairy production chains. Disease models serve as crucial tools for investigating pathology, etiology, and therapeutics. With deepening mastitis research, artificially induced infection models have gained prominence, with numerous studies employing S. aureus-induced mastitis models to explore pathogenesis and achieve significant results. These studies collectively demonstrate that S. aureus induction has become an effective methodology for investigating bovine mastitis etiology, mechanisms, and treatment efficacy. However, no studies have reported how S. aureus mastitis affects milk component synthesis or alters mammary gland fat metabolism. This study established an S. aureus bovine mastitis model to investigate fatty acid alterations, thereby addressing this research gap.

3.2 Changes in Individual Fatty Acid Component Absolute Content Pre- and Post-Infection

Yang et al. reported direct correlation between milk fatty acid content and milk fat percentage, with mastitis causing significant milk fat reduction that necessarily manifests as decreased fatty acid content. Our analysis of 34 fatty acids revealed 31 showed reduced content following S. aureus infection, likely related to the inflammatory response. BMT results indicated weak positive reactions in experimental quarters at 11 hours post-infection, progressing to clinical mastitis with intensified inflammation. Since milk fat synthesis occurs in mammary alveolar epithelial cells, mastitis impairs synthetic capacity, reducing milk fat content and consequently fatty acid content. However, the subsequent increase in these 31 fatty acids differs from Chang' s findings, primarily because this artificial induction employed highly pathogenic S. aureus that severely damaged mammary secretory cells, reducing milk fat synthesis and fat percentage. The bovine immune system' s response to intramammary infection eliminates pathogens, gradually restoring fatty acid synthesis capacity. The control group exhibited similar trends because, despite lacking typical clinical symptoms, systemic pathogen diffusion across the blood-milk barrier affected mammary cell lipid metabolism. As a mild infection, the control group' s immune response eliminated infection and restored normal non-infected status, whereas experimental group secretory cells suffered irreversible damage, preventing complete recovery despite some fatty acid content elevation that remained significantly lower than control values.

3.3 Effects of S. aureus Infection on Different Fatty Acid Categories

Our study revealed that among six fatty acid categories classified by carbon chain length and saturation, experimental group SCFA, MCFA, and LCFA ab-

solute contents were significantly reduced at 11 hours post-infection compared to 24 hours pre-infection. This aligns with Chang' s findings that healthy and subclinical mastitis milk showed no significant differences in SFA, MUFA, and PUFA absolute contents, both being significantly higher than clinical mastitis values. At 23 hours post-infection, all fatty acid categories showed higher contents than at 11 hours post-infection, demonstrating robust mammary repair capacity. Experimental group values remained lower than control group values across all three time points, particularly at 23 hours post-infection, consistent with trends observed for individual fatty acids. Invasive S. aureus severely damaged mammary secretory cells, obstructing milk component synthesis and drastically reducing milk fat percentage, though increased free fatty acids in milk may have inhibited bacterial proliferation and protected mammary tissue.

As no previous studies have reported fatty acid profiles and absolute content changes in milk from cows with artificially induced S. aureus mastitis, this research provides preliminary observations. Future investigations could employ transcriptomic sequencing and bioinformatics to analyze infection-induced fatty acid composition changes, identify fatty acid metabolism pathways and candidate genes, construct gene regulatory networks, and elucidate regulatory mechanisms of fatty acid synthesis, metabolism, and mammary immunity in S. aureus infection. This would enhance understanding of how S. aureus affects mammary lipid metabolism and other synthetic processes. S. aureus infection substantially reduces mammary fatty acid synthesis capacity, particularly for SCFA, MCFA, and SFA. However, the bovine mammary gland possesses considerable anti-stress and adaptive capacity, with fatty acid synthesis increasing substantially after infection.

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Note: Figure translations are in progress. See original paper for figures.