

Therapeutic Effect of Nisin Z on Subclinical Mastitis in Lactating Cows[∇]

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Bovine subclinical mastitis is an inflammation of the mammary gland caused by bacterial intramammary infection, accounting for large economic losses. Treatment of subclinical mastitis is not suggested for lactating cows due to the risk of milk contamination. The objectives of this study were to evaluate an antimicrobial peptide, nisin, in the treatment of subclinical mastitis in lactating cows. A total of 90 lactating Holstein cows with subclinical mastitis were randomly divided into nisin-treated ($n = 46$) and control ($n = 44$) groups. In the nisin-treated group, cows received an intramammary infusion of nisin at a dose of 2,500,000 IU once daily for 3 days while the control cows received no treatment. Milk samples were collected from the affected mammary quarters before treatment and 1 and 2 weeks after treatment for analyses of bacteria, somatic cells, and *N*-acetyl- β -*D*-glucosaminidase (NAGase). Results indicated that nisin therapy had bacteriological cure rates of 90.1% for *Streptococcus agalactiae* (10 of 11), 50% for *Staphylococcus aureus* (7 of 14), 58.8% for coagulase-negative staphylococci (7 of 17), and 65.2% for all cases (30 of 46). Meanwhile, only 15.9% (7 of 44) of untreated cows spontaneously recovered. NAGase activity in milk samples and the number of mammary quarters with a milk somatic cell count of $\geq 500,000$ /ml were significantly decreased after nisin treatment while no significant changes took place in the control group. Because of its therapeutic effects on bovine subclinical mastitis, as well as its safety in humans, nisin deserves further study to clarify its effects on mastitis caused by different pathogens.

Mastitis is the inflammation of the mammary gland in response to bacterial invasion. Clinical and subclinical mastitis are two major forms of intramammary infections (IMIs) in dairy cows. Clinical mastitis results in alterations in milk composition and appearance; decreased milk production; elevated body temperature; and swelling, redness, or heat in infected mammary glands. It is readily apparent and easily detected. However, detection of subclinical mastitis is more difficult because signs are not readily apparent. Consequently, subclinical mastitis often goes undetected with a tendency to persist, resulting in an elevated milk somatic cell count (SCC) and decreased milk production, which may lead to development of clinical mastitis and a chance for contagious bacteria to spread from infected to uninfected mammary glands. It is generally accepted that 70 to 80% of the estimated \$140 to \$300 loss per cow per year from mastitis relates to reduced milk production caused by asymptomatic subclinical mastitis (9). Antibiotic therapy is the most widely used approach to IMIs. Loss of milk due to discarding milk contaminated with antibiotics has been cited as a reason why treatment of subclinical mastitis is not suggested during lactation. Treatment is instead postponed until a clinical flare-up occurs or until dry-off, a time when antibiotics are often routinely used to treat all quarters in all cows. With increasing pressure to deliver milk with a low bulk milk SCC, it may not be viable, economically, to wait until dry-off before measures are taken to lower the prevalence of IMI. In addition, high culling rates due to high-SCC cows have been found in herds with a high milk SCC close to the upper

legal limit (1). Treatment of subclinical mastitis during lactation could represent an alternative to culling. Thus, there is an interest in searching for new drugs suitable for subclinical mastitis therapy during lactation.

Nisin is a natural antimicrobial peptide of 34 amino acids produced by *Lactococcus lactis* (4). The peptide is suggested to be effective against a wide range of gram-positive bacteria, including mastitis pathogens (6, 19, 20, 25). In the United States, nisin was confirmed to be “generally recognized as safe” in 1988 (24). Because of its high antibacterial activity and nontoxicity for humans, nisin has already been employed as a food preservative for a long time and is licensed by 48 countries around the world (5). As an active ingredient, nisin has been formulated into some commercially available products for teat dipping to prevent mastitis (20). Although antibacterial activities of nisin have been reported, few research papers have been found as of yet regarding the use of nisin for subclinical mastitis treatment. The present study was designed to evaluate a nisin-based formulation for intramammary infusion in the treatment of subclinical mastitis in lactating cows on a commercial dairy farm. Changes in SCC, IMIs, and *N*-acetyl- β -*D*-glucosaminidase (NAGase) were evaluated before and after nisin treatment. Untreated subclinical cases were used as a control.

MATERIALS AND METHODS

Cows. The study was carried out on a dairy farm in Hangzhou, Zhejiang, China, with approximate 1,000 Holstein dairy cows that were milked by machine three times daily. A cow with a milk SCC of $\geq 500,000$ cells/ml was considered to be suffering from mastitis. A total of 90 clinically healthy lactating Holstein cows with at least one quarter of natural subclinical mastitis (first lactation, $n = 16$; second lactation, $n = 21$; third lactation, $n = 20$; fourth lactation, $n = 19$; fifth lactation, 13; sixth lactation, $n = 1$) were used.

Nisin-based formulation. Purified nisin Z (18,000 IU/mg) was supplied as Silver-Elephant nisin by Zhejiang Silver-Elephant Bio-Engineering Co., Ltd.,

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Tiantai, Zhejiang, China. The nisin-based formulation mainly contained 2,500,000 IU of nisin Z. Before treatment, nisin was dissolved in 20 ml of sterile physiological saline.

Irritancy test of nisin Z on bovine mammary gland. The cow (04042) used for the irritancy test was at her first midlactation and 28 months old. The cow was clinically healthy, and all quarters were negative by the California mastitis test and bacteriological examination. The left forequarter received an intramammary infusion of physiological saline (20 ml), and the left hindquarter, right hindquarter, and right forequarter were intramammarily infused with nisin Z at 1,250,000 IU, 2,500,000 IU, and 5,000,000 IU, respectively. The infusions were administered after the morning milking. Quarter milk samples were collected 24, 48, and 72 h post-intramammary infusion for analysis of SCC and NAGase.

Treatment. Cows with subclinical mastitis were randomly divided into nisin-treated ($n = 46$) and control ($n = 44$) groups. In the nisin-treated group, the cows received an intramammary infusion of nisin at a dose of 2,500,000 IU once daily after morning milking for 3 days, while in the control group, no treatment was performed. Before nisin treatment, the diseased quarter was thoroughly milked out by hand and the teat end was cleaned using a cotton swab soaked with 70% alcohol.

Milk sample collection and analysis. Fore-milk samples were aseptically collected from the affected mammary quarters immediately before initiating treatment and 1 and 2 weeks after terminating treatments for bacteriological examination, somatic cell counting, and the NAGase test.

Bacteriological examination. The milk sample was streaked on a blood agar plate and incubated for 24 to 48 h at 37°C. After incubation, the plate was read for primary isolation of mastitis pathogens. A milk sample was considered contaminated when three or more different colony types of bacteria were detected. Then, a single colony on the blood agar was inoculated into broth medium and incubated for 18 to 24 h at 37°C. Further identification of specific bacterial species such as staphylococci, streptococci, and gram-negative bacteria was performed according to the methods described by the National Mastitis Council (14).

SCC analysis. For determination of SCC, milk samples were preserved with bronopol (2 µl/ml) after sample collection and analyzed using Integrated Milk Testing MilkoScan FT6000 (FOSS, Beijing, China).

NAGase test. Milk samples were frozen and thawed for three cycles to release NAGase within cells and then centrifuged at 3,500 rpm for 20 min to remove the cream layer. The skim milk was adjusted to pH 4.6 by the addition of 10% acetic acid and centrifuged at 3,500 rpm for 20 min to obtain whey at the top layer. The NAGase activity level in milk whey was determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) following the manufacturer's protocols. The optical density of paranitrophenol during the reaction (at 37°C) between the 4-methylumbelliferyl-*N*-acetyl-β-glucosaminide substrate and the NAGase contained in the analyzed samples was measured spectrophotometrically in triplicate at a wavelength of 400 nm. One unit of NAGase activity represents the amount of paranitrophenol released from 1 liter of whey in 15 min at 37°C.

Detection of nisin residues in milk following intramammary infusion in subclinically mastitic quarters. Two milk samples were aseptically collected from each of three subclinically mastitic cows at 24, 48, 72, and 96 h after termination of treatment with nisin at a dose of 2,500,000 IU for 3 days. One sample was from a nisin-treated quarter, and the other was from untreated quarters (composite milk). Nisin in milk samples was evaluated by the agar diffusion assay mainly as described by Pongtharangkul and Demirci (17). Briefly, S1 agar containing 1.5% (wt/vol) Tween 20 was autoclaved and cooled to 45°C. An overnight culture of *Micrococcus luteus* (NCIB 8166) was then added in a final concentration of 0.2% (vol/vol). Precisely 210 ml of this suspension was poured into each sterile petri plate (280 × 210 mm). After that, plates were kept at room temperature for 2 h to allow agar solidification. The plates were then stored at 4°C for 24 h. Holes were bored on the plates using a 7-mm-outer-diameter stainless steel borer. One hundred microliters of nisin standard solution (20, 40, 80, 160, and 320 IU/ml) as a test solution was dispensed into each well. The plates were then incubated at 30°C for a minimum of 20 h to give a well-defined inhibition zone. Seven wells were used for each nisin concentration. Using a caliper, inhibition zone diameters were measured to the nearest 0.1 mm. The mean of the largest and shortest diameters was calculated for oval inhibition zones. A regression equation was calculated for inhibition zone diameter as a function of log nisin concentration.

Test of susceptibility of *Staphylococcus aureus* isolates to antibacterial agents. Twenty *S. aureus* isolates were obtained from the mastitis cases. With a sterile loop, four to five colonies of *S. aureus* from a pure culture were picked up and suspended in 5 ml of sterile physiological saline. The bacterial suspension was adjusted to 1×10^8 CFU/ml according to the McFarland standard (2). Three hundred fifty microliters of the suspension was inoculated on a nutrient agar

plate. Susceptibility test disks were obtained commercially (Hangzhou Tian He Microorganism Reagent Co., Ltd., Hangzhou, China). After 0.5 h, disks containing penicillin G (10 µg), gentamicin (10 µg), cefamezin (30 µg), norfloxacin (5 µg), or sulfamethoxazole-trimethoprim (SMZ-TMP) (23.75/1.25 µg) were placed using a sterile forceps onto the agar surface and gently pressed down to ensure contact. Plates were incubated at 37°C for 20 h. Subsequently, the diameter of the inhibition zone around each disk was measured. Based on the diameter of the inhibition zone, the susceptibilities of the bacterial isolates to penicillin G, gentamicin, cefamezin, norfloxacin, and SMZ-TMP were classified as sensitive (≥ 29 mm, ≥ 15 mm, ≥ 18 mm, ≥ 17 mm, and ≥ 16 mm, respectively), resistant (≤ 28 mm, ≤ 12 mm, ≤ 14 mm, ≤ 12 mm, and ≤ 10 mm, respectively), and intermediate (the ranges between sensitive and resistant) as outlined by Hangzhou Tian He Microorganism Reagent Co., Ltd., Hangzhou, China.

Determination of MIC of nisin in milk. The determination of MIC of nisin in milk is based on the change of colorless tetrazolium chloride into red formazan in the presence of metabolically active bacteria. *S. aureus* isolates were inoculated on blood agar plates for incubation of 24 h at 37°C. After the incubation, a typical colony of *S. aureus* was inoculated into skim milk for incubation of 18 h, and the culture was then diluted (1:20) in skim milk. Nisin Z was dissolved in skim milk at a concentration of 640 IU per ml. Twofold serial dilutions of the skim milk were made in a 96-well polystyrene plate, and nisin-free skim milk was added in the wells on the right side as a negative control. To each well, *S. aureus* diluted in skim milk (20 µl) was added, and the well was incubated at 37°C for 2 h. After that, each well was supplemented with 6 µl of 4% tetrazolium chloride (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The color change of each well was viewed at a 30-minute interval. The MIC was expressed as the lowest concentration of nisin without color change.

Statistical analysis. SAS 9.0 software for Windows was used for statistical analysis. Chi-square analysis was used to compare the numbers of quarters with an SCC of $\geq 5 \times 10^5$ /ml between the two groups at each sampling occasion as well as the numbers of quarters with an SCC of $\geq 5 \times 10^5$ /ml and IMI between pretreatment and posttreatment in the same group. Analysis of variance was used to compare the mean activities of NAGase between the two groups at each sampling occasion and between pre- and posttreatment times in the same group. Probabilities of less than 0.05 were considered significant.

RESULTS

Irritation by intramammary infusion of nisin into bovine mammary gland. Following a single intramammary infusion, both SCC and NAGase in milk tended to increase when the dose of nisin increased from 0 to 5,000,000 IU (Fig. 1). No visible local reactions and a decreased milk yield were observed in the mammary gland infused with physiological saline (left forequarter) or infused with nisin at 1,250,000 IU (left hindquarter) or 2,500,000 IU (right hindquarter). However, the mammary quarter (right forequarter) became slightly swollen and tender with clots and flakes in the milk with apparently decreased milk production after infusion of 5,000,000 IU of nisin. The visible abnormalities persisted until 24 h after the intramammary infusion.

Persistence of milk residue following intramammary infusion of nisin for subclinical mastitis therapy. For the milk samples from nisin-treated quarters, nisin (75.8 ± 36.5 IU/ml and 5.7 ± 7.3 IU/ml) was detected 24 and 48 h, respectively, after intramammary infusion of nisin Z at the dose of 2,500,000 IU once daily for 3 days for subclinical mastitis therapy (Fig. 2). For the composite milk collected from untreated quarters, no nisin residue was detected.

Milk SCC in subclinically mastitic mammary glands treated with nisin. Mammary quarters with a milk SCC of $\geq 500,000$ cells/ml were reduced in number from 46 at pretreatment to 27 and 31 quarters at 1 and 2 weeks after nisin treatment, respectively ($P < 0.01$), while no changes were found in the control group ($P > 0.05$) (Table 1).

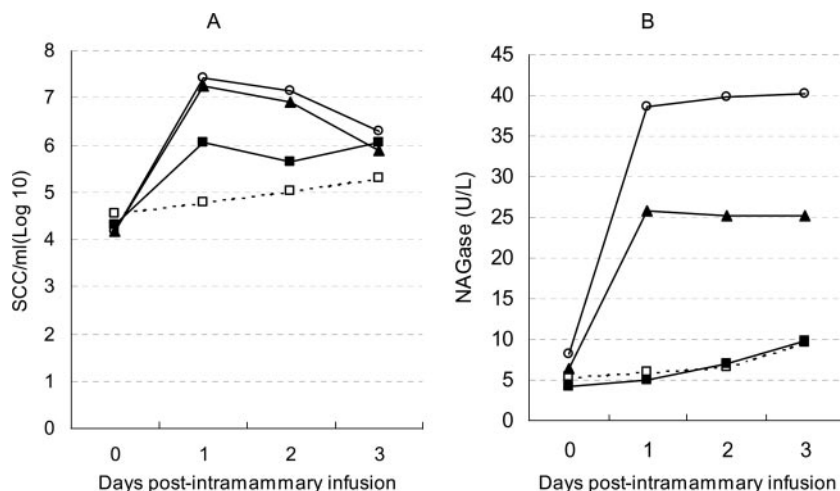


FIG. 1. SCC (A) and NAGase (B) in milk samples from four quarters of cow 04042 before and after intramammary infusion of physiological saline (□) and nisin Z doses of 1.25×10^6 (■), 2.50×10^6 (▲), and 5.00×10^6 (○) IU, respectively.

NAGase activity in subclinically mastitic mammary glands treated with nisin. NAGase activity in milk samples was reduced from 43.7 ± 35.5 U/liter at pretreatment to 26.2 ± 21.3 U/liter and 28.9 ± 23.2 U/liter 1 and 2 weeks, respectively, after nisin treatment ($P < 0.05$), while no changes were found in the control group ($P > 0.05$) (Table 2).

Changes of bacteria detected from subclinically mastitic milk samples before and after treatment. Of the bacteria isolated from milk samples, *S. aureus*, coagulase-negative staphylococci (CNS), and *Streptococcus agalactiae* were the predominant causative pathogens of subclinical mastitis, being isolated from 27.8%, 31.1%, and 33.3% of cows, respectively (Table 3), and the others accounted for only 7.8%. After intramammary infusion of nisin at a dose of 2,500,000 IU once daily for 3 days, IMI was reduced by 43.5% (20 of 46) and 65.2% (30 of 46) ($P < 0.01$) at 1 and 2 weeks after treatment, respectively. For *S. aureus* and *Streptococcus agalactiae* IMIs, 50% (7 of 14) and 90.9% (10 of 11), respectively, of the subclinical mastitis cases became bacteriologically negative ($P < 0.05$). No changes were found in the control group ($P > 0.05$).

Antibiotic resistance of *Staphylococcus aureus* isolated from mastitic cows. Of 20 *S. aureus* isolates, the isolates resistant to

penicillin, gentamicin, cefamezin, norfloxacin, and SMZ-TMP were 80%, 45%, 5%, 75%, and 90% of all isolates, respectively.

MIC of nisin for *Staphylococcus aureus* isolates in milk. The MIC of nisin for 20 *Staphylococcus aureus* isolates in milk was 75.5 ± 70.8 IU/ml.

DISCUSSION

It was previously found that infusion of nisin or another drug solution into the mammary gland in healthy cows caused irritation. Taylor et al. (22) observed irritant effects after intramammary infusion of nisin in oil in healthy cows. Hulse and Lancaster (10) found a small number of clots in milk (an indicator of irritation) after intramammary infusion of nisin (2,500,000 IU) in cows. Gill et al. (8) reported a large increase in milk SCC after a single infusion of either filter-sterilized broth lysate or a CsCl gradient-purified phage preparation. In the present study, purified nisin dissolved in sterile saline was used for intramammary infusion. Clinical signs were observed only in the mammary quarter infused with 5,000,000 IU of nisin, and no visible changes were seen in milk and mammary quarters when 2,500,000 IU or less of nisin was administered, although milk SCCs and NAGase activities were elevated in those quarters (Fig. 1).

Hulse and Lancaster (10) reported that 83 and 15 IU/ml were detected in milk samples 24 and 48 h, respectively, after

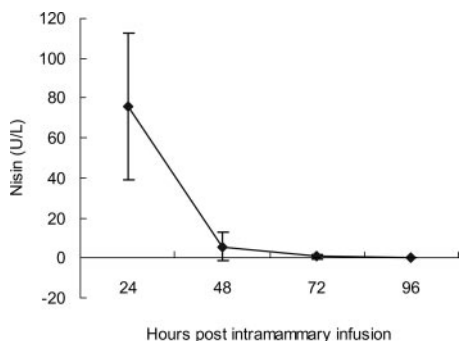


FIG. 2. Nisin Z levels (means \pm standard deviations, IU/ml) in milk samples collected from subclinically mastitic quarters ($n = 3$) 24, 48, 72, and 96 h after intramammary infusion of nisin Z at a dose of 2,500,000 IU once daily for 3 days.

TABLE 1. Quarters with SCCs of $\geq 5 \times 10^5$ /ml after nisin treatment

Group	No. (%) of quarters with SCCs of $\geq 5 \times 10^5$ /ml ^a		
	Pretreatment	Posttreatment	
		1 wk	2 wk
Nisin	46 ^A	27 (58.7) ^{Ab}	31 (70.5) ^{Ab}
Control	44 ^A	36 (81.8) ^B	40 (90.9) ^B

^a Data within the same time point with different superscript capital letters differ significantly ($P < 0.01$).

^b Significantly different compared with pretreatment values ($P < 0.05$).

TABLE 2. NAGase in milk before and after nisin treatment

Group	No. of cases	Mean concn of NAGase (U/liter of milk) \pm SD ^a		
		Pretreatment	Posttreatment	
			1 wk	2 wk
Nisin	46	43.7 \pm 35.5 ^A	26.2 \pm 21.3 ^{Ab}	28.9 \pm 23.2 ^{Ab}
Control	44	41.1 \pm 32.0 ^A	39.7 \pm 29.5 ^B	44.2 \pm 39.5 ^B

^a Means within the same time point with different superscript capital letters differ significantly ($P < 0.05$).

^b Significantly different compared with pretreatment ($P < 0.05$).

intramammary infusion of 2,500,000 IU of nisin. A similar result was observed in our present study. After intramammary infusion of the same amount of nisin once daily for 3 days for subclinical mastitis therapy, the milk from treated quarters contained 75.8 ± 36.5 IU/ml 24 h after the last treatment (Fig. 2). Forty-eight hours later the amount of nisin in milk samples ranged from 5.7 ± 7.3 IU/ml to traces. Interestingly, in another study by us of clinical mastitis treatment using nisin, only 4.5 ± 0.8 IU/ml of nisin was detected 12 h after intramammary infusion at the same dose twice daily for 3 days. The less decreased nisin residue detected in this study may be attributed to less active enzymes in subclinically mastitic milk samples than in clinically mastitic milk samples, which degrade the antimicrobial peptide. According to the Hygienic Standards for the Use of Food Additives of the Ministry of Public Health of the People's Republic of China (13), nisin used as a preservative in milk is suggested to be used at 10 IU with an upper limit of 500 IU/ml. Therefore, nisin is safe for subclinical mastitis therapy in lactating cows, as the residues detected in milk samples 24 h posttreatment were much lower than the upper limit allowed for use in preserving milk.

Treatment of subclinical mastitis once daily was clinically practical for veterinarians as this regimen was easy to practice and resulted in a concentration of 75.8 ± 36.5 IU of nisin per ml of milk 24 h later, a concentration remaining at the MIC of nisin for *S. aureus* (75.5 ± 70.8 IU/ml) as detected in this study.

Many attempts have been made to eliminate IMIs during lactation. Wilson et al. (26) infused 750 mg of florfenicol or 200 mg of cloxacillin into the mammary glands every 12 h for three times to treat IMIs. Overall cure rates were as indicated: *Streptococcus agalactiae*, 5 of 8 (63%); *S. aureus*, 5 of 54 (9%); *Streptococcus* sp., 16 of 35 (46%); *Staphylococcus* sp., 7 of 33 (21%); *Escherichia coli*, 5 of 11 (46%); *Klebsiella* sp., 3 of 6 (50%); others, 1 of 9 (11%); and all cases, 42 of 156 (27%). The authors attributed the poor therapeutic effect partly to the short duration of treatment. Oliver et al. (16) extended the duration of treatment by comparing 2-, 5-, and 8-day treatment regimens of intramammary infusion of 125 mg of ceftiofur hydrochloride. They found that the efficacy of ceftiofur therapy against all subclinical IMIs was 38.8, 53.7, and 65.8% for the 2-, 5-, and 8-day regimens, respectively, and the 8-day extended ceftiofur treatment regimen was significantly better than the 2-day treatment. Considering the residues left in milk by antibiotic therapy, some authors used biological preparations to treat subclinical mastitis. For example, Gill et al. (8) treated subclinical *S. aureus* mastitis by intramammary infusions of lytic *S. aureus* bacteriophage K once daily for 5 days and found that the cure rate was 3 of 18 quarters (16.7%) and no statis-

TABLE 3. Milk samples with positive bacteriological examination results

Bacterium	No. of positive samples in group at wk posttreatment					
	Nisin treated			Control		
	0 ^a	1	2	0 ^a	1	2
<i>S. aureus</i>	14	7 ^b	7 ^b	11	9	9
CNS	17	14	7 ^b	11	9	8
<i>Streptococcus agalactiae</i>	11	3 ^b	1 ^b	19	18	18
<i>Streptococcus dysgalactiae</i>	2	1	1	2	1	1
<i>Streptococcus uberis</i>	2	1	0	1	1	1
Total	46	26 ^b	16 ^b	44	38	37

^a Pretreatment.

^b Significantly different compared with pretreatment value within the same group ($P < 0.05$).

tically significant difference was found between the phage- and saline-treated quarters. In our present study, we treated subclinical mastitis by intramammary administration of nisin at a dose of 2,500,000 IU once daily for 3 days and obtained the indicated bacteriological cure rates: *Streptococcus agalactiae*, 10 of 11 (90.1%); *S. aureus*, 7 of 14 (50.0%); CNS, 7 of 17 (58.8%); and all cases, 20 or 30 of 46 (43.5 or 65.2%) at 1 and 2 weeks posttreatment, respectively (Table 3). Meanwhile, only 15.9% (7 of 44) of quarters spontaneously recovered in the untreated control group.

Fourteen years ago, Cai et al. (3) reported that most of the *S. aureus* isolates from mastitis cases of dairy cattle in the region where the present study was carried out were resistant to penicillin but sensitive to gentamicin. From that time on, gentamicin has become one of the choices for mastitis treatment. After extensive use of this antibiotic on farms during the past 14 years, we observed that 80% of the *S. aureus* isolates remained resistant to penicillin and 45% of them became resistant to gentamicin. Gentamicin treatment eliminated only two out of six *S. aureus* IMIs (33.3%) in another study by us (L. T. Cao et al., 2007, unpublished data).

Milk SCC consists of macrophages, polymorphonuclear leukocytes, lymphocytes, and epithelial cells (11, 12). Penetration of pathogenic bacteria into the mammary gland stimulates polymorphonuclear leukocytes to migrate from the bloodstream into the milk compartment, resulting in a rapid rise in milk SCC (21). A high SCC is usually indicative of an immune response to IMI with bacterial pathogens (7), and a low SCC ($<150,000$ cells/ml for quarter milk [21]) generally indicates freedom from such infection. In this study, intramammary infusion of nisin significantly decreased the number of quarters with high milk SCCs ($\geq 5 \times 10^5$ cells/ml) compared to the control or pretreatment SCCs, indicating the value of nisin in the treatment of subclinical mastitis for decreasing milk SCC (Table 1). This result also paralleled the significantly decreased milk NAGase activity posttreatment in the nisin-treated group compared with the control group or pretreatment, since the increased activity of milk NAGase may be observed in the inflammation of the mammary gland (15, 18, 23).

In conclusion, intramammary administration of nisin at a dose of 2,500,000 IU once daily for 3 days was effective in treatment of subclinical mastitis caused by several different

mastitis pathogens in lactating dairy cows on a commercial dairy farm. Nisin therapy resulted in the bacteriological cure rates of 90.1% for *Streptococcus agalactiae* (10 of 11), 50% for *Staphylococcus aureus* (7 of 14), 58.8% for CNS (7 of 17), and 43.5 or 65.2% for all cases (20 or 30 of 46) 1 and 2 weeks posttreatment, respectively. Meanwhile, only 15.9% (7 of 44) of quarters spontaneously recovered in the untreated control group. Mammary quarters with a milk SCC of $\geq 500,000$ cells/ml were significantly reduced in number from 46 at pretreatment to 27 and 31 quarters 1 and 2 weeks after nisin treatment, respectively, while no changes were found in the control group. NAGase activity in milk samples was significantly decreased from 43.7 ± 35.5 U/liter pretreatment to 26.2 ± 21.3 U/liter and 28.9 ± 23.2 U/liter 1 and 2 weeks after nisin treatment, respectively, while no changes were found in the control group. Following intramammary infusion, nisin was detected in milk only at 24 h (75.8 ± 36.5 IU/ml) and 48 h (5.7 ± 7.3 IU/ml), levels which were much lower than the upper limit (500 mg/ml) allowed as a preservative in milk by the Chinese authorities. Because of its efficacy in the treatment of bovine subclinical mastitis, especially drug-resistant *S. aureus*-caused IMI, as well as its safety in humans, nisin deserves further study to clarify its effects on mastitis caused by different mastitis pathogens on a larger scale.

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