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Subclinical mastitis in goats is associated with upregulation of nitric oxide-derived oxidative stress that causes reduction of milk antioxidative properties and impairment of its quality

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ABSTRACT

The aim of this study was to verify the existence of a nitric oxide (NO) cycle in goat milk and to study how changes in it affect milk composition during subclinical mastitis. Fifteen lactating dairy goats in which one udder-half was free from bacterial infection and the contra-lateral one was naturally infected with various species of coagulase-negative staphylococci were used. In comparison to uninfected glands, subclinical mastitis was associated with a decrease in milk yield, lactose concentration, and curd yield and an increase in nitrite and nitrate concentrations and with measurements reflecting increased formation of NO-derived free-radical nitrogen dioxide. The occurrence of NO cycling in goat milk was largely confirmed. The increase in the NO-derived stress during subclinical infection was not associated with significant increase in oxidatively modified substances, 3-nitrotyrosine, and carbonyls on proteins, but with increased levels of peroxides on fat. However, the relatively modest nitrosative stress in subclinically infected glands was associated with significant reduction in total antioxidant capacity and vitamin C levels in milk. We concluded that subclinical mastitis in goats caused by coagulase-negative staphylococci imposes negative changes in milk yield, milk quality for cheese production, and negatively affects the nutritional value of milk as food. Thus, subclinical mastitis in goats should be considered as a serious economic burden both by farmers and by the dairy industry.
Key words: goat, milk, subclinical mastitis, oxidative stress, antioxidant capacity

INTRODUCTION

The quality of dairy products depends on that of the raw milk, which is, in turn, primarily affected by animal health, milking intervals, milk storage, and time until processing (Roupas, 2001; Leitner et al., 2008b). Reduction in goat milk quality will have a negative influence on its industrial value, as most of the milk is processed into fermented products and cheese.

Quality and quantity of milk in dairy animals, including goats, was found to be mainly affected by subclinical IMI (Leitner et al., 2008a; Silanikove et al., 2010), mainly due to various CNS (Djabri et al., 2002; Leitner et al., 2004b; Taponen et al., 2006). In dairy cows, subclinical mastitis has only moderate projection on bulk milk because of the low increase in SCC in the infected glands (Pitkälä et al., 2004; Leitner et al., 2008b) and the dilution effect of the 4 quarters. However, the influence of the infection reflected by SCC on the animal level is relatively high in sheep and goats compared with cows because of the strong immune response to the infection and the existence of only 2 mammary glands (Leitner et al., 2011).

Milk from mastitic udders exhibits increased proteolytic activity (Le Roux et al., 1995; Leitner et al., 2011). Plasmin (**PL**) is the most important protease in milk from both healthy udders and udders with elevated SCC, but the nonplasmin proteases become more important with increased severity of udder inflammation (O'Farrell et al., 2002; Leitner et al., 2004b, 2011). Proteolysis of casein leads to a decrease in the relative proportion of caseins (especially β-CN and α_{s1}-CN) with simultaneous pronounced increase of γ-CN and proteose peptones (Silanikove et al., 2006). During the last decade, our laboratories extensively studied the physiological basis for the reductions in milk yield and its quality under exposure to IMI and stress (Silanikove et al., 2006, 2012; Leitner et al., 2011). Collectively, these studies have shown that enzymatic hydrolysis of casein by PL liberates peptides that serve as local regulators of mammary gland functions, which is reflected by simultaneous reduction in milk yield in the infected glands and the inability of the milk of those glands to coagulate (Leitner et al., 2011). Recently, we have shown that tissue type of plasminogen activator

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(**PA**) in cow milk is closely associated with the casein micelles, and that it is responsible for casein hydrolysis (Politis, 1996; Silanikove et al., 2013).

Subclinical and clinical mastitis in cows was found to be associated with an increase in the concentration of nitric oxide (NO)-derived metabolites, nitrite and nitrate (Silanikove et al., 2005, 2007). It was concluded that the NO cycle and its components in milk are important parts of the gland innate immune system in defending against invading pathogens, albeit it may be associated with nitrosative stress and impairment of milk oxidative stability (Silanikove et al., 2009, 2012), which may increase the sensitivity of milk proteins to proteolysis (Leitner et al., 2006; Silanikove et al., 2006).

The cycling of NO• in milk was demonstrated so far only in cows. The aims of the present study were to verify the existence of NO cycling in goat milk and to examine how subclinical mastitis caused by various species of CNS affects the rate of NO cycling and milk composition. In particular, we examined how subclinical mastitis affects the total antioxidant capacity and milk quality for curdling.

MATERIALS AND METHODS

Animals

Fifteen Israeli goats, mainly Saanen or Alpine × Anglo Nubian were selected from a commercial farm that was intensively monitored for research purposes. For each animal, 1 udder half was naturally infected with an identified single species of CNS and the contralateral gland was free of bacteria. Prior to animal selection, milk samples from each udder half were subjected to 3 consecutive weekly examinations to test for bacterial infection and SCC. The CNS were of various species but due to their low number, each analysis was performed across species. The selected goats were in mid lactation and their daily milk yield exceeded 2.5 L. Goats were machine milked twice daily at 0500 and 1500 h. Postmilking teat dipping was practiced, and the animals were kept in an open shelter providing 4 m^2 of shaded slatted floor and 4 m^2 of concrete-surfaced yard/goat. Goats were fed a high-quality TMR and hay (Lucerne), which provided 60% concentrate, 40% NDF, and 17% protein. Feed was offered in mangers and water in the shades was available at all time.

Milk Sampling and Analysis

On the test day, milk sampling and yield measurements were carried out during the morning milking. Yield was determined by weighing the milk of each udder half of each goat after hand milking and multiplying by 1.7. For the bacteriological tests, the teats were cleaned and disinfected and the milk was sampled and analyzed by accepted standards (Leitner et al. 2004a; Oliver et al., 2004). Three additional sets of samples were taken from each udder half and distributed for analysis as follows. One set (40 mL) was preserved by means of Broad Spectrum Microtabs II (D & F Control Systems Inc., Norwood, MA) and sent to a central laboratory (Cattle Breeders Association Laboratory, Caesarea, Israel) for analysis of the milk gross composition: protein, fat, and lactose contents (MilkoScan 6000; Foss Electric A/S, Hillerød, Denmark) and SCC (Fossomatic 360; Foss Electric A/S), all calibrated with goat milk. The second set of samples (20 mL) was used to determine rennet clotting time and curd firmness after 60 min with the Optigraph instrument (Ysebaert, Frépillon, France). A third set (300 mL) of milk was defatted at 4°C and caseins, whey proteins, proteose peptones (**p-p**), PA, plasminogen (**PLG**), and PL activities were analyzed in the skim milk (Shamay et al., 2000, 2003; Silanikove et al., 2000). The repeated addition procedure was used to measure the concentrations of free (ionized) calcium (Ca^{2+}) and the uncorrected procedure was used to determine calcium activity in these samples within 5 h of sampling by means of a specific calcium electrode (Silanikove et al., 2003). The third set was also used to analyze metabolites, the procedure of which was described in detail earlier (Silanikove et al., 2005, 2009). Nitrite was analyzed by a fluorometric method and nitrate was determined colorimetrically by the Griess reagent (Silanikove et al., 2005); uric acid was analyzed as the product of purines metabolism in milk (Silanikove et al., 2007). The measure of the activities of xanthine oxidase, lactoperoxidase, and catalase (**CAT**) was carried out as described in Silanikove et al. (2005, 2009).

The discoloration of β-carotene added to milk samples was used to determine the oxidation reaction that results from the formation of free radicals in milk. The reaction took place in a cuvette that contained 14 μ*M* β-carotene and 0.05% Tween 20 in 1.7 mL of 0.1 *M* sodium acetate buffer. The reaction was started by adding a 0.3-mL sample. The decrease in absorbance at 460 nm as measured during the first 30 s was used to calculate the rate of β-carotene bleaching.

The conversion of 5-thio-2-nitrobenzoic acid (**TNB**) into 5,5′-dithiobis-2-nitrobenzoate (**DTNB**) was used as a measure of the formation of potent radicals, such as nitric dioxide. The TNB was prepared by reduction of 1 m*M* DTNB in 100 mL of 50 m*M* sodium phosphate buffer (pH 7.4) with 4 μL of 2-mercaptoethanol. The test sample was placed in a cuvette that contained 40 μ*M* TNB in 1.8 mL of 50 m*M* sodium phosphate buffer (pH 7.4). The reaction was started by adding a 0.2-mL sample. The decrease in absorbance at 412 nm during