Small Ruminant Research 80 (2008) 87–90

Contents lists available at ScienceDirect

Small Ruminant Research

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

Short communication

Lactoferrin concentrations in goat milk throughout lactation

S. Hiss, T. Meyer, H. Sauerwein*

Institute for Animal Science, Physiology & Hygiene Unit, University of Bonn, Katzenburgweg 7-9, 53113 Bonn, Germany

ARTICLE INFO

Article history:
Received 13 December 2007
Received in revised form 18 July 2008
Accepted 24 July 2008
Available online 30 September 2008

Keywords:
Goat milk
Lactoferrin
Lactation
SCC

ABSTRACT

Lactoferrin is an iron-binding glycoprotein and is considered a major part of the non-specific disease resistance complex in the mammary gland. For cows, the influence of physiological factors on the lactoferrin concentration in milk has been reported. In addition, lactoferrin concentrations have been demonstrated to be proportional to somatic cell counts (SCC) in cows milk. In this study, we aimed to analyse the effects of lactational stage, lactation number and SCC in 19 goats throughout an entire lactational period. Lactoferrin concentrations in weekly composite milk samples were analysed with a competitive ELISA developed for caprine lactoferrin. Maximal lactoferrin concentrations were observed in the colostral samples (387 ± 69 μg/ml). In the following week, less than 20% of these concentrations were observed (62 ± 25 μg/ml) and thereafter until week 32 p.p., the weekly mean concentrations ranged between 10 and 28 μg/ml. Toward the end of lactation, approximately during the 33rd week, the concentrations began to increase and were reaching about 3.2-fold higher values in week 44 (107 ± 19 μg/ml). SCC were only available in monthly intervals and could thus not be directly related to the weekly lactoferrin recordings. When classifying the individual goats according to the median of their SCC values obtained during midlactation, the goats with SCC medians >430,000 had higher lactoferrin milk concentrations during this time than the ones with SCC below this threshold ($P < 0.05$). In addition, sampling week and parity significantly affected the lactoferrin concentration ($P < 0.05$ and $P < 0.01$, respectively). Comparing SCC and lactoferrin, both parameters are significantly affected by various physiological factors. Further studies are needed to clarify if the relationship between Lf and bacterial counts is closer compared to the relationship between SCC and bacterial counts.

1. Introduction

Lactoferrin (Lf) is an iron-binding glycoprotein and is considered a major part of the non-specific disease resistance complex in the mammary gland and other epithelial tissues (Schanbacher et al., 1993). For cows, the concentrations of the protein during colostrogenesis, lactation and involution are well documented (Smith and Schanbacher, 1977). In addition, milk Lf concentrations have been demonstrated to be proportional to somatic cell counts (SCC) in cows milk (Rainard et al., 1982; Hagiwara et al., 2003). Increasing milk Lf concentrations observed during both natural as well as experimentally induced mastitis (Harmon et al., 1976; Rainard et al., 1982; Schmitz et al., 2004) qualify Lf as an acute phase protein in bovine milk. In goats, Lf is elevated during mastitis and the close relationship between SCC and Lf is reported, too (Chen et al., 2004). However, the interpretation of SCC is different in goats as shown by the allowable maximum level of somatic cells being 1 million cells/ml as an indicator for the hygienic quality in countries such as France and the USA (Raynal-Ljutovac et al., 2005). Goat milk of high microbial quality (<1000 cfu/ml) has been associated with SCC below 1,000,000 cells/ml (Zeng and Escobar, 1995), whereas in other studies, SCC was not related to total bacterial counts in goat milk (Park and Humphrey, 1986). Therefore, other

* Corresponding author. Tel.: +49 228 732804; fax: +49 228 737938.
E-mail address: sauerwein@uni-bonn.de (H. Sauerwein).

0921-4488/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.smallrumres.2008.07.027

mastitis indices next to SCC might be useful to assess goat milk quality. Based on a methylene blue reaction test to classify milk quality, Chen et al. (2004) showed that the Lf concentrations in goat milk of low quality are higher than in high quality milk and the authors concluded that Lf might be useful in determining the infection status of udder halves. However, the literature on Lf in goat milk is limited to bulk milk and to mastitic milk and physiological factors affecting Lf concentrations have not been characterized until now. We thus aimed to evaluate the concentrations of Lf in goat milk throughout an entire lactational period taking stage of lactation, lactation number and SCC into account.

2. Materials and methods

Nineteen healthy female goats (German Improved Fawn and German Improved White, average number of lactations 2.6) milking 617 ± 146 kg on a 200-day-basis were studied during their lactation. They were housed in free stall barns with straw embeddings at the agricultural research center from the North Rhine Westphalian chamber for agriculture matters (Riswick, Kleve, Germany). The animals were fed according to the recommendations of the German Society of Nutrition Physiology (GfE, 2003) for goats. The animals had seasonal lambing (January 10 to February 6) and were milked twice daily. Sampling of milk started after lambing with the first sample being obtained within 6 h after lambing; thereafter, weekly sampling intervals were pursued until drying off. Using a milk metering device (Metatron 8, Westfalia Surge Deutschland GmbH, Boenen, Germany) during milking, a representative composite sample of the milk was obtained and was stored in 10 ml aliquots at −20 °C until further analysis. Samples from morning and evening milking were alternately used every week. Milk yield and milk composition were recorded every month by the regular milk recording organization (Landeskontrollverband Nordrhein Westfalen e.V., Krefeld, Germany). SCC was determined using the DNA-specific Fossomatic cell counter (Fossomatic 5500, Rellingen, Germany) calibrated with cows milk.

For the quantification of Lf, we developed a competitive enzyme immunoassay using the double antibody technique. Based on the protocol published for ovine Lf (Buchta, 1991), we purified caprine (c) LF from defattened goat colostrum. The identity of the protein obtained was assured by SDS polyacrylamide gel electrophoresis (SDS-PAGE) based on the protocol from Laemmli (1970). The purified Lf was used as standard, for the generation of polyclonal antibodies in rabbits and also, after biotinylation, as tracer following largely the procedures described by Hennies and Sauerwein (2003) for another protein. For the ELISA, microtiter plates (EIA Plate 9018, Corning Costar, Cambridge, MA, USA) were coated with sheep IgG (100 μl/well in 50 mM sodium hydrogen carbonate, pH 9.6, containing 150 ng anti-rabbit-Fc fragment antibodies) at 4 °C for 20 h. After coating with 300 μl 2.5% casein in 0.05 M NaCl, pH 7.4, at room temperature for 1.5 h, the plates were washed five times with washing buffer (10% PBS, pH 7.4, 0.05% Tween® 20). The plates were filled with assay buffer and stored at 4 °C for up to several weeks without appreciable loss of sensitivity. The assay buffer contained 0.1% hydrolyzed gelatin, 0.12 M NaCl, 0.02 M Na_2HPO_4, 0.01 M EDTA, 0.005% chlorhexidine digluconate (20%), 0.002% phenol red, 200 μl/l proteinase inhibitor cocktail (Complete™, Boehringer Mannheim, Germany) and 0.02% ProClin 150® (Supelco, Bellefonte, PA, USA). After removal of the assay buffer, 50 μl of prediluted Lf standard or milk samples were pipetted into the wells of the assay plates and 50 μl of antiserum diluted 1/250,000 with assay buffer were added. After preincubation at 4 °C for 1 h, 50 μl of biotinylated Lf were added and the mixture was incubated at 4 °C for another h. After three times washing using a microtiter plate washer (EL404, BIO-TEK Instruments, Winooski, VT, USA), 100 μl of a streptavidin-peroxidase conjugate solution (200 ng/ml; Sigma–Aldrich) were added per well. The plates were incubated at 4 °C for 30 min and, after 5 further washes, the wells were filled with 150 μl of a freshly prepared substrate solution containing 0.05 M citric acid, 0.055 M Na_2HPO_4, 0.05% urea hydrogen peroxide and 2% of a tetramethylbenzidine solution (12.5 mg/ml DMSO). The reaction was stopped after 45 min by the addition of 50 μl 1 M oxalic acid and the color development was determined photometrically at 450 nm on a microtiter plate reader (ELX800, BIO-TEK Instruments). To calculate the concentration in the samples from the standard curve, the four-parameter method in the MikroWin 3.0 Program was used.

For the statistical evaluation of the Lf data, we used the SPSS program (Version 12.0). The general linear model included sampling week, number of lactation ($n = 10$ goats in their 2nd lactation ($n = 10$) versus goats from higher numbers of lactation (3rd: $n = 7$, 4th and 5th lactation: $n = 1$ each)), SCC group and the corresponding interactions as fixed effects. Sampling week was considered as repeated effect. SCC effects were analyzed in midlactation (6–27 week) only. For testing the hypothesis that goat with higher SCC may have higher Lf concentrations, we subdivided the animals according to their SCC values: for all individual animals, the median SCC value from the recordings during midlactation was used to calculate the median overall animals (=430,000 cells/ml). Animals with an individual median above or below this threshold were allocated to either a SCC group < or > 430,000.

Data given are means ± S.E.M.; P-values <0.05 were considered as significant.

3. Results and discussion

The assay developed herein allowed for a valid quantification of cLf. Parallelism of the standard curve and different dilutions of goat milk was established. The recovery of defined amounts of purified cLf spiked into goat milk samples was 106 ± 8% ($n = 6$). The minimal detectable dose in the assay was 0.2 ng/ml; the intra-assay coefficient of variation was 4.1% ($n = 27$) and 9.3% ($n = 20$) for the interassay variation.

From the 19 animals studied, one goat was leaving the herd at week 26; the remaining 18 animals maintained lactation at least for 32 weeks. After this time, the animals were dried off by and by. SCC was 1,109,000 ± 643,009 cells/ml in colostrum, 486,930 ± 111,132 cells/ml in the 5th week of lactation and started to increase in the 31th week of lactation (1,030,170 ± 187,271 cells/ml) up to 4,295,200 ± 954,393 cells/ml in the 43th week of lactation. The basal level SCC in bacteria-free udders of goats is about 300,000 cells/ml (Raynal-Ljutovac et al., 2007), and milk with <800,000 SCC/ml can be related to a whole flock-infection rate of about 25% (Leitner et al., 2008). Coagulase negative staphylococci (CNS) appear to be the most prevalent pathogens and account for 58–93% of the bacteria causing intramammary infections in goat across various countries and geographical zones (Raynal-Ljutovac et al., 2007). However, the incidence of intramammary infections during established lactation in goats, as in cows, is low (Leitner et al., 2007). In view of these reports, the infection rate in our study, although not monitored, can be assumed to be well below 25%. The increase of SCC at the end of the lactational period is in accordance with previous findings (for review see Haenlein, 2002).

The concentrations of Lf in goat milk throughout the lactational period are shown in Fig. 1. Maximal concentrations were observed in the colostral samples (387 ± 69 μg/ml). In the following week, less than 20% of these concentrations were observed (62 ± 25 μg/ml) and thereafter until week 32 p.p., the weekly mean concentrations ranged between 10 and 28 μg/ml. Towards the end of lactation, approximately during the 33rd week, the concentrations began to increase and reached about 3.2-fold higher values in week 44 (107 ± 19 μg/ml). Both, sampling week and lactation number significantly affected the Lf concentration ($P < 0.05$ and $P < 0.01$, respectively).