The lactoferrin content variation and its related factors in milk of Xinong Saanen goats

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ABSTRACT

Lactoferrin (LF) is a prominent protein in milk, and is present in many other secretory fluids and white blood cells. The variation of LF content in milk is possibly regulated by multiple environmental factors such as milk composition and endogenous factors. In this study, we analysed the variations of LF in both milk and plasma of 20 Xinong Saanen goats and its related factors. Firstly, LF content in colostrum was 222.6 ± 41.57 μg/mL, and then throughout the lactation period the mean concentrations remained stable, ranged between 34.61 and 51.94 μg/mL. The LF concentration in plasma fluctuated between 173.56 and 246.20 μg/mL during the entire lactational period. There was no correlation between the milk and plasma LF concentrations (P > .05). However, LF in milk was closely correlated with milk lactose, protein and SNF contents (P < .01), but not with milk fat and total solids (P > .05). In addition, LF concentration in milk was correlated with the lactation stage (P < .01). Further studies are required to investigate these factors that cause LF variations in the milk of Xinong Saanen goat.

Introduction

Lactoferrin (LF) is an iron-binding glycoprotein in the transferrin family, and found in milk, many other secretory fluids and white blood cells (Gonzálezchávez et al. 2009; Ammons and Copié 2013). LF has multiple functions, such as acting as a growth factor to stimulate cell proliferation and differentiation (Legrand et al. 2008). Notably, LF is considered a major part of the non-specific disease resistance complex in the mammary gland and other epithelial tissues (Benissa et al. 2005). LF can directly kill bacteria by binding to bacterial surface to reduce its tolerance to environmental factors (Soukka et al. 1991; Yen et al. 2011).

Because of the wide array of important biological functions, LF and its related affecting factors are greatly investigated (Chen and Mao 2004). Harmon (1994) reported that LF concentrations in milk relate to somatic cell counts (SCC), stage of lactation and milk yield (private communication). Milk LF concentrations have been demonstrated to be correlated with parity of cattle, for example primiparous cows have lower LF concentrations in milk than multiparous ones (Kume and Tanabe 1993). In goats, LF is elevated during the process of mastitis and has a close relationship with SCC (Chen et al. 2004).

Milk ing frequency may be one of the main factors which have influence on the LF concentration. In dairy cows, short-term changes of milking frequency during early lactation have been shown not only to produce an immediate and a long-term effect on milk yield in stall-fed cows but also affect proliferation of mammary cells as well as expression of the major milk protein genes. The abundance of αS1-casein, β-casein, α-lactalbumin and β-lactoglobulin mRNA was higher in tissue samples from cows milked four times (4×) compared with once (1×) a day, whereas LF mRNA abundance was lower in 4× udder halves (Murney et al. 2015). Milking frequency and the globo-lousness of the udder in Tinerfena breed goats were correlated with milk yield and milking ability (Capote et al. 2006). It is shown in the literature that LF in goat milk is limited to bulk milk and to mastitic milk only. Physiological factors that affect LF concentrations need to be characterized. Thus, the goal of this investigation is to establish a possible relationship between LF content in milk and in plasma. Additionally, we also take lactation stage and milk composition into account.

Material and methods

Animals and feeding

Twenty healthy female Xinong Saanen dairy goats with similar body condition score, parity, milk yield and delivery time from one herd kept in a pasture-based farming system were used for this study. Goats were housed in free barns at the Xinong Saanen Dairy Goat Breeding Farm (Yangling, Shaanxi, China). Those animals were fed according to the recommendations for nutrient requirements by the National Technical Committee for Standardization of Animal Husbandry (NY/T 2835-2015).

Sample collection and storage

Colostrum sample was harvested within 24 h after kidding, and then milk samples from individual does were obtained every 4 weeks until the late lactation (about 31 weeks). On the day of milk sampling, goats were milked at 08:00 and 20:00 and milk samples were aseptically taken. Two samples were then
pooled. An aliquot of milk sample was used for analysis of milk compositions using the multifunctional dairy analyser (MilkoScan FT2, Foss Electric analyzer, Denmark), including fat (Fat), protein (Pro), lactose (Lac), total solids (TS) and solids-not-fat (SNF). Another aliquot for LF content determination was skimmed at 10,000×g for 15 min. Then, the skimmed milk was drained into tubes marked with each goat’s ID number and stored at −20°C until analysis.

Blood samples were drawn from the jugular vein at 08:00 per 4 weeks, and sodium citrate was chosen as the anticoagulant. The sample was centrifuged at 3000 rpm for 20 min to harvest plasma, and the plasma was stored at −80°C.

Quantification of LF

The concentrations of LF in the colostrum and milk samples were determined using ELISA kit (Yuanye Bio-Technology Co., Shanghai, China) according to the method described by Chen et al. (2004). Briefly, the testing sample, the standards and the HRP-labelled antibody were successively added into the wells that were coated with the LF antibodies. After incubation and thoroughly rinsing, the stop solution was added. The intensity of colour was positively correlated with LF concentrations in the sample. The intensity of colour was recorded at 450 nm using a spectrophotometer, which was positively correlated with LF content in the sample. The concentration of LF was calculated based on the calibration curve. The accuracy of the assay was judged by the linear correlation coefficient (the R-value) of the simple linear regression, which was greater than 0.99 in this experiment. The detection limit of assay was 1.0 μg/mL, and both the intra- and inter-assay coefficients of variation were lower than 15%.

Statistical analysis

All statistical analyses were performed using SPSS 17 for Windows (SPSS Inc., Chicago, IL). The distribution of LF in the whey was analysed by Chi-square test, and Spearman correlation analysis was used to analyse the relevance between different division factor and index. Homogeneity analysis of the LF data in each group was made by using Bartlett’s test and normality test. Afterwards, the difference of LF data (log) in each group was analysed by one-way ANOVA. P-values < .05 were considered as significant, and P < 0.01 as extremely significant.

Results and discussion

The LF concentrations in milk and in plasma are displayed in Table 1. The concentrations of LF in goats varied from 34.61 to 222.6 μg/mL, and the maximal concentrations were observed in the Colostrum samples. The LF concentrations in milk samples ranged between 34.61 and 51.94 μg/mL. The plasma LF concentrations throughout the lactational period fluctuated between 173.56 and 246.20 μg/mL, with individual variations from 102.57 to 384.13 μg/mL. The LF concentration reached the bottom of 173.56 ± 36.73 μg/mL in the seventh week of lactation. Throughout 31 weeks of lactation, the LF concentrations in plasma remained relatively stable.

There was no significant correlation in the LF concentration between milk and plasma (P > 0.05) Table 2. In contrast, a strong relevance between the two indicators under the immune condition has been reported (Chen et al. 2004), indicating that blood may be a source of LF for milk under immune conditions. It would thus be helpful to detect mammals’ physiological alterations or infection degree by monitoring the LF concentration in milk or in blood. Bovine mammary epithelial cells can produce LF. By detecting the expression of LF mRNA in the mammary gland, it was discovered that LF expression in normal lactation period was much lower than during the dry period, which may be due to the down-regulation of the genes (Goodman and Schanbacher 1991). In addition, the expression of LF associated with cancer is multifactorial and may include gene structural changes as well as altered regulation, which has been found in leukaemia and breast cancer (Panella et al. 1991).

As we know, LF plays an important role in immunity (Baveye et al. 1999; Valenti and Antonini 2005; Luzi et al. 2017). The highest LF concentrations in the Colostrum may indicate that LF has multiple functions for newborn infants and their mothers. This high concentration reinforces the idea of Colostrum importance (Hernández-Castellano et al. 2014). The LF in milk may be recruited from the bloodstream, while it is also synthesized in the mammary gland itself (Molenaar et al, 1996). Yang et al. (2000) suggested that serum and milk LF concentrations, although closely correlated, might be regulated through independent systems activated simultaneously.

Considering LF concentrations in milk are influenced by various factors (Cheng et al. 2008), milk compositions and the relationship with the corresponding LF concentration were analysed, and the results are shown in Tables 3 and 4. There were strong correlations between the LF concentration and lactation stage, milk protein, lactose and SNF, whereas no correlations were found with the total solids and milk fat.

| Weeks of lactation | LF concentrations in milk (μg/mL) | LF concentrations in plasma (μg/mL) |
|--------------------|----------------------------------|-----------------------------------|
| 0                  | 222.60 ± 41.57                  | 206.41 ± 26.08                   |
| 1                  | 51.94 ± 7.13                    | 240.42 ± 40.42                   |
| 3                  | 45.30 ± 9.28                    | 225.43 ± 61.88                   |
| 7                  | 35.59 ± 1.64                    | 173.56 ± 36.73                   |
| 11                 | 34.61 ± 7.31                    | 206.22 ± 27.77                   |
| 15                 | 40.64 ± 5.84                    | 223.86 ± 39.46                   |
| 19                 | 40.93 ± 5.32                    | 206.08 ± 27.83                   |
| 23                 | 41.28 ± 4.39                    | 221.16 ± 19.06                   |
| 27                 | 37.01 ± 5.39                    | 227.24 ± 25.63                   |
| 31                 | 35.44 ± 6.93                    | 246.20 ± 38.71                   |

Notes: Data are presented as mean ± SD. The different superscripts within the same column indicate significant difference among the weeks (P < .05).

| LF concentrations in milk (log) | Correlation coefficient (R-value) | Significant coefficient (P-value) |
|---------------------------------|-----------------------------------|----------------------------------|
| Plasma (log)                    | 0.061                             | 0.392                            |

Note: 200 milk samples and 200 plasma samples from 20 individual goats were analysed.
Both LF concentration and milk compositions are influenced by various factors such as blood biochemical indexes and health conditions, whereas LF can also be synthesized in the mammary gland itself. So far, the relationship between LF in milk and these factors has not been fully established. However, the significant correlations between LF concentration in milk and the stage of lactation have been stated in all cases. The LF content gradually increases with the course of lactation, but the changes in LF content are highly dependent on breeds of cows, and there are strong interactions between breed and stage of lactation (Król et al. 2013). In buffaloes, milk LF concentrations increased with the increase in days of milking, at a rate of 0.0015 g/L daily during lactation (Giacinti et al. 2013). In buffaloes, milk LF concentrations increased with the increase in days of milking, at a rate of 0.0015 g/L daily during lactation (Giacinti et al. 2013). In buffaloes, milk LF concentrations increased with the increase in days of milking, at a rate of 0.0015 g/L daily during lactation (Giacinti et al. 2013). In buffaloes, milk LF concentrations increased with the increase in days of milking, at a rate of 0.0015 g/L daily during lactation (Giacinti et al. 2013). In buffaloes, milk LF concentrations increased with the increase in days of milking, at a rate of 0.0015 g/L daily during lactation (Giacinti et al. 2013). In buffaloes, milk LF concentrations increased with the increase in days of milking, at a rate of 0.0015 g/L daily during lactation (Giacinti et al. 2013).

Expression of LF gene in a variety of tissues is regulated differentially (Teng 2010). Besides protein–protein interaction among the transcription factors (Nelson et al. 1991), LF can also be induced by retinoic acid and estrogens (Geng et al. 1998). Those studies demonstrated that LF synthesis is responsive to multiple signalling pathways and can be regulated either directly or indirectly by many effectors.

Conclusion
The LF concentration in milk and plasma varied throughout the lactation period; however, there was no association between them. Strong correlations between the LF concentration and the concentrations of protein, lactose and SNF in milk were found. As LF is regulated by both extrinsic (nutrition, development and growth factors) and intrinsic factors (transcriptional factors and nuclear receptors), further studies are needed in these areas.

Disclosure statement
No potential conflict of interest was reported by the authors.

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