

Determination of Lactoferrin in Goat Milk by HPLC Method

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Abstract: The aim of this study was the determination of lactoferrin in goat milk using HPLC method. Milk samples were collected at a goat farm in the South Moravia Region, the Czech Republic. It were established bulk tank samples of raw milk ($n = 24$) and pasteurised milk ($n = 27$) that were collected during lactation. Lactoferrin contents were analysed by reverse phase high-performance liquid chromatography (RP-HPLC) with diode-array detector PDA 2996. Detection was carried out at the wavelength 205 nm. The average concentration of lactoferrin in goat milk was 120 ± 18 $\mu\text{g/ml}$. The lactoferrin content was increasing within the lactation period in the ranges of 98 ± 170 $\mu\text{g/ml}$ in April to 149 ± 19 $\mu\text{g/ml}$ in November. The heat treatment (pasteurisation at 72°C for 20 s) resulted in no significant effect on the lactoferrin content. No statistically significant differences ($P = 0.05$) were found between the values of raw and pasteurised goat milk.

Keywords: lactoferrin; milk; RP HPLC; goat

INTRODUCTION

Interest in dairy goats and goat milk products is a part of the recent trend in health food demand. There is also a renewed interest in goat milk as a substitute for those who suffer from allergies or intolerance against cow milk. The major nutrient composition of goat milk resembles cow milk, whereas goat milk has its unique chemical, biochemical, physical, and nutritional characteristics compared to other species' milk (PARK 2006).

Lactoferrin is a mammalian cationic iron-binding glycoprotein belonging to the transferring family which was discovered 70 years ago. It is widely distributed in all biological fluids and is also expressed by immune cells (PIERCE & LEGRAND 2009). Many biological functions have been ascribed to lactoferrin. One of the functions is the transport of metals, but lactoferrin is also an important component of the non-specific immune system, since lactoferrin has antimicrobial properties against bacteria, fungi and several viruses (VAN DER STRATE *et al.* 2001).

Its extensive antimicrobial activities were originally attributed to its ability to sequester essential iron, however, it is now established that it possesses bactericidal activities as a result of direct interaction between the protein or lactoferrin-derived peptides (FARNAUD & EVANS 2003). Lactoferrin is also implicated in protection against cancer development and metastasis (PIERCE & LEGRAND 2009). From nutritional point of view, lactoferrin is of interest as a dietary source of amino acids and for the bioavailability of iron (KORHONEN & MARNILA 2003).

The objective of our investigation was the determination of lactoferrin in goat milk using RP-HPLC method.

MATERIALS AND METHODS

Goat milk samples. Milk samples were collected at a goat farm in the South Moravia Region, the Czech Republic. It were established bulk tank

samples of raw milk ($n = 24$) and pasteurised milk ($n = 27$) ($72^{\circ}\text{C}/20\text{ s}$) that were collected during lactation from April to November.

Sample preparation. The fat was removed from milk by centrifugation at 3000 g of 15 min. In order to separate the whey, the samples were precipitated with 1 mol/l HCl to pH 4.6 and centrifuged at 3000 g at 15 min again. The samples of whey were stored at -18°C to the analysis. All samples were analysed in duplicates.

HPLC method. For HPLC determination of the lactoferrin separation module Alliance 2695 with diode-array detector PDA 2996 (Waters, Millford, USA) were used. Detection was carried out at the wavelength 205 nm. Separation was performed on a chromatographic column Poroshell 300SB-C8, $2.1 \times 75\text{ mm}$, $5\ \mu\text{m}$ particle size (Agilent, Santa Clara, USA). Linear gradient and flow rate 1 ml/min were used. Mobile phase A consisted of water/acetonitrile/trifluoroacetic acid (95:5:0.1) and mobile phase B water/acetonitrile/trifluoroacetic acid (5:95:0.1). The column temperature was set at 45°C and injection volume was $10\ \mu\text{l}$. Data were collected and evaluated by software Empower (Waters, Millford, USA). An external standard method for quantification analytes was used.

Statistical evaluation. The statistical significance of the differences was established using a statistical and graphic system STAT Plus (MATOUŠKOVÁ *et al.* 1992). Evaluation of the data was done with a pair t -test.

RESULTS AND DISCUSSION

Optimisation of the HPLC analysis was carried out using a standard solution of lactoferrin

(Sigma, Milwaukee, USA). The calibration curve was extrapolated in the concentration range of $30\text{--}300\ \mu\text{g/ml}$ ($y = 15\ 077x - 261\ 163$; $R^2 = 0.9999$). The method sensitivity was found to be a slope of the calibration curve of lactoferrin. The repeatability was found from the results of multiple measurements of one sample and it was found to be a 3% RSD. Limit of detection (LOD) was found to be 3 S/N (signal/noise ratio) $4.5\ \mu\text{g/ml}$. Limit of quantification (LOQ, found at 10S/N) was $15\ \mu\text{g/ml}$. The method recovery was 88%. The chromatogram of the goat milk sample is presented in Figure 1.

Average values of lactoferrin in raw and pasteurised goat milk were presented in Table 1. The lowest value was obtained in April, the highest in November at the end of lactation. The similar results in raw goat milk were found by HISS *et al.* (2008). These authors observed maximal lactoferrin concentration in the colostrum samples ($387 \pm 69\ \mu\text{g/ml}$). In following week concentrations decreased and 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 ($107 \pm 19\ \mu\text{g/ml}$). From the results in Table 1 is obvious that heat treatment has no effect on lactoferrin content. No statistically significant differences ($P = 0.05$) were found between the values of raw and pasteurised goat milk. The same results were found by KORHONEN and MARNILA (2003). The authors described that according to many experimental studies, the standard pasteurisation regime (72°C for 15 s) used in the dairy industry has practically no effect on lactoferrin structure. Also, preheating at 70°C for 3 min followed by UHT treatment (130°C for 2 s) led to only 3% loss in residual iron-binding capacity.

Table 1. Average values of lactoferrin in goat milk ($\mu\text{g/ml}$)

Month	Raw goat milk	Pasteurised goat milk
April	98 ± 17	87 ± 12
May	114 ± 16	106 ± 10
June	104 ± 18	92 ± 17
July	124 ± 15	121 ± 20
August	130 ± 19	121 ± 18
September	144 ± 17	100 ± 15
October	145 ± 22	104 ± 20
November	149 ± 19	103 ± 17

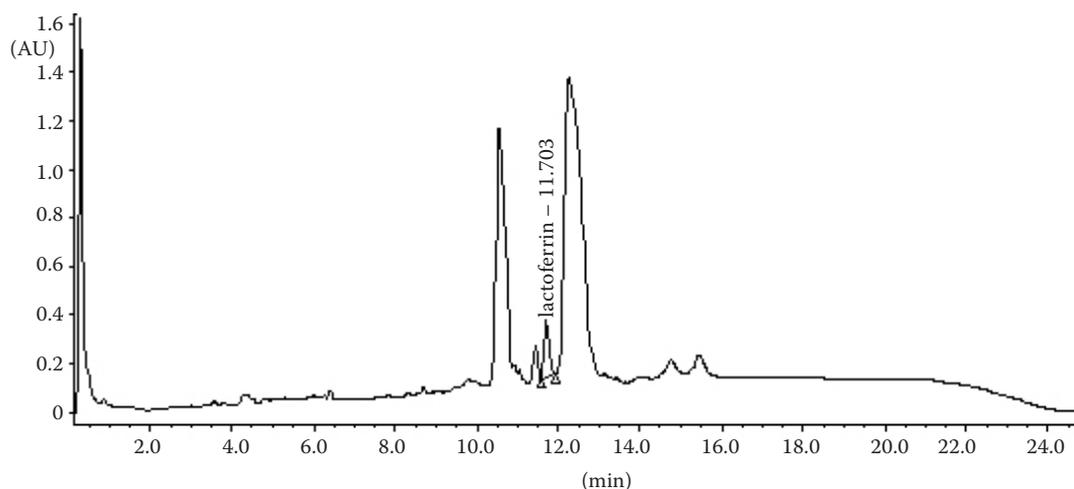


Figure 1. Chromatogram of raw goat milk sample

CONCLUSIONS

RP HPLC method for the determination of lactoferrin in goat milk was applied. Concentrations levels of lactoferrin ranged in raw goat milk from 98 ± 17 to 149 ± 19 $\mu\text{g/ml}$ during the lactation. Heat treatment had no significant effect on the lactoferrin content, values in pasteurised milk (72°C for 20 s) ranged between 87 ± 12 – 121 ± 19 $\mu\text{g/ml}$.

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