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Lactoferrin and IgG levels in ovine milk throughout lactation: Correlation with milk quality parameters

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ABSTRACT

The aim of this work has been to determine the levels of lactoferrin and IgG in ovine milk during the two first months of lactation and to compare with some milk quality parameters. Milk samples were collected from ewes of an Assaf flock. The levels of lactoferrin were determined by radial immunodiffusion. For this purpose, lactoferrin was isolated from ovine milk by chromatographic methods and polyclonal antibodies against it were obtained in rabbits. IgG levels were determined by a commercial sandwich ELISA. The mean concentration value of lactoferrin at the first day of lactation was 0.74 mg/ml and that for IgG of 9.25 mg/mL. The levels dropped sharply during the first three days of lactation and at the third week of lactation, the concentration in mature milk was of 0.07 mg/ml and 0.25 mg/ml for lactoferrin and IgG, respectively. There was no correlation between the levels of lactoferrin and IgG concentrations in ovine milk samples with those of total protein and bacterial count. A low positive correlation between lactoferrin and somatic cell counts (SCC) was found, which was more evident when samples were classified according to SCC levels. However, no significant differences were found with respect to IgG levels in milk samples, for high or low levels of SCC. Consequently, it seems that only high lactoferrin levels could be used as an indicator of subclinical mastitis.

1. Introduction

Milk proteins have important nutritive and biological functions for the newborn. There is a group of milk proteins that exert defensive properties, some with specific activity, such as immunoglobulins, and others with non-specific activity, such as lactoferrin, lactoperoxidase and lysozyme (Hettinga et al., 2011). Lactoferrin is an 80 kDa iron-binding glycoprotein with two iron binding sites of high affinity. This protein is not only present in exocrine fluids such as colostrum, milk, tears and saliva, but also in polymorphonuclear neutrophil leukocytes (Farnaud and Evans, 2003). Lactoferrin exerts multiple biological functions: antimicrobial activity, regulation of intestinal iron uptake, growth promotion and modulation of defense systems related to inflammation in the epithelial mucosa (Adlerova et al., 2008; Sánchez et al., 1992a). Lactoferrin receptors have been found in intestinal tissues, monocytes, macrophages, neutrophils, lymphocytes, platelets and also in some bacteria (Gray-Owen and Schyvers, 1996; Legrand et al., 2006). The mechanisms by which lactoferrin develops antibacterial activity, depend on the type of microorganism. These mechanisms are based on iron deprivation by lactoferrin from the environment where bacteria live, and also on its interaction with the bacterial membrane, causing disturbance on their metabolism (Farnaud and Evans, 2003).

The levels of lactoferrin in normal milk vary considerably between species. Human milk contains a high concentration of lactoferrin (1.0–6.0 mg/ml) (Montagne et al., 2001), whereas in other species the levels are relatively low, as in cow, goat, and sow (0.01–0.1 mg/ml) or in rabbit, rat, and dog (below 0.05 mg/ml) (Masson and Heremans, 1971). Intermediate levels of lactoferrin are found in mouse, guinea pig, and mare milk, being of about 0.1–1 mg/ml. In dairy cows, the

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regulation of lactoferrin synthesis in the mammary gland contrasts with that of other milk proteins. Thus, bovine lactoferrin mRNA levels are relatively low in the lactating gland and increase remarkably during mammary involution that takes place after the cessation of milking (Goodman and Schanbacher, 1991). These high concentrations are probably related with a role of lactoferrin in the defense of the mammary gland against infections (Shimazaki and Kawai, 2017).

On the other hand, immunoglobulins (IgG) are transferred to the newborn by colostrum in the first hours after delivery in ruminants, as they cannot cross the placental barrier, constituting the main passive defense mechanism (Hurley and Theil, 2011). Transfer of IgG to newborn lamb during the first 24 h after birth is essential, before the maturation of intestinal cells (Domínguez et al., 2001). IgG are the main class of immunoglobulins in ruminant milk secretion, in which they are present at high concentration, around 40–50 mg/ml in cow colostrum and from 0.2 up to 10 mg/ml in mature milk (Conesa et al., 2005; Murphy et al., 2005). The levels of IgG in colostrum have been reported to be between 48 mg/ml and 28 mg/ml in goats (Romero et al., 2013; Rudovsky et al., 2008). The presence of high levels of IgG in milk can be of biological value for the suckling animal because they are considered to participate in the protection of intestine against infections. However, the presence of IgG is not desirable when milk is going to be transformed as a liquid product for consumption, or as a dairy product. The addition of colostrum to milk for human consumption is not permitted in some countries due to hygienic reasons. However, the main reason to avoid the addition of colostrum into milk comes from technological issues related with fouling of the equipment used for heat treatment (Bansal and Chen, 2006; Jeurnink, 1995). The presence of colostrum in milk may also impair the sensorial characteristics of dairy products and the renneting time in cheese manufacture, depending on its amount (Feagan, 1979; Raynal-Ljutovac et al., 2005). The levels of IgG are controlled in some countries, because of the problems that their presence may cause in milk, and are included in the quality based milk payment system (Raynal-Ljutovac et al., 2005). It has been reported that IgG levels can vary in ewe's milk along seasons due to the concentration of deliveries in a few months, when their levels will be higher than average (Galan-Malo et al., 2014).

Although it is well known that the concentration of some milk proteins decreases during lactation, no systematic study has been made comparing lactoferrin and IgG concentrations in ewe's milk throughout lactation. The aim of this work has been to determine the levels of those proteins in ovine milk during the two first months of lactation and to compare them with other milk quality parameters. This study can contribute with basic knowledge to establish objective milk quality parameters and new criteria for the payment in relationship to quality of sheep milk.

2. Materials and methods

2.1. Milk sample collection

The concentration of lactoferrin was determined in milk obtained from the experimental flock of Assaf sheep of the University of Zaragoza, Spain (41°41′N). The Ethics Committee for Animal Experiments at the University of Zaragoza approved all the procedures performed in the study. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Ewes used in this experiment were provided by a certified commercial farm, where all ewes with signal of clinical or subclinical mastitis are retired from milking. Therefore, the animals used in this study had not suffered from any previous mastitis. The ewes were in the second or third lactation.

Individual milk samples were collected from 30 ewes at least once a week, until the eighth week of lactation. For reasons related to animal management, it was not possible to collect milk samples right after delivery from all the ewes, but only from five animals. The skimmed milk samples were analyzed immediately after collection or stored at −20 °C until analysis.

Compositional parameters of milk samples were analyzed by the Asociación Interprofesional Lechera de Aragón (Movera, Spain). Total protein, lactose and fat were determined by using a Milkoscan, and somatic cell counts and total bacteria counts by Bactoscan FC (FossElectric, Denmark).

2.2. Immunochemical techniques

2.2.1. Obtaining antiserum against ovine lactoferrin

Whey was prepared from ewe's milk by chymosin coagulation, dialyzed and subjected to cationic exchange chromatography to isolate lactoferrin, as described previously (Navarro et al., 2015). Lactoferrin was obtained with a purity degree higher than 90%.

Polyclonal antiserum against ovine lactoferrin was obtained by immunizing rabbits with isolated lactoferrin as described previously (Conesa et al., 2008). The presence of antibodies against ovine lactoferrin was confirmed by double immunodiffusion and by immunoelectrophoresis as described by Sánchez et al. (1992b). The samples were allowed to diffuse for 24–48 hours and immunoprecipitates were stained with Coomassie blue G-type at a concentration of 250 mg/l in methanol, distilled water and acetic acid (45:49:6, v/v) by immersion for one hour, and distained with a mixture of methanol, acetic acid, glycerol and distilled water (250:8:2:65, v/v).

2.2.2. Western-blotting

Polyacrylamide gel electrophoresis with SDS (SDS-PAGE) of fractions obtained during lactoferrin isolation from ovine whey by chromatography was performed in 7.5% polyacrylamide gels in a PhastSystem (GE Healthcare, Uppsala, Sweden). Transference of proteins from electrophoretic gels to nitrocellulose was performed by using a MilliBlot-SDE Transfer System (Millipore); Western-blotting was carried out as described by Franco et al. (2010).

2.2.3. Quantification of lactoferrin by radial immunodiffusion

The concentration of lactoferrin present in ewe's milk samples throughout lactation was performed by radial immunodiffusion according to Mancini et al. (1965) adapted by Sánchez et al. (1992b). Gels of 1.5% agarose were prepared in 0.025 M diethyl-sodium barbiturate buffer, pH 8.24, containing 0.3 M NaCl. For every 25 ml of agarose warmed at 50 °C, a volume of 125 µl of rabbit polyclonal antiserum against ovine lactoferrin was added. The gel with the specific antiserum was placed on glass plates of 9 × 12 cm and allowed to settle. The gel with 2 mm of thickness was perforated with 20 wells of 3 mm diameter and 8 µl of the samples were applied per well. The standards consisted of isolated ovine lactoferrin at 0.3; 0.2; 0.15 and 0.075 mg/ml. The samples were allowed to diffuse for 72 h at room temperature in a humid chamber with thymol as preservative. The gels were then washed, stained and distained as indicated in the previous section. The diameter of the immunoprecipitates corresponding to standard samples was measured and the values represented on a standard curve. The concentration of protein in samples was determined by interpolating the values of their diameters in the standard curve.

2.2.4. Quantification of immunoglobulins by sandwich ELISA

IgG in ewe's milk were analysed using the test Calokit-Sheep (ZEULAB, Zaragoza, Spain) as described in Galan-Malo et al. (2014). Milk samples were diluted according to the stage of lactation in order to adapt the IgG levels to the working range of the ELISA test. Absorbance was read at 450 nm in a Multiskan microplate reader (Labsystems, Helsinki, Finland). A quadratic calibration curve was obtained by plotting absorbance readings against concentrations of IgG standards. Then, the concentration of IgG in milk samples was directly calculated