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Blood-derived proteins in milk at start of lactation: Indicators of active or passive transfer

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ABSTRACT

Colostrum has a different composition compared with milk in established lactation. This difference is in part due to the partially open blood-milk barrier, which, when closed, is designed to prevent the interdiffusion of blood and milk components. In the first days of lactation, α-lactalbumin (α-LA), a milk protein, is typically present in blood and several blood-derived proteins are also present in milk, such as IgG_1, IgG_2, serum albumin (SA), and lactate dehydrogenase (LDH). With the exception of IgG_1, which is known to be transferred by active transcellular transport, the other proteins are thought to pass paracellularly through the temporarily open barrier. Along with an exchange of blood and milk components, somatic cell count (SCC) is typically high in colostrum. The decline of these proteins and SCC can be used as indicators to determine transcellular or paracellular transport. Two hypotheses were tested. The first hypothesis was that the decline curve for a protein or SCC would be the same as IgG_1, indicating transcellular transport, or the decline curve would be different than IgG_1, indicating paracellular transport. The second hypothesis was that the decline curves of SCC and all proteins that are thought to have paracellular transport would be the same. Ten Holstein cows were milked at 4 h after parturition, the next 5 consecutive milkings, and the afternoon milking on d 5, 8, 10, and 14 of lactation for a total of 10 milking time points, and sequential jugular blood samples were also taken. Blood and milk samples were analyzed for the concentrations of LDH, SA, IgG_1, IgG_2, and α-LA and milk samples were measured for SCC. Protein concentration and SCC curves were generated from all 10 time points and were evaluated using the tau time constant model to determine the rate of decline of the slope of each protein. When examining the first hypothesis, the concentration of IgG_1 declined significantly faster in the milk than the proteins IgG_2 and LDH, but declined at the same rate as SA. Immunoglobulin G_1 also declined significantly faster than SCC and α-LA in plasma. The second hypothesis showed that IgG_2, LDH, and SA in milk were declining at the same rate, but were declining significantly faster than SCC and α-LA in plasma. These results indicate that only active transcellular transport of IgG_1 occurred, with a sharp decline at parturition, compared with IgG_2, SA, LDH, α-LA, and SCC, which are likely following paracellular transport.
Key words: colostrum, blood-milk barrier, blood-derived protein, immunoglobulin, dairy cow

INTRODUCTION

The blood-milk barrier is crucial for the function of the mammary gland to prevent the interdiffusion of blood and milk components, and therefore is highly impermeable during lactation in healthy animals. Most importantly, the blood-milk barrier prevents milk constituents from diffusing into blood, thus allowing the uptake of water from blood plasma into milk driven by an osmotic gradient. Conversely, the barrier prevents blood constituents from being lost by the lactating animal. During colostrogenesis and establishment of lactation, the barrier is not fully functional and can be leaky, allowing for blood components to enter the milk and vice versa (Nguyen and Neville, 1998). Under hormonal regulation of lactogenesis, the blood-milk barrier eventually becomes fully functional and stops the free transfer of blood and milk components (Morgan and Wooding, 1982); however, the precise time when this change occurs is unknown.

Colostrum, or the first milk after parturition, has a different composition than milk in established lactation. Colostrum contains higher concentrations of sodium and chloride, lower concentrations of lactose and potassium, and higher concentrations of immunoglobulins, especially IgG_1 (Nguyen and Neville, 1998; Baumrucker et al., 2010), compared with normal milk. One reason for the differences in colostrum and milk could be the open blood-milk barrier during early lactation, allowing the proteins and ions to pass freely by paracellular

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means (Linzell and Peaker, 1974). Also in contrast to established lactation, the SCC is high during the colostral period, regardless of the infection status of the mammary gland (Dohoo and Meek, 1982). The SCC can remain elevated for up to 2 wk after parturition (Natske et al., 1972).

Another reason colostrum has a different composition than mature milk is the active transcellular transport of proteins. This is the case for IgG_1, which is the most important immunoglobulin for the passive immunization of the newborn calf through colostrum uptake (Weaver et al., 2000). In cows, IgG_1 is transferred from blood into colostrum via specific transcytosis with the aid of FcRn receptors that are located on the basolateral surface of alveolar mammary epithelial cells (Kemler et al., 1975; Butler, 1983).

Besides IgG_1, several other blood-derived proteins are present in colostrum. These proteins, serum albumin (**SA**), lactate dehydrogenase (**LDH**), and IgG_2, are thought to reach the milk through passive paracellular transport as long as the blood-milk barrier is leaky. The function of both SA and LDH in the mammary gland is unclear, but they are indicators of blood-milk barrier permeability (Stelwagen et al., 1994). Immunoglobulin G_2 is an important immune protein but, unlike IgG_1, no specific transfer receptor is known thus far.

During establishment of lactation, the mammary-specific protein α-LA can be found in blood and this is thought to occur by passive paracellular transport (McFadden et al., 1987). α-Lactalbumin is a component of the enzyme lactose synthase that quantitatively determines the biosynthesis of lactose in the mammary gland.

Currently, no studies have investigated the specific decline patterns of proteins and SCC during the first days of lactation compared with the active transfer of IgG_1. The aim of the present study was to examine the decline of proteins in colostrum or plasma during the first days of lactation and use these proteins as indicators of active transcellular transport and passive paracellular transport. The first hypothesis was that the decline curve for a protein or SCC will be the same as IgG_1, indicating transcellular transport, or the decline curve will be different from IgG_1, indicating paracellular transport. The second hypothesis was that the decline curves of SCC and all proteins that are thought to have paracellular transport will be the same.

MATERIALS AND METHODS

Animals

All animal trials were approved and permitted by the Committee of Animal Experiments, Canton of Fribourg, Switzerland (permit no. 2013–01-FR). Ten Holstein dairy cows [Holstein-Friesian (n = 5), Red Holstein (n = 5)] were selected. Parities of experimental cows ranged from 2 to 5 and no signs of clinical mastitis were noted in any cow during the experimental period. Cows were housed at the Agroscope research station (Posieux, Switzerland) and transferred to straw-bedded calving pens approximately 7 d before parturition. Dry cows were fed roughage ad libitum and 0.5 kg of mineral feed daily until calving. After calving, cows were daily fed roughage, 0.3 kg of mineral feed, 2 kg of protein concentrate, and energy concentrate was fed at 0.5 kg for the first week of lactation and 2 kg the second week of lactation. From d 2 after calving on, cows were allowed to commingle with the herd either in freestall housing or on pasture, and were machine milked regularly (twice daily, 0530 and 1630 h) in the milking parlor between samplings. Cows received 2 oral calcium boluses, the first immediately after calving and the second 4 h after calving. Calves were immediately separated after parturition and were not allowed to nurse their dams.

Experimental Procedures

Beginning 4 h after calving, milk and blood samples were taken. Samples were taken for the first 6 consecutive milkings (morning and afternoon) and during the afternoon milking on d 5, 8, 10, and 14 of lactation. Milking time points were determined according to the milk yield curve published by Kessler et al. (2014). Cows were machine milked completely at every sampling time point and the milk yield was recorded. The milking time was also recorded to determine the time between the sampling and calving. Twenty minutes after the start of milking, cows were restrained and blood samples were taken from the jugular vein with evacuated tubes containing EDTA (Vacuette, Greiner Bio One, Kremsmünster, Austria). Blood was stored on ice until centrifugation at $2{,}500 \times g$ for 20 min at 4°C to obtain plasma. Milk and plasma samples were stored at −20°C until analysis.

SCC

Milk samples were processed for SCC using a DeLaval cell counter (DCC, DeLaval, Tumba, Sweden) according to the manufacturer's protocol. Samples were diluted 1:10 in commercially available milk if the cell count was $>3 \times 10^6$ because the detection limit for the cell counter is between 3×10^6 and 4×10^6 cells/mL. First colostrum samples were also diluted 1:10 in commercially available milk when viscosity of samples was too high to be read by the cell counter.