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Longer Milking Intervals Alter Mammary Epithelial Permeability and the Udder's Ability to Extract Nutrients

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ABSTRACT

Increasing the milking interval decreases milk yield and modifies milk composition. To gain a clearer understanding of the regulation of milk yield and composition, a study was conducted to establish the response curves of nutrient extraction by the mammary gland and mammary epithelial permeability in response to increasing milking intervals. Four multiparous lactating dairy cows were milked at 8-, 12-, 16-, or 24-h intervals over a period of 7 d using a Latin square design. Between the 8- and 24-h milking intervals, milk yield and milk protein levels fell curvilinearly from 38.2 to 29.2 kg/d and from 1,086 to 827 g/d, respectively. Milk fat yield decreased linearly from 1,475 to 1,235 g/d. Indicators of the opening of tight junctions increased linearly with increasing milking intervals: milk BSA increased from 148 to 207 mg/L and plasma lactose increased from 22.9 to 32.0 mg/L. The mammary gland's ability to extract nutrients decreased with increasing milking intervals. Extraction rates of glucose, β-hydroxybutyrate, and total glycerol decreased significantly (from 27.2 to 23.3%, from 42.3 to 34.4%, from 36.6 to 30.8% between 8- and 24-h milking intervals, respectively), and not significantly for α-amino nitrogen (from 23.2 to 20.0%). The extraction rate of acetate remained constant. Moreover, the extraction of milk fat precursors appeared to be less regulated than those of the precursors of milk protein and lactose, which could partly explain why milk yield and milk protein yield decreased more than milk fat yield. The arteriovenous differences of β-hydroxybutyrate and total glycerol remained constant, whereas those of glucose decreased significantly from 0.98 to 0.87 ± 0.05 mmol/L and not significantly from 0.74 to 0.64 ± 0.12 mmol/L for α-amino nitrogen. As a result, the mammary gland's ability to extract nutrients appears to be downregulated explaining partly the decrease in daily milk yield observed in response to increased milking intervals.

Key words: dairy cow, milking frequency, mammary nutrient extraction, mammary epithelium permeability

INTRODUCTION

Changes in the milking frequency modify milk yield and composition to a more or less marked extent depending on the component (lactose, fat, or protein). Switching cows from thrice-daily to twice-daily milking results in a decrease in milk yield of 10 to 15% on average (Pearson et al., 1979; DePeters et al., 1985; Szûcs et al., 1988; Barnes et al., 1990). Although DePeters et al. (1985) did not see any variation in the milk fat and protein contents, other authors have reported an average increase in the milk fat content of 2 g/kg (Pearson et al., 1979; Szûcs et al., 1988; Barnes et al., 1990) when milking was reduced to twice daily. Throughout the lactation period, milk protein content was slightly affected; Sorensen et al. (2001) observed a –0.3 g/L fall in protein levels, whereas Klei et al. (1997) reported an increase of 0.9 g/kg. When the milking frequency is reduced from twice to once a day, loss of milk yield and changes in milk composition are more accentuated than when reducing from 3 to 2 milkings per day. Milk yield falls by 35 to 50% throughout lactation and by an average of 20% over periods of 1 to 12 wk (Davis et al., 1999). During most of those studies, the milk fat content rose because of a concentration effect by an average of 2.8 g/kg, although this increase could be as much as 6.4 g/kg (Rémond and Pomiès, 2004). Protein content tends to rise by an average of 1.5 g/kg (Davis et al., 1999; Rémond and Pomiès, 2004).

Milk yield and composition are partly linked to the quantity of nutrients used by the mammary gland. Although this quantity is a function of the nutrient flux into the gland, it also depends on the gland's ability to extract and use the nutrients to synthesize and secrete milk constituents. Modifications of the nutrient extraction ability of the mammary gland could be one of the mechanisms implicated in the reduction in milk component production in response to increasing milking intervals. In fact, variations in the mammary nutrient extraction could depend on the milking interval to which animals are subjected and on the type of nutrient considered. These differences could explain why the fall in

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milk production is more marked during a change from twice-daily to once-daily milking than from thrice-daily to twice-daily milking and why milk fat content varies differently from the milk protein content. Indeed, after a 2-d interruption of milking in the goat, Fleet and Peaker (1978) observed a reduction in the arteriovenous difference (**AVD**) of glucose and acetate. In the dairy cow milked twice daily, Thivierge et al. (2002) observed that the AVD of amino acids and lipogenesis, estimated from the respiratory quotient, peaks during the first 8 h after milking and then declines.

Tight junctions are complexes located at the apex of the lateral membranes of epithelial cells. Tight junctions ensure the cohesion of epithelial cells and thus guarantee a relative impermeability of the mammary epithelium. Barrier and fence functions have been attributed to tight junctions. In barrier function, tight junctions regulate the paracellular movement of ions and small solutes across the epithelial or endothelial cell bed. In fence function, tight junctions separate the plasma membrane into basolateral and apical domains and participate in maintaining the asymmetric distribution of ion channels, pumps, and carriers in the plasma membrane (Schneeberger and Lynch, 1992). As the milking frequency decreases, tight junctions open causing an increase in the mammary epithelium permeability, which allows the passage of ions (Na^+, K^+) and solutes (lactose, BSA) according to the law of mass action (Schneeberger and Lynch, 1992). According to Davis et al. (1999) and Stelwagen (2001), these tight junctions may be involved in regulating milk production in dairy ruminants. In the goat milked once daily or after a 36-h interval, the increase in the mammary epithelial permeability coincides with a reduction in milk secretion (Stelwagen et al., 1994). In addition, the artificial opening of the tight junctions by a calcium chelating agent (EGTA, ethylene glycol bis(2-aminoethyl ether)-N,N,N′,N′-tetraacetic acid) in the goat causes a 15% decrease in milk yield, which is comparable to the one observed when animals are subjected to once-daily milking (Stelwagen et al., 1995). In dairy cows milked once a day, the opening of the tight junctions has clearly been demonstrated (Davis et al., 1999; Stelwagen, 2001). Tight junctions could also be affected in cows milked 3 times in 2 d (Rémond and Boît, 1997). However, no clear evidence was provided about the role of tight junctions in the regulation of milk yield in this species.

An experiment was conducted to get a better understanding of the role of the mammary gland's ability to extract nutrients and of the mammary epithelial permeability when daily milk production is reduced by increased milking intervals from 8 to 24 h in dairy cattle. The response curves of mammary gland extractions of nutrient precursors for milk components in relation to the opening of tight junctions were established in dairy cows receiving 4 types of milking frequency.

MATERIALS AND METHODS

Treatments, Cows, and Experimental Design

Treatments consisted of 4 milking frequencies under a constant level of feeding: thrice-daily milking, twice-daily milking, milking 3 times in 2 d, and once-daily milking, corresponding to 8-, 12-, 16-, and 24-h milking intervals, respectively.

Four multiparous Holstein cows (635 ± 30 kg of BW) in their second or third lactation at 72 ± 3 d postpartum at the beginning of the experiment were used. The cows were surgically prepared to estimate the mammary extraction of nutrients on the left half udder of each cow, according to the methods described by Guinard et al. (1994). Arterial blood was drawn from the carotid, which is more accessible than the external pudic artery. The venous blood of each half-udder returns toward the heart through 3 veins: the external pudic vein, the subcutaneous abdominal vein, and the perineal vein. This last vein carries less than 10% of the blood leaving the udder and may be neglected according to Linzell (1974). The return of the venous blood toward the heart also depends on the animal's physical position. In cows during second lactation or beyond, as in this study, the venous blood returns toward the heart principally via the subcutaneous abdominal vein when the animal is in an upright position. In a supine position, the venous blood returns toward the heart via the external pudic vein (Linzell, 1974). For these reasons, the venous blood was drawn from the subcutaneous abdominal vein, which was more accessible when cows were standing. Surgical preparation procedures were reviewed and approved by the Animal Care Committee of the French Ministry of Agriculture. One month before the beginning of the experiment, 2 permanent catheters (polyurethane catheter tubing, i.d. 1.0 mm, o.d. 1.7 mm; UNO, Roestvaststaal B.V., Netherlands) were inserted into the left carotid and subcutaneous abdominal vein, against the bloodstream, to measure the AVD in nutrient concentrations. The arterial and venous catheters were protected by silastic tubing (Silclear grade medical silicone tubing, i.d. 1.57 mm, o.d. 3.18 mm; VWR International SAS, Briare, France). A Dacron ring (Mersutures, TS53, Ethicon, Issy Les Moulineaux, France) was fixed around the catheters as they exited the body to prevent infection.

The experiment was conducted using a Latin square design with 4 cows and 5 periods. A fifth period was subsequently added because of a damaged venous catheter in 1 cow during the first period. Another cow was