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Brief Communication

Microcirculation in the Bovine Teat Skin, Measured by Laser Doppler Flowmetry

Congestion and odema are microcirculatory phenomena which occur during the early stages of inflammation (*Higgins & Lees* 1984). After machine milking, congestion and/or oedema in the teat tissue are common, especially in the teat end. This could impair the defence mechanisms of the teat and thereby increase the risk of infection (*Hamann* 1989). When designing milking equipment and milking routines, the degree of machine-induced congestion and oedema must be minimized. Methods to measure the teat tissue conditions are therefore essential. A number of such methods has been described (*IDF* 1987), for example the use of a cutimeter to measure teat end thickness as an indicator of congestion and oedema (*Hamann & Mein* 1988).

Laser Doppler flowmetry is a non-invasive method for continuous blood flow evaluation in microvascular studies (*Öberg et al.* 1983). The laser Doppler instrument measures a variable which can be defined as the product of the number of red blood cells and their mean velocity in the measuring volume (~/mm^2).

The laser Doppler flowmetry could be useful as a new method to measure teat tissue conditions. In a preliminary experiment, laser Doppler flowmetry was used to study the microcirculation in the bovine teat skin, before and after conventional machine milking, and during endotoxin-induced inflammation in the teat cistern.

Three multiparous cows, 7–10 years old, of the Swedish Red and White Breed were used to study the skin microcirculation in the distal teat end before and after one occasion of machine milking. The cows were in midlactation and produced 24 l milk/day, on average. The skin blood flow (ml/min/100 g) in the distal teat end was measured by attaching the laser probe (ALF 21, Advance Company, Ltd, Tokyo, Japan) to the teat skin at the cranial part of the distal teat end. Care was taken to place the probe at the same spot on each measurement occasion and not to touch the teat during the measurements. When a stable basal flow was obtained, the flow was recorded on a polygraph for at least 2 min and the average flow was calculated. Measurements were made on the left front and the left rear approximately 30 min before and immediately after afternoon machine milking (Duovac, Alfa Laval, Tumba, Sweden).

One primiparous, non-lactating, non-pregnant cow of the Swedish Red and White Breed was used to study microcirculatory changes in the bovine teat during endotoxin-induced inflammation. The teat and udder cisterns were surgically separated from each other (*Persson & Åström* 1989) to enable studies of reactions in the teat cistern without influence from the gland tissue.

To remove accumulated cells, the teat cistern was flushed with 10 ml sterile physiological saline 1 h before the start of the experiment. Teat cistern samples, for estimation of total somatic cell counts (SCC), were taken by infusing the teat cistern with 3.5 ml saline via an infusion cannula inserted into the teat canal. The saline was immediately strip-milked into a sterile test tube. Samples were taken just before (0 h) the infusion of 20 µg *Salmonella typhimurium* SH 4809 endotoxin in 1 ml saline, and at 1 and 7 h post infusion (p.i.). The SCC was used as an inflammatory marker and was determined microscopically in duplicate after staining with Türk's reagent.

Skin blood flow measurements (ALF 21) were made just before infusion, every 30 min up to 3 h p.i., and thereafter at 4 and 6 h p.i. The laser probe was attached to the teat skin at a cranio-lateral position at the middle of the teat cistern. Care was taken to put the probe in the same place on each measurement occasion, and to avoid touching the teat during the measurement. When a stable basal flow level was obtained, four flow values with 15 s in between, were noted and an average flow value was calculated. At 3, 4 and 6 h p.i. the flow was also recorded on a polygraph for at least 2 min.

Normally occurring teat contractions remove the interstitial fluid from the teat via the lymphatic vessels. During the milking phase in conventional milking, the vacuum applied to the teat will disturb the teat contractions, and fluid will accumulate in the teat tissue (*IDF* 1987). During the milking massage phase a compressive load will work on the teat end. This will facilitate the venous flow and removal of the interstitial fluid. During low or no flow rate periods the removal of blood and interstitial fluid will be reduced and congestion, possibly also oedema, may occur (*IDF* 1987). In the machine milking experiment one teat was excluded due to incorrect measurement premilking. The blood flow in the teat skin before milking varied between 1.6 to 3.9 ml/min/100 g (Table 1). After machine milking, the flow was markedly increased (1.8 to 2.6 times) in all the teats (n = 5). This could indicate an increase in the blood flow rate and/or congestion in the teat end after machine milking.

Endotoxin infusion into the teat cistern caused inflammation, as measured by clinical symptoms and SCC. The teat was warm and swollen on palpation from 2 h after the infusion of endotoxin in the teat cistern. No cells were found in the teat cistern samples at 0 and 1 h, while the total number of somatic cells recovered at 7 h was 79.3×10^6.

The teat skin blood flow before infusion was 1.2 ml/min/100 g (Table 2). It increased rapidly after the infusion of endotoxin and reached a maximum flow level (6.5 ml/min/100 g) at 2 h post infusion. At 2.5 h p.i. the flow was markedly decreased, remained at the same level at 3 and 4 h, but increased again at 6 h post infusion. *Dhondt et al.* (1977) found similar results when studying the mammary blood flow (MBF) after intramammary infusion of endotoxin in goats and cows. They found a biphasic pat-

Table 1. Distal teat skin blood flow (ml/min/100 g, (SD)) before (BM) and after (AM) machine milking.

Cow no.	100		118		168
teat[a]	LF	LR	LF	LR	LF
BM	2.4 (0.4)	1.6 (0.3)	2.0 (0.4)	3.9 (0.5)	2.5 (0.3)
AM	6.2 (0.4)	3.6 (0.1)	3.7 (0.4)	9.1 (0.5)	4.6 (0.7)

[a] LF = left front, LR = left rear.

Table 2. Teat skin blood flow (ml/min/100 g) during endotoxin-induced inflammation.

Time[a]	Flow (SD)	Time[a]	Flow (SD)
0	1.2 (0.3)	2.5	2.9 (0.2)
0.5	2.6 (0.1)	3	3.0 (0.4)
1	4.4 (0.6)	4	2.7 (0.1)
1.5	3.6 (0.3)	6	4.2 (0.2)
2	6.5 (0.4)		

[a] hours post infusion.

tern in the MBF with the first and greatest peak at 2 to 3 h p.i. and a smaller, second peak at 9 to 11 h post infusion. The first peak coincided with swelling of the gland. In the present experiment, swelling of the teat was noted in connection with the peak flow. The mechanisms underlying the changes in the blood flow during endotoxin-induced inflammation are not fully understood, but the initial increase in blood flow was probably initiated by local chemical mediators, giving rise to vasodilatation which resulted in hyperaemia of the capillary network. The hyperaemia resulted in congestion followed by exudation due to increased vessel permeability (*Higgins & Lees* 1984).

The results presented in this paper indicate that the laser Doppler flowmetry method could be of value in the study of microcirculatory phenomena occurring in the teat tissue, and could possibly also be used as a complement to the cutimeter method (*Hamann & Mein* 1988) for assessing teat tissue conditions. More experiments are, however, needed to evaluate the variability and repeatability of laser Doppler blood flow measurements in the teat skin, and to develop suitable standard procedures.

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