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How teat canal keratin depends on the length and diameter of the teat canal in dairy cows

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The teat canal is an invagination of the outer teat surface. Its epithelial constitution is, however, highly specialized as indicated by its high turnover rate, its unique function in trapping bacteria, and in sealing the teat canal between milkings. The keratin of the teat canal is in a dynamic state of generation and degradation. Repeated removal during milking of keratin contaminated with or colonized by bacteria plays a significant role in preventing mastitis (Murphy, 1959; Capuco et al. 1992). To study the biology of the keratin lining, e.g., its turnover, and its relation to mastitis defence, reliable methods of collecting keratin *in vivo* for quantitative and qualitative analysis are necessary. Bright et al. (1990) compared methods for keratin collection *in vivo* and suggested that a tapestry needle was a suitable tool for collecting repeatable, representative samples of keratin for lipid analysis from single teat canals of living cows.

Theoretically, one can construct a model of stratum corneum making up the keratin layer, of evenly distributed and stacked cells lining the teat canal lumen along its full length. The thickness of this cylindrical layer depends on the number of cells on top of each other, while the total mass of such a cylinder may be calculated only if the lumen diameter and cylinder length are known. Therefore, theoretically, the amount of keratin present in the teat canal depends on both teat canal length (TCL), teat canal diameter (TCD), and the thickness of stratum corneum. Senft & Kemper-Krämer (1983) found convincing evidence for an influence of TCL and TCD on the amount of keratin recovered from excised teats. They removed teats from 304 cows of various breeds immediately after slaughter. TCL and proximal, medial and distal TCD were measured and the amount of keratin present in the teat canal was determined. The objective of the present study was to investigate whether TCL and TCD influence the dry weight of keratin removed *in vivo* by a tapestry needle using the procedure suggested by Bright et al. (1990).

Materials and Methods

During two consecutive calving seasons, data were collected from a total of 36 primiparious Danish Holsteins in the dairy herd at Research Centre Foulum, Denmark ($n=15$ and $n=21$, in year 1 and year 2 respectively). In year 1, samples were collected from two randomly chosen teats from each cow approximately 3 weeks *post partum* ($n=30$). In year 2, samples were collected on three occasions (weeks 1, 3 and 6 *post partum*, total $n=120$). On each occasion samples were taken from two randomly chosen teats from each cow. California Mastitis Test (CMT) scoring was performed after each keratin sampling. If CMT score was above 3, the keratin sample was excluded. Keratin sampling was performed with a size 14 tapestry needle, as described by Bright et al. (1990). Before teat preparation, teat ends were gently scrubbed with ethanol. Then, a sterile pre-weighed size 14 tapestry needle (Prym®, 52220 Stolberg, Germany) was inserted approximately 20 mm into the teat canal, rotated three times, and gently withdrawn. Needles containing keratin samples were then immediately frozen at −20 °C. To determine dry weight of the keratin, samples were later freeze-dried and weighed again.

Before teat preparation all teats were subjected to ultrasonography in order to detect abnormalities and to determine teat canal length. Ultrasonographic scans were carried out with an ALOKA Echo Camera model SSD-500 mounted with a 7·5-MHz ultrasound probe by submerging teats in a water-filled (35 °C) plastic cup as described by Spencer et al. (1996). Images were stored on a video recorder and TCL was measured on a monitor.

TCD was measured with a spenometer as described by Grøndahl (1975). The spenometer, consisting of a spring-loaded conical probe, was inserted into the teat canal before preparation, while the TCD was recorded.

The dry weight of recovered keratin, in each sample within cow was analysed by the following model:

$$\begin{aligned}\text{KERATIN} = {} & \text{TCL} + \text{TCD} + \text{TCD*TCD} + \text{TCD*TCD*TCL} \\ & + \text{QUARTER} + \text{YEAR} + \text{TCL*YEAR} \\ & + \text{TCD*YEAR} + \text{TCD*TCD*YEAR} \qquad (1)\end{aligned}$$

where the fixed effects were: TCL, teat canal length; TCD, teat canal diameter; TCD*TCD, teat canal area; TCD*TCD*TCL, teat canal volume; QUARTER, the four teats; and YEAR, year 1 or year 2. A mixed model (PROC MIXED; SAS, 1996) was used to allow inclusion of random effects

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Table 1. Means of teat canal length (TCL), teat canal diameter (TCD) and dry weight (DW) of keratin removed during sampling

Variable	TCL (mm ± SD)	TCD (mm ± SD)	Keratin (mg DW ± SD)
Year 1	10·2 ± 2·2 (n=30)	3·2 ± 0·3 (n=30)	2·18 ± 0·8[a]† (n=30)
Year 2	11·3 ± 1·7 (n=120)	3·2 ± 0·2 (n=114)	2·99 ± 1·1[b] (n=120)

† Means within columns with different superscripts differ ($P<0·05$)

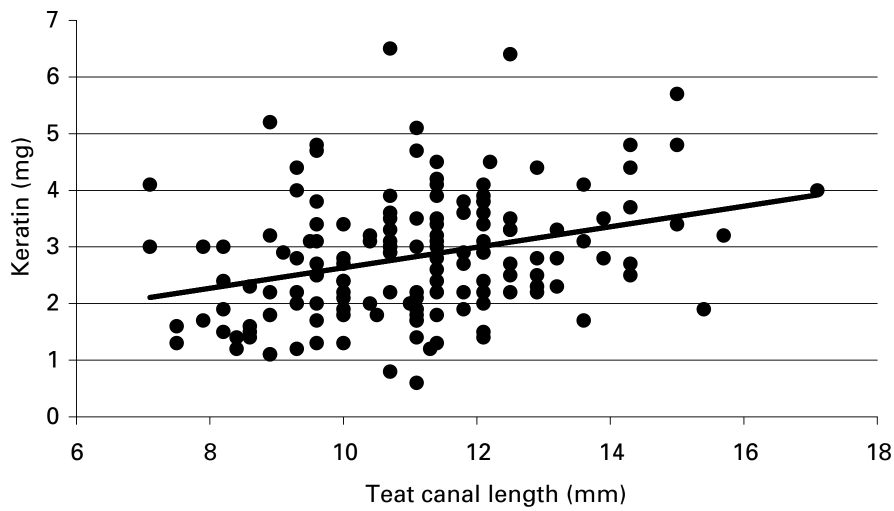

Fig. 1. The relation between the dry weight of keratin and teat canal length. Results are for year 1 and year 2 combined.

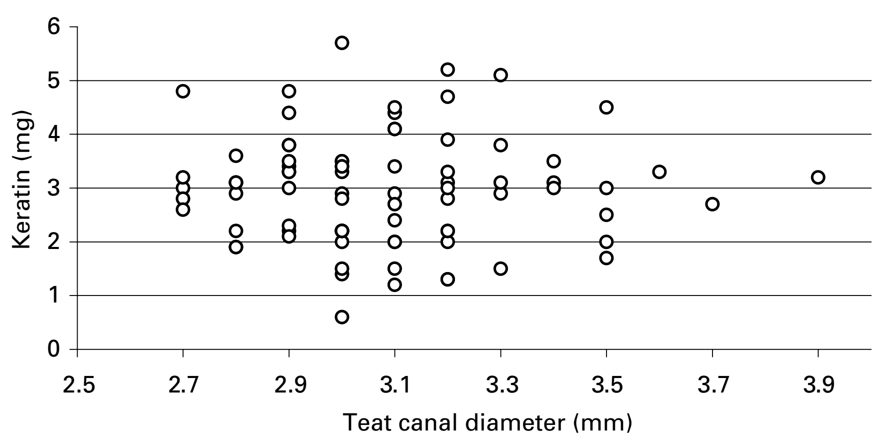

Fig. 2. The relation between the dry weight of keratin removed and teat canal diameter. Results are for year 1 and year 2 combined.

of COW∗YEAR and to allow repeated measurements of QUARTER within COW. Nonsignificant fixed effects were successively removed from the model according to level of significance.

Results are presented as least squares means.

Results and Discussion

No teats with hyperkeratosis or otherwise abnormal appearance were detected. TCL averaged 10·2 and 11·3 mm in year 1 and year 2, respectively (difference non significant) (Table 1). In year 2, TCL did not depend on weeks *post partum*. The dry weight of keratin recovered from the teat canals was slightly less in year 1 than in year 2 (2·26 and 2·94 mg, respectively, $P=0·05$) and was significantly related to TCL ($P=0·002$). In year 2, dry weight of keratin increased significantly during the first 6 weeks of lactation ($P<0·01$). Approximately 11% more keratin was recovered from rear teat canals than from front teat canals ($P=0·05$). Rear teat canals were also approximately 3% longer ($P<0·01$) than front teat canals. The linear relationship

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between the sample values of dry weight of keratin (mg) and TCL (mm) was computed as: dry weight of keratin (mg)=0·18*TCL+0·83 (r^2=0·10, Fig. 1). TCD averaged 3·2 mm and decreased between time of sampling from 3·3 mm in the first week, to 3·2 mm 3 weeks later, and finally to 3·1 mm at 6 weeks *post partum* (P<0·001). Our results do not support the hypothesis that the amount of keratin recovered from teats depends on TCD (Fig. 2).

There are two possible reasons why our results support a relation between the amount of keratin recovered and TCL but not TCD. First, the amount of keratin recovered by this method may not represent the true amount of keratin actually present in the teat canal. That would contradict Bright et al. (1990) who stated that 70·3±3·4% of the keratin in lactating cows was removed by reaming the teat with a tapestry needle. However, Bright et al. (1990) based their conclusions on a very limited number of samples. Bright et al. (1990) reamed the left teats of eight lactating cows (16 teats) and thereafter excised teats and scraped keratin from teat canals to determine the percentage of total keratin removed by reaming. Those 16 teat canals may have been similar in length and diameter, which would have led to the reported low variability in keratin wet weight. Measures of teat canal length and diameter were, however, not reported. The same workers examined 14 teats from dry cows and reported that 85·6±5·3% of the keratin was removed by reaming with a tapestry needle. The second explanation is simply that the amount of keratin present in the teat canal in our experiments did not depend on teat canal length and diameter which would contradict the convincing results reported by Senft & Kemper-Krämer (1983).

Consequently, the true amount of keratin present in the teat canal does not equal the amount of keratin that can be retrieved with the tapestry needle sampling procedure. A second possible theoretical model of keratin removal by the tapestry needle may explain the difficulties in correlating TCD with the reamed amount of keratin. Consider the keratin layer in the teat canal as a cylinder with an inner and outer diameter and a certain length. The total amount of keratin depends on all three measures. Now, by inserting the needle with its static diameter, one would expect the amount of keratin removed to increase with decreasing TCD, the keratin removed would be the keratin from teat canal lumen and as far out as the needle permits. We know, however, that the method removes keratin from the teat canal even if TCD exceeds the diameter of the needle. This may have to do with the elasticity of the teat canal, which may depend on the degree of relaxation of the teat sphincter. Or in other words, the influence of TCD on the amount of keratin removed with the tapestry needle may depend on whether or not the teat is foremilked; whether or not the cow is milked; and finally, on the presence of noradrenaline and dopamine mediators acting on the α-adrenergic receptors located on the smooth muscle cells of teat tissues.

Our results support the hypothesis that TCL influences the amount of keratin removed by reaming the teat with a tapestry needle. Including TCL may therefore be considered in the evaluation of quantitative data from experiments involving this kind of keratin collection procedure. Our results did not support the hypothesis that the amount of keratin recovered depends on TCD. It seems that the effect of TCD is complicated and needs further investigation.

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References

Bright SA, Bitman J, Capuco AV, Wood DL & Miller RH 1990 Methods of collection and lipid composition of teat canal keratin in dry and lactating cows. *Journal of Dairy Science* **73** 98–106

Capuco AV, Bright SA, Pankey JW, Wood DL, Miller RH & Bitman J 1992 Increased susceptibility to intramammary infection following removal of teat canal keratin. *Journal of Dairy Science* **75** 2126–2130

Grøndahl J 1975 Milking-out characteristics in the cow in relation to teat canal diameter. Teat canal measured with the spenometer in the living cow, milk flow registered with an electronic miligraph. Thesis, Oslo, Norges Veterinaerhoegskole, Inst. for Obstetrikk, 125 pp.

Murphy JM 1959 The effect of certain milk stresses to the bovine teat canal on infection with *Streptococcus agalactiae*. *Cornell Veterinarian* **49** 411–415

SAS 1996 *SAS/STAT® Software: Changes and Enhancements, Release 6.12*, 158 pp. Cary, NC: SAS Institute Inc

Senft B & Kemper-Krämer G 1983 [Investigations on teat canal keratin in cows]. *Züchtungskunde* **55** 330–336

Spencer SB, Griel LC & Goldberg JJ 1996 The use of ultrasonography to measure teat congestion. *Proceedings of the Annual Meeting of the National Mastitis Council* **35** 172–173