The Diagnosis of Subclinical Mastitis Using a Coulter Counter TA II

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Introduction

Mastitis in the dairy cow generally is associated with a reduction in yield in the affected quarter, a change in milk composition and an increase in the somatic cell content. A wide variety of tests have been employed in an attempt to diagnose subclinical mastitis using indirect or direct measurements of the physiological effects of udder damage. Cell counting of bulked milk is an established method of monitoring subclinical mastitis in a herd. The results are widely used in National Schemes to advise farmers of the level of mastitis in their herds (Sheldrake 1973) but is not an accurate indicator of the number of cows affected. In milk recording schemes, samples are taken from the total udder milk of individual cows for fat, protein and yield. These samples may be used for lactose determination and cell counting for monitoring individual cow's udder health status. Many factors influence the interpretation of results since only a low correlation has been found between the bulked milk cell content and the number of quarters affected (Brolund 1985).

The objective of this study was to confirm the accepted variation in individual cell count levels, and observe the changes associated with diagnosed clinical mastitis. In addition to examine the use of total udder milk data, somatic cell content, yield, lactose, concentration, as a mastitis management tool within a single herd and recorded over a period of time.

Materials and Methods

Experimental animals and their management

The herd comprised 50 Friesian cows predominantly in first to third lactation. Data files were established on each cow in one herd free from bacteriological evidence of mastitis at the commencement of lactation. In order to reduce some variable influence due to managerial factors cows were housed continuously and fed indoors on a complete diet. The mean bulked milk cell content of the herd remained less than 200,000 cells/ml throughout the trial.

Age Distribution of the Herd

Parity	1	2	3	4	5	6	7	8
No. of cows	11	14	11	4	5	3	1	1

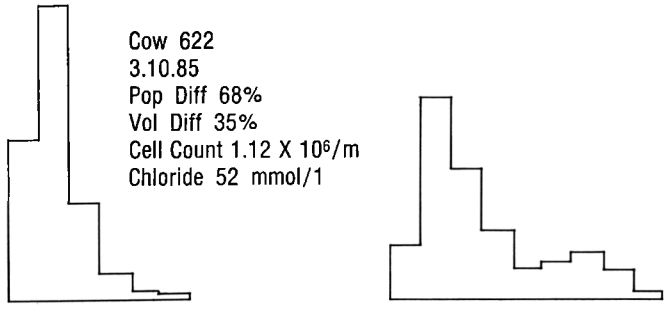
Sample procedure

Samples of udder total milk were taken at weekly intervals throughout the lactation at the evening and morning milking. In order to take into account diurnal variation cell counts were corrected for yield differences. Samples were divided into two 10 ml portions. One was preserved by the addition of 0.1 ml Somafix[1]. The other was preserved with potassium dichromate (Lactabs)[2] for subsequent chemical analysis.

Cell counting procedures

Somatic cell estimation and analysis were carried out using a Coulter[1] TA II cell counter with a population count accessory programmed to gather total somatic cell population, differential population count, and differential cell volume estimation. This instrument was precalibarated, using Somacount[1] reference particles, for particle sizes ranging from 4.0 micrometres (μm) equivalent spherical diameter (esd) in channel 6 to 40 μm esd in channel 16. Channel 6 corresponded to a particle size equivalent to a polymorphonuclear leucocyte demonstrated by reference to blood white cell counts, and channel 16 to macrophage and plasma cell nuclei observed by microscopic examination. An XY recorder which plotted a histogram of cell volume against cell count and cell count against cell diameter (range 4μm-10 μm) was linked to the TA II analyser to provide a visual record for each cow sample. (Figs 1-4)

FIGURE 1. Mastitis Positive



[1]*Coulter Electronics, Luton, Bedfordshire*
[2]*Thompson & Capper, Runcorn, Cheshire*

FIGURE 2. Non Mastitis

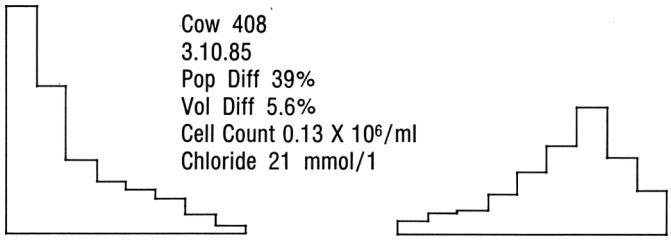


FIGURE 3. Late Lactation: No Infection.

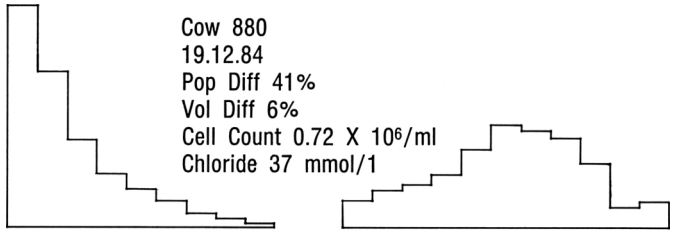
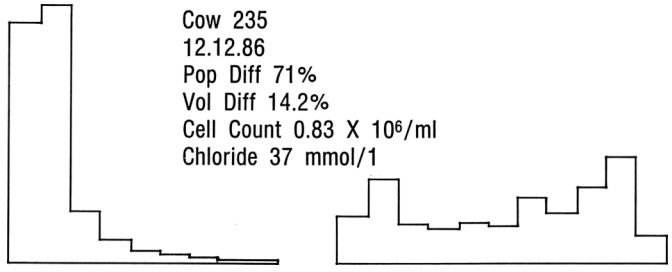


FIGURE 4. Mastitis: Late Lactation



Chemical procedures

Dichromatic preserved samples were analysed for fat, protein and lactose percentage using a FOSSMATIC MILKOSCAN 208. Chloride content was measured using a CORNING 920 chloride meter and expressed in mmol/litre.

Statistical recording

All results were retained on a Winchester disc drive COMMART CP 2452 programme added to a data file and put into a central file store. The total cell counts, percentage leucocytes, fat percentage, protein percentage, lactose percentage, yield in kilograms, chloride content in mmol/litre were recorded and totals, means and standard deviations calculated at weekly intervals, summarised each four weekly period. At the end of each lactation the complete lactation record reproduced, with totals, means and standard deviations calculated.

TABLE 1. Mean Total Cell Counts 1000s/ml.

Parity	1	2	3	4	5	6
a.m.	150	140	170	140	280	710
p.m.	200	190	230	170	300	700

Results and Discussion

A summary of the cell count averages in lactation age groups is in Table I for morning and evening milking.

The cell counts show an inverse relationship to yield in their diurnal variation suggesting that the physiological mechanisms involved is the effect of yield and dilution. The general trend of increasing cell count with lactation age regarded by some workers (Dohoo et al 1981) as significant was not evident in the first four lactation age groups. It has been suggested (Schultze 1977) that any increase was independent of infection. Results show that there is a rise in the percentage leucocytes with increasing age (Table II).

TABLE 2. Leucocytes as a % of the Total Cell Count.

Parity	1	2	3	4	5	6
a.m.	44.5	46.1	47.0	46.4	52.7	64.3
p.m.	43.4	47.2	49.6	48.7	52.9	67.4

In estimating the percentage leucocytes the influence of the yield variable is largely removed.

TABLE 3. Distribution of the Lactation Mean Leucocyte Counts.

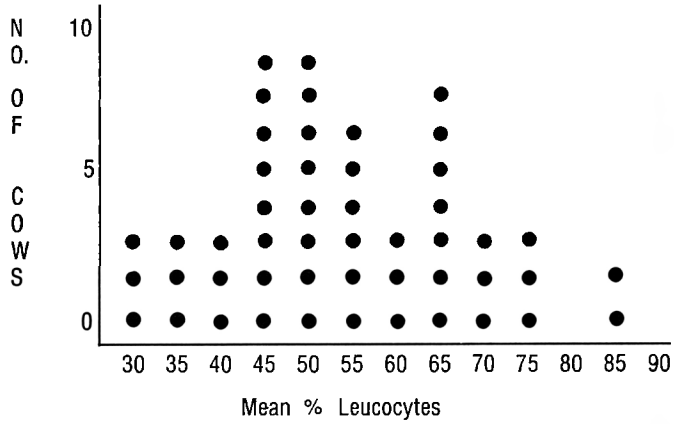


Table III shows the distribution of the leucocyte percentage of the individual cows, this approximates to a more normal distribution in contrast to the total cell count. A cow with a mean milk leucocyte content of more than 60% of the total cells may be regarded as having a chronic inflammatory process irrespective of the total cell content. In most cases a rise in the leucocyte percentage preceeded a rise in the total cell content.

A comparison of average yields and mean leucocyte percentages lactose percentage and chlorides of those cows showing pathogens in the milk on one or more occasions revealed no significant difference in yield, lactose or chloride but a significantly higher leucocyte level than animals remaining uninfected.

	% leucocytes	Yield 1	Lactose %	Chloride mmol/1
Infected (16)	58	20.5	4.69	26.8
Uninfected (34)	44	20.5	4.85	24.3

TABLE 4. Distribution of Mean Cell Counts.

Cell count cells/ml	Infected (16)	Uninfected (33)
Less than 150,000	—	8
16 — 200,000	—	19
21 — 250,000	1	4
26 — 300,000	5	2
31 — 350,000	3	
36 — 400,000	2	
41 — 450,000	1	
46 — 500,000	—	
Over 500,000	4	

The difference in the mean cell content of those infected compared with the uninfected was highly significant. Uninfected cows have a mean cell count of 174,000 cells/ml whereas infected cows had a mean of 415,000 cells/ml, however the mean was considerably influenced by several extreme values. Table IV gives the distribution of the cell content of infected and uninfected cows.

If a threshold of 300,000 cells/ml is established as the linear score criterion for infection 6 cows or 12% would be classified as negative, however the mean leucocyte percentage in those animals exceeded 50% indicating infection.

Our results suggest that an analysis of the milk incorporating a differential count reduces the number of cows falsely classified as negative and improves the precision of diagnosis. Workers have suggested that an age correction is established for the threshold values of infection. From an epidemiological point of view the probability of infection is more important than the threshold particularly early in lactation. The demonstration of a high leucocyte count will confirm infection even if the cell content is below the accepted threshold. It is suggested that a threshold of 50% leucocytes and a total cell count threshold of between 250,000 to 300,000 cells would reduce the percentage of false negative in any control scheme. For the purposes of any control scheme there is no question that quarter samples give a more accurate picture giving less false negative results and is more than anticipated when using composite milk samples. By using a combination of lactose percentage and leucocyte percentage the precision afforded can offset the extra expense required for quarter sampling and a satisfactory control scheme may be established using composite milk samples.

References

1. Brolund L, 1985, Cell counts in bovine milk. Acta Vet. Scand. supplement No. 80, p97. 2. Dohoo IR, & Meek AH, 1982, Somatic cell counts in bovine milk. Can. Vet. J. 23, 119. 3. Dohoo IR, Meek AH & Martin SW, 1984, Somatic cell counts in bovine milk: Relationships to production and clinical episodes of mastitis. Can. J. Comp. Med., 48, 130. 4. Dohoo IR, Meek AH, Martin SW & Barnum DA, 1981, Use of total and differential somatic cell counts from composite milk samples to detect mastitis in individual cows. Can. J. Comp. Med., 45, 8. 5. Reneau JK, 1986, Effective use of dairy herd improving somatic cell counts in mastitis control. J. Dairy Sci., 69, 1708. 6. Sheldrake RF, Hoare RJT & McGregor GD, 1983, Lactation stage, parity, and infection affecting somatic cells, electrical conductivity and serum albumin in milk. J. Dairy Sci., 66, 542.