

Cyclic fluctuations in acetone concentrations in the blood and milk of clinically healthy dairy cows

HANLIE E.K. WINTERBACH, P.J. APPS, W. GIESECKE, and INGE-MARIE PETZER
Food Hygiene Division, Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa

ABSTRACT

WINTERBACH, HANLIE E.K., APPS, P.J., GIESECKE, W. & PETZER, INGE-MARIE 1993. Cyclic fluctuations in acetone concentrations in the blood and milk of clinically healthy dairy cows. *Onderstepoort Journal of Veterinary Research*, 60, 247–255

Milk samples were taken daily or twice weekly, and blood samples twice weekly, from six clinically healthy dairy cows. Acetone concentration was determined by a new headspace gas-chromatographic method that proved to be suitable in terms of practicality, sensitivity and precision. The concentration of acetone in milk was closely correlated with that in blood ($r^2 = 0,967$). There was no relationship between lacteal acetone concentration and either somatic cell count or bacterial infection. In both blood and milk there were fluctuations in acetone concentration that were synchronous between the six cows. The fluctuations were apparently cyclic, with a period of approximately 10 d. Such fluctuations have not previously been reported.

INTRODUCTION

Clinical ketosis affects about 2–15% of dairy cows in the United States of America and Europe (Schultz 1974; Baird 1982; Gröhn, Saloniemi & Syvajarvi 1986). No data are available from South Africa. Borderline ketosis may affect as many as 50% of high-producing dairy cows (Schultz 1971; Emery, Burg, Brown & Blank 1964) and those with symptoms are probably a minority of the affected animals. Because it affects more cows, the total economic losses due to subclinical ketosis may be greater than those due to its clinical form. Weigt (1983) identified ketosis as a definite predisposing factor for coliform mastitis and, in an epidemiological survey, Gröhn, Erb, McCulloch & Saloniemi (1989) found that more diseases were associated with clinical ketosis than with any

other. Plainly, ketosis is a problem with substantial economic impact.

The onset of clinical ketosis is gradual (Schulz 1971), which raises the possibility of preventive treatment if the pre-clinical stages can be adequately monitored. Using a colorimetric test, Emery, Bell & Thomas (1968) established the practicality of weekly monitoring of milk ketone bodies, but not its utility in forecasting and treating cases of clinical ketosis.

A variety of assays have been used to investigate changes in the concentration of ketone bodies during lactation. Results are available for both long-term changes—with sampling at weekly or monthly intervals (Andersson 1984; Knodt, Shaw & White 1942; Schultz & Myers 1959)—and diurnal variations with 4-h sampling intervals (Andersson & Lundstrom 1984). Curiously, day-to-day variations over periods of more than 2 weeks (Knodt *et al.* 1942) have been neglected. Mills, Beitz & Young (1986) assayed blood beta

hydroxybutyrate every 3 d, but presented no time courses for changes in concentration.

The aims of this study were as follows:

- To test the suitability of a new headspace gas-chromatographic analysis of milk (Winterbach & Apps 1991) for routine monitoring of the metabolic status of dairy cows.
- To compare the analytical variability of the method with the biological variation between cows, and from day to day.
- To provide data on the day-to-day changes in the acetone concentration of milk.

METHODS AND MATERIALS

Animals

Specimens of milk and blood were obtained from six clinically healthy, multiparous Friesian cows from the Onderstepoort Veterinary Institute dairy herd. The cows had calved between 29 April 1989 and 16 May 1989 (Table 1). The herd was maintained on a zero-grazing system, with concentrates (protein 150 g/kg, fat 25 g/kg, fibre 90 g/kg, calcium 15 g/kg, phosphorous 6 g/kg) supplied according to milk yield, and lucerne hay *ad libitum*.

Sampling

Between 17 May 1989 and 17 July 1989 milk samples were taken from two of the cows (999 and 1595) on every working day, and at weekends if possible, and, with adjustments for public holidays, from the other four on every Monday and Friday.

Milk samples were taken for determination of acetone, and for bacteriology and somatic cell counting. Before routine morning milking each subject's udder was washed with water and dried with a clean paper towel. Quarter samples of approximately 50 ml of foremilk were drawn into glass bottles with plastic caps. The teats were then disinfected with a methylated spirits swab, and samples were taken for udder health examination by somatic cell counting and bacteriology (Toole 1971; International Dairy Foundation 1981a; 1981b). If the teats are sterilized before milk is taken for acetone determination methanol appears as an interfering peak on the chromatogram (Winterbach 1989). The cows were then machine-milked as usual.

Blood samples were collected from the jugular vein in 10-ml heparinized vacuum tubes each week on Monday and Friday, immediately after morning milking.

The samples were frozen at -20°C until analysis. Samples were analysed by equilibrium headspace gas-chromatography, for acetone and acetoacetate,

as acetone, (Winterbach & Apps 1991). To eliminate the effects of variation in analytical sensitivity, the samples were analysed in an erratic order which was not the same as that in which they were collected. Analyses were completed by September 1989.

Milk and blood samples were tested with Ketostix (Miles Laboratories, Stoke Poges, Slough SL2 4LY, UK) according to the manufacturer's instructions.

Data processing and statistical calculations were carried out using the SYSTAT and SYGRAPH software packages (Systat Inc., Leland, Illinois, USA).

RESULTS

Acetone concentrations

The acetone concentration in 636 milk samples and 100 blood samples was determined. In five of the six cows, milk and blood acetone concentrations in the milk and blood samples showed marked changes with time (Fig. 1). Fluctuations between the quarters of individual cows and between the cows were synchronized. In the sixth cow (cow 972) the fluctuations were less marked, but they were still synchronized with those in the rest of the group (Table 2).

The mean of the acetone concentrations in milk from each of the quarters was very closely correlated with the acetone concentration of blood drawn from the same cow on the same day (Table 1). In addition, fluctuations in the acetone concentration of a cow's milk were synchronized with equivalent fluctuations in the acetone concentration of her blood. Exceptions were milk-acetone peaks in cow 1595 on days 8 and 18, and in cow 999 on day 18. These peaks were preceded by 1 d by peaks in blood acetone (Fig. 2).

The ratio of acetone concentration in milk to that in blood, had a maximum of 1,656, a minimum of 0,114 and a mean of $0,481 \pm 0,277$ S.D.

The difference between the concentration of acetone in blood and milk samples was very closely correlated to the acetone concentration in blood, with the exception of a single outlying point for cow 1200 (Table 1).

Both blood and milk samples always tested negative with Ketostix.

Udder health

Bacterial infection

The milk samples were cultured on agar plates. All samples that grew colonies indicating environmental contamination were excluded from the analysis. Milk-borne bacteria were cultured from 41 of the remaining 288 samples.

TABLE 1 Statistics of acetone concentrations in the blood and milk of six clinically healthy cows

Cow no.	Calving date	AI date	Blood-milk acetone concentration correlation	Correlation of difference between blood-milk: blood acetone concentration
972	29-04-89	14-07-89	0,852**	0,984**
999	13-05-89	14-07-89	0,761*	0,956**
1200	12-05-89	21-09-89	0,980**	0,991**#
1215	10-05-89	03-10-89	0,721*	0,836**
1595	16-05-89	04-09-89	0,932**	0,980**
1631	10-05-89	21-09-89	0,992**	0,987**
Pooled data			0,967**	0,981**#

* P = 0,001

** P < 0,001

Single outlier point deleted

TABLE 2 Temporal synchrony of peaks in lacteal acetone concentration among six clinically healthy dairy cows

Date	Trial day	Cows measuring peaks on trial days					
		972	999	1200	1215	1595	1631
Tue 23-05-89	7				✓		✓
Wed 24-05-89	8					✓	
Fri 26-05-89	10	✓	✓				
Fri 02-06-89	17	✓		✓	✓		
Sat 03-06-89	18		✓			✓	
Mon 12-06-89	27	✓	✓	✓	✓	✓	✓
Mon 19-06-89	34			✓			
Fri 23-06-89	38	✓	✓		✓		✓
Mon 03-07-89	48	✓	✓	✓	✓	✓	✓
Thu 13-07-89	58		✓				

In cow 972 (t-test; $t = -5,406$, $P < 0,001$) and cow 1200 ($t = 2,106$, $P = 0,043$) the acetone concentration of the milk from infected and healthy quarters differed significantly. In cow 972 the acetone concentrations in the infected quarters were higher than in the healthy quarters, but in cow 1200 the concentrations in the infected quarters were lower than in the healthy quarters.

There was no significant difference between the somatic cell counts of milk containing bacteria and uncontaminated milk (t-test; $t = -1,489$, $P = 0,144$).

The presence of bacteria coincided only twice with a peak in SCC, and one of these occasions was in a cow (cow 1631) with chronic subclinical infection.

Somatic cell counts (SCC)

Somatic cells were counted in 563 milk samples.

There was no relationship between SCC and acetone concentrations in the pooled data, or for any of the cows individually. Only twice did a peak in the SCC in at least one quarter coincide with a peak in the concentration of acetone.

On day 38, two of the three quarters of cow 999 were infected (the sample from the fourth quarter was contaminated). One of the two samples had an elevated SCC and there was a peak in the concentration of acetone in the milk and blood.

The range of the concentration of acetone from a cow's quarters on a particular day was independent of SCC or bacterial infection.

DISCUSSION

Suitability of the method

Although the headspace gas-chromatographic method used is, in principle, sufficiently quick and simple for monitoring on farms, the high cost of the equipment and consumables is likely to restrict it to the research laboratory.

This study confirms Winterbach & Apps' (1991) finding that the headspace method is sufficiently sensitive for the differences in ketone concentration between body fluids to be ignored when the body fluid

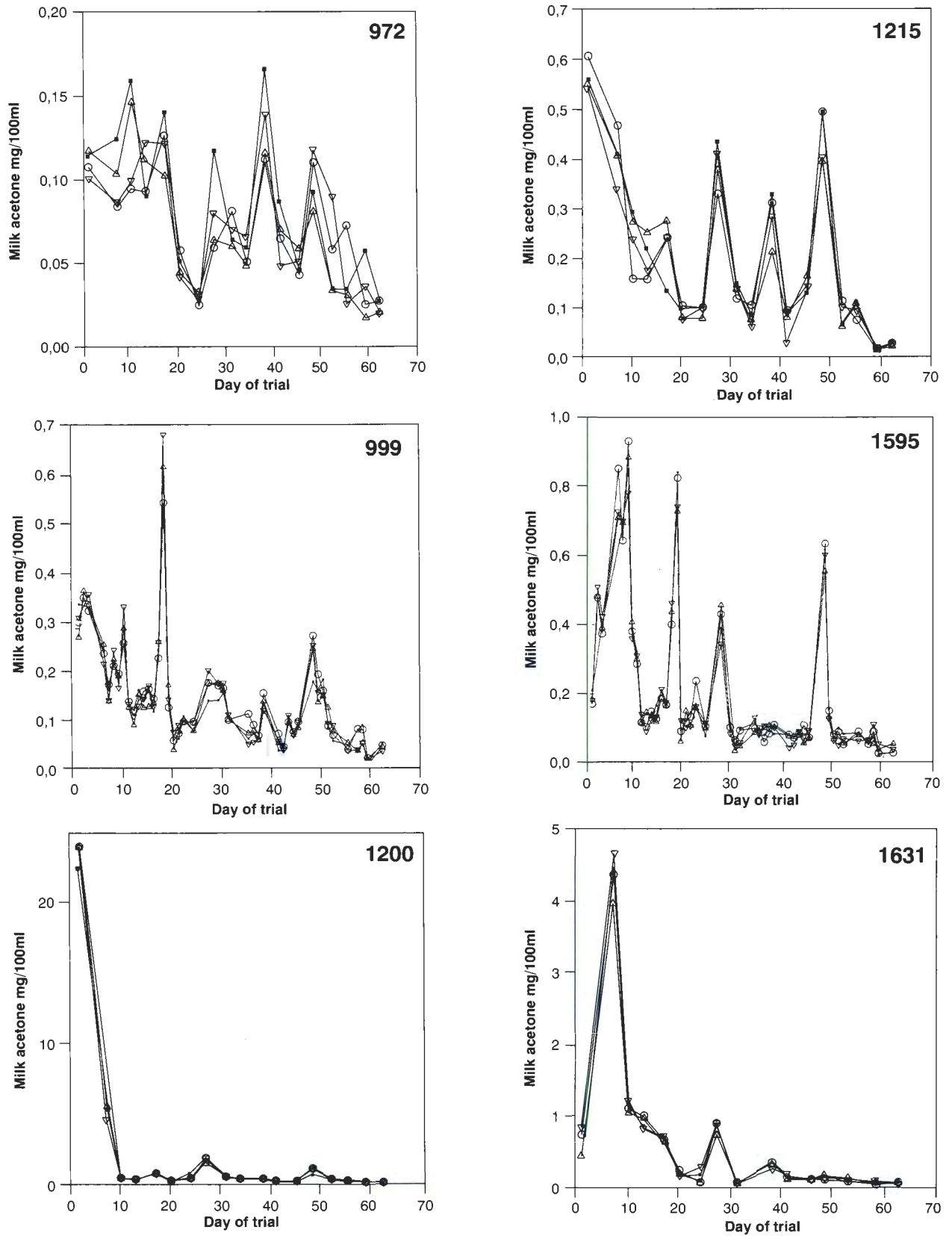


FIG. 1 Changes in acetone concentrations in milk from individual quarters of six clinically healthy Friesian dairy cows. Udder quarters are symbolized as follows: •—right front, o—right hind, Δ—left-front, ▽—left hind

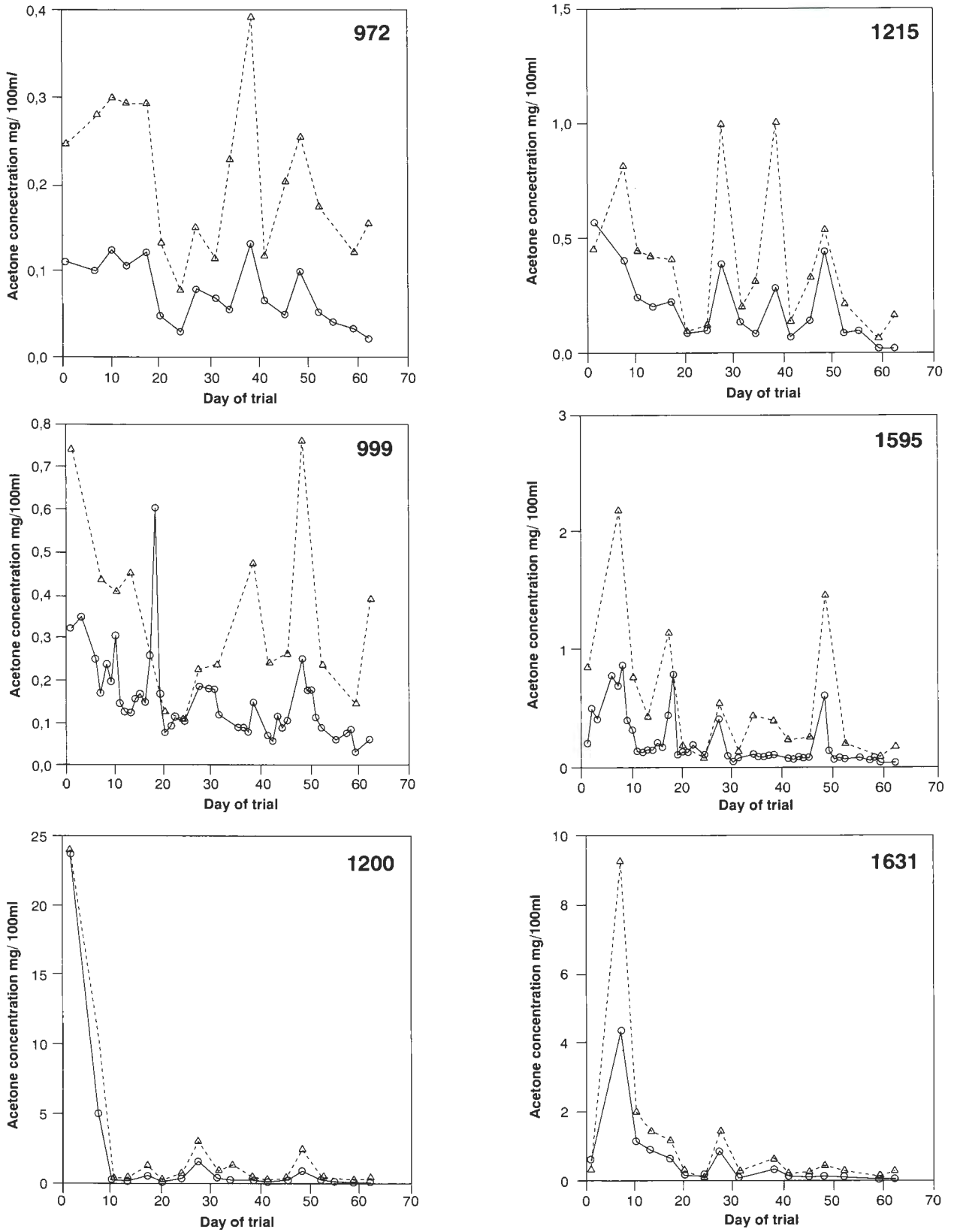


FIG. 2 Changes in acetone concentrations in blood (Δ) and milk averaged over four quarters (o) from six clinically healthy Friesian dairy cows

for use in acetone determination is selected. Milk ketones are approximately half as concentrated as blood ketones (Schultz & Myers 1959). Ketone body concentrations in urine are up to four times higher than those in milk (Schultz 1971).

The average throughput of the headspace gas-chromatography method was 9,6 samples/h, and there were no samples with acetone concentrations below the method's limit of quantitation (Winterbach & Apps 1991). Marstorp, Anfalt & Andersson's (1983) flow-injection analysis handles up to 300 samples/h, but its lower detection limit, at four times noise (i.e. a coefficient of variation of 25%) is 0,58 mg/100 ml. This would have been too high to trace the day-to-day variations in cows 972, 999, 1215 and 1595, rendering the method unsuitable for monitoring the metabolism of healthy cows. The upper concentration limit of the flow injection method is 29 mg/100 ml, which is below the maximum of 40–50 mg/100 ml reached in clinical ketosis (Thin & Robertson 1953).

How frequently a quarter's acetone concentration had a particular rank in relation to the concentration from the other three quarters was tested by Chi-squared. The null hypothesis was that the chance of a quarter having a particular rank was 0,25. In only one of the cows (cow 1631) ($\chi^2 = 25,5$, d.f. = 9, $P = 0,002$) did one quarter consistently deliver milk with an acetone concentration higher or lower than that of milk from the other three. Consequently, in an ANOVA to test the significance of day-to-day variation against inter-quarter variation, "quarter" was replaced as a factor by that quarter's rank in relation to the other three on the same day. The ANOVA showed that in only one cow (cow 972) differences between quarters were significantly large compared to day-to-day variation ($F = 3,068$, $P = 0,034$) (see Fig. 1). It would therefore be possible to monitor a cow's metabolic status accurately from a pooled milk sample because, in addition, each cow's quarters were synchronized in their changes in acetone concentration, and the mean acetone concentration between quarters was closely correlated with blood acetone concentration.

The headspace method's lower detection limit in relation to sample concentration, its ability to track daily changes in ketone concentration (Fig. 1) and its rate of sample throughput combine with the possibility of pooling milk from the four quarters of a single cow to make it a powerful method for monitoring normal and subclinical ketone concentrations in dairy cows. If the new method had not been sensitive or precise enough to monitor daily changes in acetone concentration, the data would not have shown the synchronized fluctuations in acetone concentration between quarters and between cows, or the close relationship between blood and milk ketone levels.

Acetone concentrations

Although the acetone concentrations in milk sometimes exceeded the expected threshold for clinical ketosis of 2–4 mg/100 ml (Schultz & Myers 1959; Emery *et al.* 1964; Andersson 1984), the cows did not show clinical symptoms—perhaps because the peaks of acetone concentration were of short duration.

The correlation between milk and blood acetoacetate plus acetone (0,967) found in this study, is closer than the 0,87 found by Schultz & Myers (1959) and the 0,615 found by Andersson & Lundström (1984), and very similar to the 0,974 found by Andersson (1984), probably due to improvements in analytical precision. With such close correlations, milk acetone concentrations provide a very accurate measure of ketone concentrations in blood (Andersson 1984; Andersson & Lundström 1984). The correlation between the ketone concentrations in milk and blood is considerably closer than that of 0,5115 between urine and blood (Knodt *et al.* 1942). The acetone concentration in milk samples therefore provides a more precise measure of blood ketones than that in urine samples. Milk samples are also much easier to collect than urine samples.

The close linear regression of the milk-blood difference in acetone concentration on the blood acetone concentration confirms that the diffusion coefficient of acetone from blood to milk is not affected by acetone concentration—at least up to concentrations characteristic of subclinical ketosis. The single outlier from cow 1200 lies at the highest acetone concentration measured in this study, and could represent a change in the properties of the blood-milk barrier, or the production and secretion of acetoacetate by the udder (Kronfeld, Raggi & Ramberg 1968; Schwalm, Waterman, Shook & Schultz 1972). This is consistent with the results of Andersson & Lundström (1984) and Andersson (1984) which suggest that acetone is the only ketone body that diffuses passively from blood to milk. The secretory epithelium uses 3-hydroxybutyrate (Palmquist, Davis, Brown & Sachan 1969) and, in rats, acetoacetate (Williamson, Mc Keown & Ilic 1974) as metabolic substrates, and may produce acetoacetate in ketotic cows (Kronfeld *et al.* 1968; Schwalm *et al.* 1972). This study confirms the finding of Andersson & Lundström (1984) that the determination of acetone and acetoacetate together as acetone is both practical and informative.

Udder health

White & Rattray (1968) showed that, *in vitro*, acetoacetate at 10 mg/100 ml reduced the ability of leucocytes to slow the growth of pathogens in milk. At such a ketone concentration a cow would be clinically ketotic, which suggests a relationship between clinical ketosis and udder health. In this study the SCC and acetone concentrations found in healthy

cows were independent of each other. In only two of the cows (cow 972 and cow 1200) was there a significant relationship between bacterial status and ketone concentration, and in them the effects were opposite. Apart from the difficulties of diagnosing sub-clinical mastitis (Giesecke & Van den Heever 1974), the absence of a consistent relationship between ketosis and udder health may have been a consequence of the ketone concentrations being low, or of any bacterial responses to the effects of ketones on milk leucocytes lagging behind the sharp fluctuations in ketone concentration.

In studies of other parameters it has been found that milk from quarters infected with mastitis is more likely to differ from that in uninfected quarters than to fall outside a "normal" range (Maatje & Rossing 1991). Had this been the case in the present study, where none of the cows had clinical mastitis, there would have been a relationship between the range of acetone concentrations found in the four quarters, and either SCC or bacterial infection. No such relationship was found.

In general, the detection and interpretation of associations between acetone concentrations, SCCs and bacterial infections will be confounded by the delays with which any of them can be expected to exert its effects on the others.

In contrast to the lack of effect of acetone concentration on SCC found in the healthy cows in this study, Lotthammer, Boehnke & Morawietz (1988) found that high SCCs were more common in clinically ketotic cows than in normal ones.

Cyclic changes

Andersson & Lundström (1984) found diurnal changes in ketone concentration that covered, at most, a doubling in concentration. Knodt *et al.* (1942) found irregular day-to-day fluctuations in milk acetone concentration, but they did not remark on a synchronization between cows. Although Procos (1961) mentions transient, strongly positive Ketostix reactions in urine, the occurrence of sharp, synchronized peaks in the acetone concentrations of blood and milk was entirely unexpected. Consequently, in searching for an explanation of why they occurred we are hampered by a lack of experimental data, as opposed to observational data, and by a sampling schedule that, in hindsight, is seen to partially obscure the pattern of the fluctuations.

In the milk samples collected at least every weekday from cows 999 and 1595, four of the 11 peaks were on a Monday, three on a Friday, and two on a Saturday. For blood, and milk from the other four cows, peaks could occur only on the first and last working days of a week (usually Mondays and Fridays) because those were the only days on which samples

were taken. It must be kept in mind that, with sampling only on weekdays, what is apparently a peak on a Monday or Friday could be a point on the rising or falling slope of a fluctuation reaching a maximum on the Saturday or Sunday. The peaks in milk acetone for cows 999 and 1595 on days 8 and 18 illustrate this. If milk samples had not been taken on days 8 and 18 the peaks would have appeared on days 7 and 17, and would then have been synchronized with peaks in blood acetone. This has no bearing, however, on the occurrence of the peaks on the same days in different cows.

Peaks in acetone concentration on Mondays and Fridays could have been associated with the drawing of blood on those days, which involved extra handling and restraint of the cows. Handling animals increases the fatty acid content of their blood (Bowden 1971). Fox (1971) asserts that any stress predisposes a cow to the development of ketosis.

An additional influence on acetone concentrations on Mondays, Fridays and Saturdays could have been the change in milking routine at weekends, when the afternoon milking was 2 h early and the morning milking was sometimes late. The extended overnight milking interval could well be stressful for the cows. Van der Iest & Hillerton (1989) found an increase in SCC when milking intervals were changed. The high acetone concentrations on Mondays could have been produced during recovery from the stress of the weekend, but for the weekend routine to increase acetone concentrations on Fridays would require anticipation by the cows. Similarly, for the stress of blood sampling to have affected acetone concentrations, the cows would have to have anticipated it, because milk samples were taken before blood was drawn, and foremilk is secreted several hours before its removal. Cows are able to anticipate unpleasant events; Heeschen, Reichmuth & Hamann (1991) found peaks in SCC that they attributed to the stressful anticipation of a painful injection.

Any explanation invoking changes in routine, including blood sampling, fails to account for the lack of acetone peaks on some of the Mondays and Fridays. An alternative interpretation is that the acetone concentrations in all the cows showed a regular cyclic fluctuation lasting 9–11 d, with the apparent length of each cycle depending on the coincidence of a sampling day with a fluctuation in acetone concentration.

The synchronization between cows, of the fluctuations in blood and milk acetone concentrations, suggests that the cause of the fluctuations was exogenous, or that some form of cycle entrainment had occurred. The acetone peaks were more closely synchronized in time since the beginning of the trial than in time since calving, an observation that strongly supports entrained or external cycles over endogenous ones. Influences such as temperature, feeding or

handling would be expected to produce synchronized changes, though not necessarily on a regular cycle, but none of the management factors affecting the herd had a 10-d period. There was some synchrony of oestrous cycles; cows 972 and 999 were artificially inseminated on the same day (14 July 1989, day 59 of the trial), as were cows 1200 and 1631 (21 September 1989, after the end of the trial) (Table 1).

There is no known cyclic fluctuation of about 10 d in dairy cow physiology. A cow's oestrous cycle lasts 21 d, and is divided equally between the follicular and the luteal phases. A 10-d cycle of acetone concentration could arise if acetone concentrations rose (or fell) during each change between phases. The link between acetone and hormone cycles is given some support by there being a peak in acetone on day 58, 1 d before cows 972 and 999 were artificially inseminated.

A 10-d cycle in milk or blood acetone concentrations has not previously been detected and, if the cycle is real, it has very important implications for dairy cow physiology and susceptibility to disease. At the very least, peaks in acetone concentration would confound the interpretation of weekly test results in preventive monitoring (Emery *et al.* 1968). The need to confirm the existence of such a cycle in a larger sample of cows, and the need to identify its causes and consequences, are compelling reasons for further work in this area.

ACKNOWLEDGEMENTS

We would like to thank Dr D. Verwoerd, Director of Onderstepoort Veterinary Institute for his support of this work.

REFERENCES

ANDERSSON, L. 1984. Concentrations of blood and milk ketone bodies, blood isopropanol and plasma glucose in dairy cows in relation to the degree of hyperketonaemia and clinical signs. *Zentralblatt für Veterinärmedizin Series A*, 31:683–693.

ANDERSSON, L. & LUNDSTRÖM, K. 1984. Milk and blood ketone bodies, blood isopropanol and plasma glucose in dairy cows; methodological studies and diurnal variations. *Zentralblatt für Veterinärmedizin Series A*, 31:340–349.

BAIRD, G.D. 1982. Primary ketosis in the high-producing dairy cow: clinical and subclinical disorders, treatment, prevention, and outlook. *Journal of Dairy Science*, 65:1–10.

BOWDEN, D.M. 1971. Non-esterified fatty acids and ketone bodies in blood as indicators of nutritional status in ruminants: a review. *Canadian Journal of Animal Science*, 51:1–13.

EMERY, R.S., BELL, J.W. & THOMAS, J.W. 1968. Benefits derived from routine testing for milk ketones. *Journal of Dairy Science*, 51:867–868.

EMERY, R.S., BURG, NANCY, BROWN, L.D. & BLANK, G.N. 1964. Detection, occurrence and prophylactic treatment of borderline

ketosis with propylene glycol feeding. *Journal of Dairy Science*, 47:1074–1079.

FOX, F.H. 1971. Clinical diagnosis and treatment of ketosis. *Journal of Dairy Science*, 54:974–978.

GIESECKE, W.H. & VAN DEN HEEVER, L.W. 1974. The diagnosis of bovine mastitis with particular reference to subclinical mastitis: a critical review of relevant literature. *Onderstepoort Journal of Veterinary Research*, 41:169–212.

GRÖHN, Y., SALONIEMI, H. & SYVAJARVI, J. 1986. An epidemiological and genetic study on registered diseases in Finnish Ayrshire cattle. *Acta Veterinaria Scandinavica*, 27:209–222.

GRÖHN, Y.T., ERB, H.N., McCULLOCH, C.E. & SALONIEMI, H.S. 1989. Epidemiology of metabolic disorders in dairy cattle: association among host characteristics, disease, and production. *Journal of Dairy Science*, 72:1876–1885.

HEESCHEN, W., REICHMUTH, J. & HAMANN, J. 1991. Application of BST and somatic cell counts in milk. *IDF Mastitis News*, 16:7.

INTERNATIONAL DAIRY FEDERATION 1981a. Laboratory methods for use in mastitis work. *Bulletin of the International Dairy Federation*, 132. IDF, Brussels.

INTERNATIONAL DAIRY FEDERATION 1981b. Bovine mastitis: definitions and guidelines for diagnosis. *Bulletin of the International Dairy Federation*, 211. IDF, Brussels.

KNODT, C.B., SHAW, J.C. & WHITE, G.C. 1942. Studies on ketosis in dairy cattle. II. Blood and urinary acetone bodies of dairy cattle in relation to parturition, lactation, gestation, and breed. *Journal of Dairy Science*, 25:851–860.

KRONFELD, D.S., RAGGI, FIORA & RAMBERG, C.F. 1968. Mammary blood flow and ketone body metabolism in normal, fasted and ketotic cows. *American Journal of Physiology*, 215: 218–227.

LOTTHAMMER, VON K.-H., BOEHNKE, H.-J. & MORAWIETZ, M. 1988. Beziehungen zwischen verschiedenen Blutparametern als Kriterien für Stoffwechselstörungen und dem Milchzellgehalt bei Milchrindern. *Deutsche Tierärztliche Wochenschrift*, 95:379–384.

MAATJE, K. & ROSSING, W. 1991. The efficacy of in-line measurement of quarter electrical conductivity. *IDF Mastitis News*, 16:6–7.

MARSTORP, P., ANFALT, T. & ANDERSSON, L. 1983. Determination of oxidised ketone bodies in milk by flow injection analysis. *Analytica Chimica Acta*, 149:281–289.

MILLS, S.E., BEITZ, D.C. & YOUNG, J.W. 1986. Characterization of metabolic changes during a protocol for inducing lactation ketosis in dairy cows. *Journal of Dairy Science*, 69:352–361.

PALMQUIST, D.L., DAVIS, C.L., BROWN, R.E. & SACHAN, D.S. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of D(-)-B-hydroxybutyrate. *Journal of Dairy Science*, 52:633–638.

PROCOS, J. 1961. Modification of the spectrophotometric determination of ketone bodies in blood enabling the total recovery of B-hydroxybutyric acid. *Clinical Chemistry*, 7:97–106.

SCHULTZ, L.H. 1971. Management and nutritional aspects of ketosis. *Journal of Dairy Science*, 54:962–973.

SCHULTZ, L.H. 1974. Ketosis, in *Lactation. A Comprehensive Treatise*, edited by B.L. Larson & V.R. Smith. London: Academic Press: 317–353.

SCHULTZ, L.H. & MYERS, MARGO. 1959. Milk test for ketosis in dairy cows. *Journal of Dairy Science*, 42:705–710.

- SCHWALM, J.W., WATERMAN, R., SHOOK, G.E. & SCHULTZ, L.H. 1972. Blood metabolite interrelationships and changes in mammary gland metabolism during subclinical ketosis. *Journal of Dairy Science*, 55:58–64.
- THIN, C. & ROBERTSON, A. 1953. Biochemical aspects of ruminant ketosis. *Journal of Comparative Pathology*, 63:184–194.
- TOOLE, A. 1971. A monograph on bovine mastitis—Part 1. Economics, aetiology and diagnosis. *Annual Bulletin of the International Dairy Federation*, 2:1–23.
- VAN DER IEST, RENEE & HILLERTON, J.E. 1989. Short-term effects of frequent milking of dairy cows. *Journal of Dairy Research*, 56:587–592.
- WEIGT, URSULA 1983. Clinical aspects of coliform mastitis in the bovine. *Veterinary Research Communications*, 7:253–257.
- WHITE, R. & RATTRAY, E.A.S. 1968. The in vitro effect of ketone bodies on the growth curves of mastitis organisms in milk. *Journal of Comparative Pathology*, 78:179–188.
- WILLIAMSON, D.H., McKEOWN, STEPHANIE R. & ILIC, VERA 1974. Metabolic interactions of glucose, acetoacetate and insulin in mammary-gland slices of lactating rats. *Biochemical Journal*, 150:145–152.
- WINTERBACH, HANLIE E.K. 1989. Development of a gas-chromatographic headspace method for the analysis of acetone in milk, blood and urine in sub-clinically and clinically ketotic dairy cows. Research Report, Department of Botany, University of Pretoria.
- WINTERBACH, HANLIE & APPS, P.J. 1991. A gas-chromatographic headspace method for the determination of acetone in bovine milk, blood and urine. *Onderstepoort Journal of Veterinary Research*, 58:75–79.