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Short term changes in selected metabolites in pasture fed dairy cows during peak lactation

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ABSTRACT

The objectives of this study were to measure within day variation and day-to-day variation in selected metabolites in serum and milk samples obtained from animals in a herd of 20 Holstein-Friesian cows which grazed ryegrass/white clover pastures during peak lactation in October.

In the first trial, the 20 cows were milk and blood sampled at 0630 h, 1030 h and 1430 h on 3 October and at 0630 h on the following day. Average metabolite concentrations in serum were within the ranges indicated by a regional laboratory as being normal for healthy cattle, but significant differences were associated with sampling time and among individual animals within this range. Blood and milk urea nitrogen (BUN and MUN) and blood beta hydroxybutyrate (BOH) concentrations increased by 1.82 ± 0.14 , 1.37 ± 0.17 and 0.45 ± 0.04 mmol/l, respectively (mean change \pm sed) from the first 0630 h sampling to the 1430 h sampling. Glucose and non-esterified fatty acid (NEFA) levels decreased by 1.03 ± 0.06 and 0.13 ± 0.03 mmol/l, respectively during this period. Albumin remained constant.

Average daily concentrations for each of these metabolites, except NEFA differed significantly in samples obtained from 12 animals over a period of 21 days from the 7 to 27 October 1991. The largest proportional changes in daily concentration were in BUN and MUN (ranging from 4.7 to 7.5 mmol/l).

Sampling regimes which could be used to identify factors contributing to sample variation will require the same representative portion of a herd to be sampled at the same time at frequent (daily) intervals in the case of BUN and MUN. Either blood or milk may be sampled as the concentrations of urea N were similar (5.64 ± 1.05 (sd) and 5.70 ± 1.10 mmol/l, respectively) and strongly correlated ($R^2 = 96.6\%$). Daily sampling would be less critical for albumin, BOH, glucose and NEFA, but it should include the same animals being sampled at the same time of day.

Keywords: Metabolite, variability, dairy cow, pasture.

INTRODUCTION

The use of blood and milk metabolites which indicate the metabolic status of dairy herds have frequently been used in American and European studies to assess the effects of different feeding regimes on production and reproduction (Blowey *et al.*, 1973; Payne and Payne, 1970; Payne *et al.*, 1974; Rowlands, 1980; Rowlands *et al.*, 1974). Beta hydroxybutyrate (BOH) and non-esterified fatty acids (NEFA) have been suggested as indicators of tissue mobilisation and glucose as an indicator of energy status. Short term changes in protein status can sometimes be reflected by changes in blood and milk urea nitrogen (BUN and MUN) whereas albumin is associated with longer term changes (Rowlands, 1980; Payne and Payne, 1987). Published concentrations for each of these constituents are available for cows fed defined rations (Payne and Payne, 1987). Limited comparable data are available for late winter calving cows of high genetic merit offered a sole ration of pasture, partly because its nutrient composition cannot be controlled and may differ because of individual animals grazing selectiveness.

Several New Zealand reports with dairy cows (Fisher *et al.*, 1975; McClure, 1970; Moller, 1991; Williamson and Fernandez-Baca, 1992) have shown that the feeding value of a pasture can affect metabolite concentrations. The two most recent of these reports showed that BUN concentrations dur-

ing early or peak lactation exceed the average classified as normal by Ruakura Animal Health Laboratory (RAHL). These elevated concentrations may be associated with reduced milksolids production and reproductive performance, even though they were still within the range defined as normal by this laboratory. In contrast McClure (1968) suggested that lower than average concentrations of serum glucose in pasture fed cows were contributing to reduced productive and reproductive performances. None of these studies had systematically measured the extent of variation in metabolite concentrations associated with sampling time, sampling day or among the sampled animals.

Consequently, the objectives of the present study were to measure these sources of variation in pasture fed dairy cows during peak lactation and to identify suitable sampling regimes for future work.

MATERIALS AND METHODS

Trial 1

Blood and milk samples were obtained from each of 20 Holstein-Friesian cows at 0630 h, 1030 h and 1430 h on 3 October 1991 and at 0630 h the following day. These times were selected as approximately those times when samples are commonly taken for submission to a diagnostic laboratory ie.

the morning milking, mid-morning or the afternoon milking. The selected cows had an average breeding index (BI) of 130 and were in one herd which rotationally grazed 13 paddocks of permanent pasture of predominantly perennial ryegrass/white clover at a stocking rate of 3.8 cows/ha. Visual estimates of pre- and post-grazing herbage levels were used to calculate the average daily herbage intakes (kg DM/cow/day). The herd included four, two-year-old cows and 16 older cows.

Each blood sample (10 ml) was collected from a jugular vein after fore milk (30 ml) sampling. Blood samples were collected into plain glass tubes and allowed to clot at room temperature for 60–90 minutes before centrifuging at 2800 rpm for 15 minutes. This procedure was adopted following consultation with RAHL (R.S. Ellison *pers. comm.*). Serum was submitted for all metabolite analyses, as opposed to plasma, as this sample form is usually submitted by veterinarians after sampling cows in commercial herds. In addition, results from a concurrent trial which compared blood collection methods (S. McDougall *pers. comm.*) indicated no difference in bovine glucose concentrations in blood collected in a plain vacutainer compared to collection in a vacutainer containing 10 mg potassium oxalate and 12.5 mg sodium fluoride.

RAHL analysed each serum sample for urea (BUN), albumin, glucose, BOH and NEFA using reagent kits (Boehringer Mannheim; Auckland, New Zealand) with a Hitachi 717 auto-analyser. Fore milk samples were assayed for urea (MUN). Laboratory quality assurance reports indicated inter assay CV's of <5%.

Trial 2

Results from Trial 1 were used to select 12 of the 20 cows as having average BUN concentrations above (high) or below (low) the herd average. These cows were paired (one high and one low BUN cow per pair). Two pairs of cows were sampled for blood and milk every day at 0800 h (4 cows/day) from 7 October to 27 October. During the trial, every pair of cows was sampled with every other pair at least once and each pair of cows was sampled seven times. Any particular pair was sampled at a minimum interval of 2 days. These 12 cows remained as part of the original herd. This design was used to accommodate ethical issues involving repeated sampling from the coccygeal vein over an extended period. There were no other impositions on the normal management of the cows in this herd.

Metabolite concentrations were determined on all samples collected with the exception of NEFA during the first 9 days.

Statistical analysis and result interpretation

Changes in metabolite concentrations between each sampling time within day (Trial 1) were compared by t test as the data involved repeated measures of selected animals. Day to day variation was analysed using Genstat REML estimating the variance components for cow and time. Results are expressed as mean \pm se and the significance level is 5% unless expressed otherwise. Mean MUN and BUN concentration of individual cows in Trial 2 were compared using linear regression analyses.

The RAHL routinely provides a range in the concentrations of each metabolite as a guide to interpret the results. Each range has been derived by calculating the 95% confidence limits about the mean of samples specifically submitted from clinically normal New Zealand herds in the region, combined with values from the international literature.

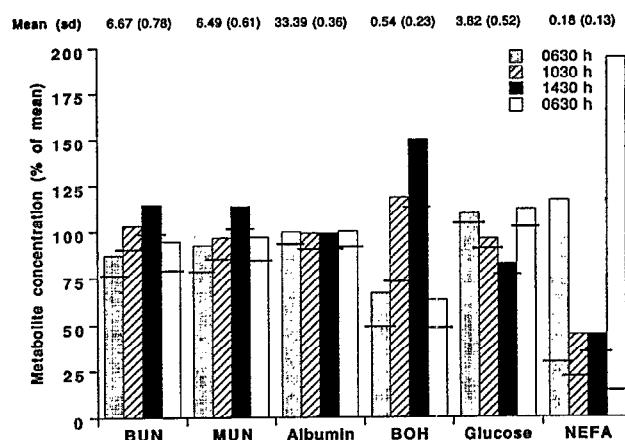
RESULTS

During Trial 1 and 2, the average daily production was 18.8 kg milk/cow/day (1.5 kg milksolids/cow/day). They also gained an average of 10.3 kg Iwt/cow. Pre- and post-grazing pasture levels were 2450 and 1340 kg DM/ha respectively and the average herbage intake was estimated at 13.6 kg DM/cow/day.

Trial 1

Average concentrations of BUN (7.65 mmol/l), MUN (7.38 mmol/l) and BOH (0.81 mmol/l) were highest in samples taken at 1430 h (Fig. 1) whereas glucose concentrations were lowest (3.15 mmol/l).

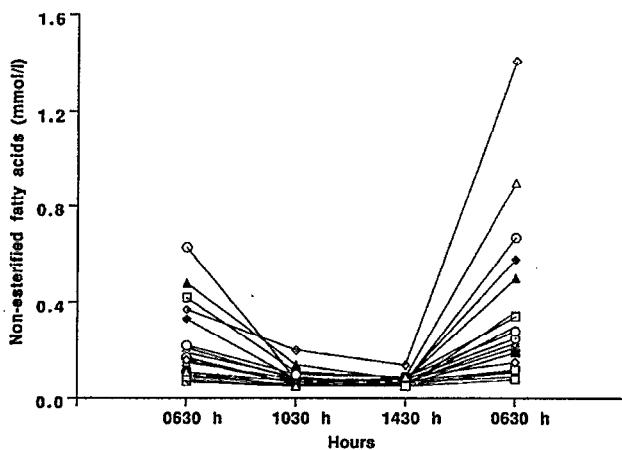
FIGURE 1: Proportional differences around the overall mean concentrations (indicated as 100%) of blood urea nitrogen (BUN) (mmol/l), milk urea nitrogen (MUN) (mmol/l), albumin (g/l), beta hydroxybutyrate (BOH) (mmol/l), glucose (mmol/l) and non-esterified fatty acids (NEFA) (mmol/l) for the respective mean concentrations of samples collected at 0630 h, 1030 h and 1430 h on 3 October and at 0630 h on 4 October as well as the standard deviation of each of these means expressed as a negative percentage (Trial 1).



The magnitude of the significant changes in BUN, MUN, BOH and glucose from 0630 h to 1430 h were 1.82 ± 0.14 , 1.37 ± 0.17 , 0.45 ± 0.04 , and 1.03 ± 0.06 mmol/l respectively, followed by significant changes of 1.32 ± 0.14 , 1.08 ± 0.23 , 0.47 ± 0.04 and 1.11 ± 0.08 mmol/l by 0630 h the next day. When this variation is expressed as a proportion of the respective mean concentrations, BUN, MUN, BOH and glucose concentrations changed by 27, 21, 83 and 27% between 0630 h and 1430 h (Fig. 1). Only average BUN concentrations differed significantly between the two 0630 h sampling times (5.84 vs 6.33 mmol/l). Significant differences among sampling times in concentrations of BUN, MUN, BOH and glucose occurred as well as consistent differences among the individual animals. None of the interactions be-

tween sampling time and individual animals was significant. Mean albumin concentrations did not change significantly between sampling times (Fig. 1). Although the mean NEFA concentration at the 0630 h sampling time was up to twice as great as the overall mean (Fig. 1), concentrations only varied by 0.1 mmol/l for 15 of the 20 animals (Fig. 2). The remaining five animals had elevated concentrations at both 0630 h samplings (0.33 to 0.63 and 0.34 to 1.41 mmol/l) but lower concentrations at 1030 h and 1430 h (0.07 to 0.2 mmol/l).

FIGURE 2: Changes in the concentrations of non-esterified fatty acids (NEFA) in serum (mmol/l) obtained from 20 cows at 0630 h, 1030 h and 1430 h on 3 October and at 0630 h on 4 October (Trial 1).



Trial 2

Mean levels of BUN, MUN, BOH, glucose, NEFA and albumin were 5.64 ± 1.05 (sd), 5.70 ± 1.10 , 0.34 ± 0.08 , 4.06 ± 0.25 , 0.18 ± 0.09 mmol/l and 33.14 ± 1.72 g/l, respectively.

TABLE 1: Mean concentrations of blood urea nitrogen (BUN), milk urea nitrogen (MUN), albumin, beta hydroxybutyrate (BOH), glucose and non-esterified fatty acids (NEFA) and estimated variance both among the 12 individual animals (Cow) and the 21 days of sampling (Day) as well as the unexplained variation (Error) (Trial 2).

	BUN mmol/l	MUN mmol/l	Albumin g/l	BOH mmol/l	Glucose mmol/l	NEFA mmol/l
Mean	5.64	5.70	33.14	0.34	4.06	0.18
Cow	0.70	0.74	1.90	0.0013	0.018	0.06
Day	0.32	0.37	0.38	0.0018	0.016	0.02
Error	0.19	0.20	0.62	0.0038	0.033	0.06

Sample time and individual cow metabolite differences were both significant sources of variation (Table 1). This is illustrated in Fig. 3 by comparing the minimum and maximum concentrations of each metabolite for averages for individual days or cows.

Daily averages for each metabolite except NEFA varied significantly from day-to-day over 3 weeks of sampling. The daily patterns for BUN and MUN are shown in Fig. 4. The maximum changes in BUN and MUN on consecutive days during the 3 weeks were 1.90 and 2.24 mmol/l respectively, which were equivalent to changes of 34 and 40% of the respective means (Fig. 4).

FIGURE 3: Proportional differences around the overall mean concentrations (indicated as 100%) of blood urea nitrogen (BUN) (mmol/l), milk urea nitrogen (MUN) (mmol/l), albumin (g/l), beta hydroxybutyrate (BOH) (mmol/l), glucose (mmol/l) and non-esterified fatty acids (NEFA) (mmol/l) for the respective minimum and maximum concentrations of each metabolite for averages for individual days or cows in samples collected daily from 7 October to 27 October (Trial 2).

Mean (sd) 5.64 (1.05) 5.70 (1.10) 33.14 (1.72) 0.34 (0.08) 4.06 (0.25) 0.18 (0.09)

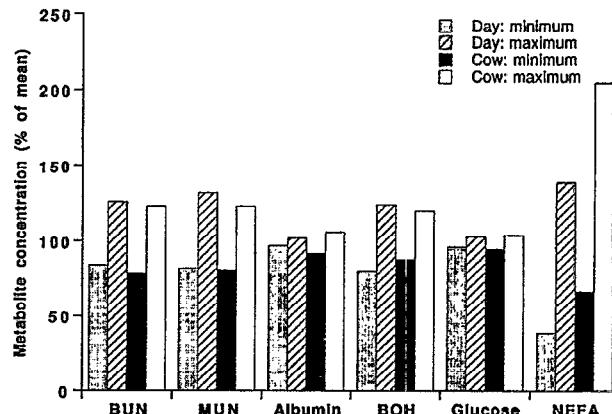
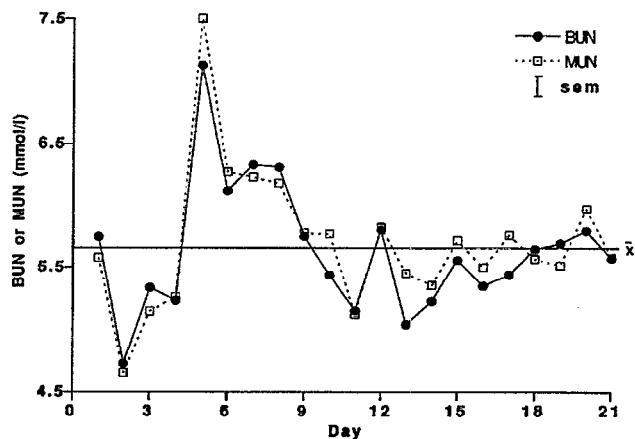


FIGURE 4: Average daily concentrations of blood urea nitrogen (BUN) (mmol/l) and milk urea nitrogen (MUN) (mmol/l) in samples collected from 7 October (Day 1) to 27 October (Day 21).



BOH, albumin and glucose concentrations were less variable with the greatest changes on days 7, 20, and 16, respectively.

Mean concentrations of BUN and MUN of individual cows were closely correlated ($R^2 = 96.6\%$, rse = 0.15; $P < 0.0001$) and described by the following regression equation:

$$\text{BUN} = 0.28 + (0.94 \text{MUN}) \text{ (Fig. 4)}$$

The coefficients of variation for BUN, MUN, albumin, BOH, glucose and NEFA were 19.5, 20.1, 5.1, 24.3, 6.4 and 2.1%, respectively.

Average concentrations for each metabolite varied significantly between animals with similar ranges from the minimum to maximum averages to those recorded for the daily averages (Fig. 3). Animals selected in the group with a high BUN concentration remained significantly higher (BUN: 1.26 ± 0.33 mmol/l sed, MUN: 1.35 ± 0.31 mmol/l sed) than animals in the low urea level group throughout the sampling period. There were no sampling day x group interactions.

DISCUSSION

The BUN concentrations measured in this group of cows during October 1991 confirm the field observations of Moller (1991) and Williamson and Fernandez-Baca (1992) that high levels can occur in spring. However, the glucose levels obtained were higher than those reported by McClure (1970). The interpretation of field results may not always take account of the significant variation identified in Trial 1 and 2 in relation to time of sampling during the day, differences among sampling days or the significant variation among animals even after taking account of sampling time and sample day.

The results from the sampling schedule used in Trial 1 demonstrated that metabolite concentrations change significantly throughout the day, with the exception of albumin (Fig. 1). These time effects for individual animals were comparatively consistent except for NEFA (Fig. 2). In this case the difference in mean concentrations were mainly associated with the greater variability in samples from five of the 20 animals at both of the 0630 h samplings (Fig. 2).

The results indicate that a selected group of animals should be sampled at a standard time when measuring changes in BUN, MUN, BOH and glucose over several days. This decision will also influence the variation in sample concentrations in NEFA due to the differing pattern of change amongst individual cows during the day. Sampling time is less critical for measuring albumin but the same animals should be sampled each day. Changes in the concentrations of some of these metabolites during the day have been reported by others (Allcroft, 1933; Bowden, 1971; Coggins and Field, 1976; Fisher *et al.*, 1975; Hove and Blom, 1973). The changes of 27, 83 and 27% in the mean concentrations of BUN, BOH and glucose, respectively in Trial 1, were greater than the post-feeding metabolite variation reported by Coggins and Field (1976). They found BUN and ketone bodies concentrations increased 16 and 38% and glucose decreased 11%. The relative importance of this source of variation has been debated (Manston *et al.*, 1981; Payne and Payne, 1987) and appears to largely depend on the amount and frequency of feeding. This emphasises the need to select a standardised sampling time within a management system where feeding and milking times are not varied.

All metabolites except NEFA varied significantly from day to day, especially in BUN, MUN and BOH concentrations (Fig. 3, 4). Daily changes were often rapid. For example the lowest and the highest daily mean BUN/MUN concentrations during the 3 week sampling period occurred over 3 days and represented a change of 43 and 50% of the overall mean BUN and MUN values. These two parameters followed similar patterns of daily change and their mean concentrations did not differ significantly. A similar relationship between individual cows has also been observed by others (Ide *et al.*, 1966; Journet *et al.*, 1975; Oltner and Wiktorsson, 1983). Daily fluctuations in albumin and glucose were significant, but less than BUN and MUN.

The causes of the day-to-day variation in metabolites could not be identified. Rowlands (1980) briefly suggested potential influences as being fluctuations in diet composition, changes in health, changes in weather and analytical variation

which can occur from day to day. Comparable studies have not focused on interactions between these factors among cows under conditions involving the rotational grazing of pasture. Some effects of weather may be short term with adverse weather resulting in lower herbage intakes; or long term if pasture growth, digestibility and carbohydrate or protein contents were influenced. These interactive environmental factors appeared to influence concentrations of BUN and MUN to a greater extent than the other metabolites and the corresponding changes in metabolite concentration were sometimes rapid (Fig. 4). McClure (1968), and recently Moller (1991) and Williamson and Fernandez-Baca (1992) have suggested that pasture nutrient content has affected blood metabolite concentrations and consequently herd fertility. These field trials reported that the higher BUN concentrations were associated with pasture of a higher protein content. Our results indicate that frequent sampling of the same animals at a set time will best reflect these concentration changes. The fluctuations in BUN over time emphasise the need for extended periods of sampling to look at long term trends.

The potential for using a bulk milk sample from the farm milk vat for measuring corresponding concentrations of blood metabolites, whether it be for monitoring disease incidence in the herd or as an indicator of nutrition, is indicated by the close relationship between BUN and MUN (Fig. 4) and needs to be developed. This composite sample would meet the requirements of sampling the whole herd at a standard time and at frequent intervals by an easy, non-invasive method.

BUN, MUN and BOH had larger coefficients of variation than those for albumin, glucose and NEFA, presumably reflecting the difference in degree of homeostatic control exerted by the cow. A large proportion of BUN and MUN variation was due to differences between sampling days as well as among cows, whereas random error made a large contribution to variability in BOH (Table 1). These proportions of variance may only be indicative of the patterns in a normal population as the 12 cows sampled over 3 weeks were selected as having above or below average concentrations of BUN among the original herd of 20 cows. Comparing the variance components of the population sample of 20 cows used in Trial 1 with those for the 12 selected cows in Trial 2 indicated that the between cow variance component may have been overestimated by approximately 10% in the case of BUN, MUN, glucose and NEFA and underestimated in the case of albumin and BOH.

In the present trials, only 5% of the samples had a concentration outside of the respective ranges for each of the metabolites, classified by the RAHL. There were significant time and animal differences within these ranges. BUN and MUN concentrations in the samples from the animals in Trial 2 were within the range classified as 'normal' by RAHL (3.3 - 8.1 mmol/l c.f. 2.7 - 12.3 mmol/l). Albumin levels were similar (29 - 36 g/l c.f. 25 - 40 g/l), BOH levels at the lower end of the range (0.2 - 0.6 mmol/l c.f. 0.2 - 1.0 mmol/l), and glucose at the higher end of the range (3.47 - 4.62 c.f. 2.5 - 4.1). NEFA levels ranged from 0.09 - 0.43 mmol/l but no normal range was indicated by RAHL. The standardised sampling time of 0800 h used in Trial 2 would have been earlier than when most field samples would be collected.

Given the metabolite concentration changes observed during the day in Trial 1, this difference in sampling time could contribute to differences between observed metabolite levels and the range of concentrations classified as normal by RAHL.

In conclusion, the results from this study indicate significant variability within day and from day-to-day in most of the blood metabolites which were measured. Future trials designed to identify the effects of management on blood metabolites should incorporate sampling of specific animals in a herd on consecutive days at a standard time each day.

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