

Intraday variation of metabolic key indicators in serum of dairy cows between week 2 antepartum and week 12 postpartum

S. WIEDEMANN¹, K. HORSTMANN², M. PIECHOTTA², U. MEYER³,
G. FLACHOWSKY³, M. KASKE²

¹Institute of Animal Breeding and Husbandry, Christian-Albrechts-University Kiel, Kiel, Germany

²Clinic for Cattle, University of Veterinary Medicine, Hannover, Germany

³Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute of Animal Health, Braunschweig, Germany

ABSTRACT: Metabolic diseases during early lactation in dairy cows can be routinely diagnosed assessing key indicators in blood. The objectives of the present study were to characterize the impact of interindividual along with intraday variation on specific metabolites and to investigate the effect of the sampling time point relative to calving. Serum samples of four high-yielding, clinically healthy, multiparous dairy cows (body weight 589 ± 27 kg) were obtained in 3-h intervals during 24-h intervals throughout the transition period and early lactation (week -2 antepartum (ap), weeks 1, 2, 3, 5, 7, and 12 postpartum (pp)). The lowest intraday variation (less than 15%) as indicated by relative coefficients of variation (CV) was found for glucose, cholesterol, and aspartate aminotransferase (AST). Intraday variation characterized by a CV between 15 and 30% was typical of urea, β -hydroxybutyrate (BHB), total bilirubin, and non-esterified fatty acids (NEFA). The highest intraday variation (CV > 30%) was assessed for insulin. Week relative to calving had significant influence on interindividual means of BHB, NEFA, insulin, and cholesterol in blood, but did not affect the interindividual variation of all parameters investigated. No significant intraday variation patterns were found. It is concluded that the considerable intraday variation of especially BHB and NEFA has to be taken into account in herd health monitoring for estimating the proportional outcome in respect to animals exceeding thresholds for specific metabolic key parameters.

Keywords: blood metabolites; lactation; twenty-four-hour pattern

Ketosis, fatty liver, and a concomitant lipomobilization syndrome are often the consequence of the metabolic challenge in high-yielding dairy cows in the first weeks of lactation (Herdt, 2000; Bobe et al., 2004). In the case of metabolic disturbances, the risk of further production diseases (e.g. displaced abomasum, metritis, and mastitis) is considerably increased (Cameron et al., 1998; Duffield, 2000).

Monitoring herds for metabolic diseases by assessing metabolic key indicators in the blood or milk of cows at risk is an established tool in herd medicine (e.g. reviews by Oetzel, 2004; LeBlanc,

2010). Thereby, high risk herds can be identified and by appropriate management the economic impact of a high incidence of subclinical diseases can be avoided (e.g. loss of peak milk production, reduced fertility performance, and increased risk of periparturient diseases).

However, metabolic parameters used in herd medicine are known to be influenced considerably by the stage of lactation, feed intake, milk yield, and individual factors. Accordingly, there is a risk of misinterpretation of single values which is reduced in practice by collecting blood from many animals (Oetzel, 2004).

Unfortunately, the intraday variation of respective parameters under common feeding conditions is often unclear and not taken into consideration by veterinarians. Knowledge on variation, on the other hand, represents a prerequisite to identify robust parameters minimally affected by external factors, to assess the significance of a single value of each parameter of an individual animal, and, finally, to calculate the number of analyzed samples being needed to get valid information about the status of a herd.

Thus, it was the objective of this study (*i*) to quantify the interindividual intraday variation of metabolic key parameters, and (*ii*) to investigate whether the intraday variation is affected by the sampling time point relative to calving.

MATERIAL AND METHODS

Experiments were approved by the Animal Welfare Committee of Lower Saxony (regulation No. Az. 2 509.42502/09-A-03.02) and were carried out at the experimental station of the Institute of Animal Nutrition of the Friedrich-Loeffler-Institute (Germany).

Animals

Four multiparous Holstein-Friesian cows (body weight 589 ± 27 kg (mean \pm SD), one animal in the 2nd, two animals in the 3rd, and one animal in the 6th lactation, milk yield during last lactation 8901 ± 766 kg) were used. During the dry period, the cows were housed together with 28 other cows in a conventional free stall with 29 cubicles and a concrete slatted floor. After calving, cows were moved into a comparable pen with the same stocking density. Cows were milked twice daily at 6:00–8:00 h and 15:00–18:00 h in a 2 \times 5 tandem milking parlour.

Feeding

Dry cows were fed a mixture of corn silage and grass silage at 9.30 h (65 : 35 w/w; 5.8 MJ NEL/kg DM, 120 g crude protein (CP)/kg DM, 233 g crude fibre (CF)/kg DM) intended to offer *ad libitum* intake. Feed was pushed in front of cows at approximately 11:30, 14.30, and 18:30 h. A total of 2 kg concentrates per day offered at automatic feeding stations (0.2 kg of concentrate I (DM 89.4%,

7.8 MJ NEL/kg DM, CP 228 g/kg DM) and 1.8 kg of concentrate II (DM 88.8%, 8.3 MJ NEL/kg DM, CP 252 g/kg DM)).

After calving, cows were fed a mixture of corn silage and alfalfa silage (65 : 35 w/w; 6.3 MJ NEL/kg DM, CP 127 g/kg DM, CF 214 g/kg DM). Food was freely available in single feeding troughs which were filled at 9.30 h with 50 kg of fresh feed (wet weight). Within the first week after calving, an additional 1 kg concentrate I and 4 kg of concentrate II were offered per day at automatic feeding stations. In the following weeks the amount of concentrate II was adjusted twice weekly to the individual milk yield (0.5 kg/kg FCM) for the milk yield above 10 kg/day. The daily amount of concentrate II was available for the cows in eight portions, i.e. in 3-h intervals. As a result, cows' consumption of concentrates at sampling time points during weeks 1, 2, 3, 5, 7, and 12 postpartum was 5.1 ± 0.2 , 6.9 ± 0.4 , 9.4 ± 0.3 , 11.7 ± 0.2 , 12.0 ± 0.15 , and 11.5 ± 0.15 kg, respectively (mean \pm SE). In each pen, two water troughs provided free access to water.

Blood sampling

Intraday patterns of blood parameters were assessed seven times in each of the four cows two weeks antepartum (ap) and in weeks 1, 2, 3, 5, 7, and 12 postpartum (pp).

At 7.30 h of the respective day, a clinical investigation was carried out (Dirksen et al., 2012) revealing an undisturbed general condition. Thereafter, blood samples were collected by puncture of the jugular vein (1.5 mm) (Braun, Melsungen, Germany) in 3-h intervals over a period of 24 h starting at 8:00 h after fixing the animal with a halter. Prior to these experiments, animals were accustomed to fixing and handling regularly. Therefore, all blood samples were taken with minimal disturbance of the cow's well-being. Blood was drawn into disposable tubes containing EDTA and sodium fluoride as anti-coagulant for plasma collection, respectively. Blood for serum analysis was collected into a tube containing clot activator. Within 4 h after collection serum was obtained after centrifugation at 1700 g for 15 min. Aliquots were stored at -20°C until analysis.

Blood analyses

An automatic analyser Cobas Mira Plus[®] (Roche Diagnostics, Basel, Switzerland) was used to assess

the serum activity of aspartate aminotransferase (AST) (A11A00017; ABX Diagnostics, Montpellier, France) and the serum concentrations of β -hydroxybutyrate (BHB) (RB 1008; Randox Laboratories GmbH, Krefeld, Germany), cholesterol (401-25P; Sigma Diagnostics, Deisenhofen, Germany), glucose (A11A00116; ABX Diagnostics, Montpellier, France), non-esterified fatty acids (NEFA) (99475409; Wako Chemicals GmbH, Neuss, Germany), total bilirubin (550-A; Sigma Diagnostics, Deisenhofen, Germany), and urea (A11A00075; ABX Diagnostics, Montpellier, France). The within-run precision ($n = 18$) calculated as coefficients of variance were 1.4, 7.4, 2.1, 4.0, 2.4, 3.0, and 3.1% for AST, BHB, cholesterol, glucose, NEFA, total bilirubin, and urea, respectively.

Serum insulin was quantified applying a commercial radioimmunoassay (Insulin-RIA; Biermann, Bad Nauheim, Germany) based on the double antibody method according to Kaske et al. (2001b). Each sample was measured in duplicates.

Statistical analysis

Statistical evaluation of the data was performed using SAS software (Statistical Analysis System, Version 8.2, 2001). Normal distribution of data was checked. Means and standard deviations (SD) of respective value were calculated with all values of all animals within one sampling point. The interindividual variation of the nine single values obtained over each period of 24 h in each cow was expressed as the relative coefficient of variation (CV) calculated as $CV = SD/mean \times 100$. Means

of CV were calculated over all animals for each sampling point. Differences between weeks relative to calving were checked by One-Way Repeated Measures ANOVA. P -values of < 0.05 were considered as significant.

RESULTS

Cows' intraday means of serum glucose concentrations did not vary significantly during late pregnancy (week 2 ap) and later stages of lactation (week 12 pp) displaying levels of 3.3 mmol/l (Table 1). However, in the 5th week pp, a nadir of 2.8 ± 0.4 mmol/l was found. Interindividual CVs obtained by calculating the variation within 24 h intervals did not depend on week relative to calving ($P = 0.79$) (Table 2). Furthermore, the sampling time of the respective day had no effect on values ($P = 0.41$).

The mean intraday levels of β -hydroxybutyrate (BHB) serum concentrations across all animals did not depend on week relative to calving (Table 1). Interindividual variation was not affected by the week relative to calving; the respective CV varied between 14 and 27% (Table 2). Time of sampling during the day had no influence on results ($P = 0.13$).

Week relative to calving had a significant effect on mean non-esterified fatty acids (NEFA) levels within 24 h, as NEFA markedly increased after parturition from 232 μ mol/l at week -2 ap compared with values of 773 μ mol/l (week 1 pp; $P = 0.02$) and decreased later on to 139 ± 32 μ mol/l (week 12 pp) (Table 1, Figure 1A). The interindividual

Table 1. Mean serum concentrations (\pm SD) for glucose, β -hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), insulin, cholesterol, total bilirubin (TB), aspartate aminotransferase (AST), and urea of four monitored cows

Week (ap/pp)	Glucose (mmol/l)	BHB (mmol/l)	NEFA (μ mol/l)	Insulin (mU/l)	Cholesterol (mmol/l)	TB (μ mol/l)	AST (U/l)	Urea (mmol/l)
-2	3.3 ^a \pm 0.1	0.63 ^a \pm 0.06	232 ^{abc} \pm 68	3.5 ^{ab} \pm 2.2	2.4 ^a \pm 0.4	2.9 ^{ab} \pm 0.4	27.1 ^a \pm 2.5	4.1 ^a \pm 1.1
1	3.1 ^{ab} \pm 0.6	0.67 ^a \pm 0.21	773 ^d \pm 163	1.7 ^b \pm 0.4	2.0 ^a \pm 0.3	7.1 ^c \pm 3.9	43.3 ^b \pm 8.2	3.0 ^b \pm 1.7
2	3.1 ^{ab} \pm 0.4	0.65 ^a \pm 0.30	722 ^{de} \pm 558	3.0 ^{ab} \pm 0.3	2.1 ^a \pm 0.4	6.1 ^c \pm 3.1	50.6 ^c \pm 1.7	2.3 ^b \pm 0.8
3	3.2 ^{ab} \pm 0.2	0.53 ^a \pm 0.10	397 ^{bc} \pm 134	6.9 ^{ac} \pm 1.2	2.5 ^a \pm 0.3	2.1 ^{ad} \pm 0.3	45.8 ^{bc} \pm 8.5	2.3 ^b \pm 0.3
5	2.8 ^b \pm 0.5	1.41 ^b \pm 0.60	355 ^{cc} \pm 157	4.8 ^a \pm 2.6	3.3 ^b \pm 0.5	3.1 ^b \pm 0.2	40.1 ^b \pm 4.8	2.7 ^b \pm 1.0
7	3.1 ^{ab} \pm 0.2	0.68 ^a \pm 0.26	178 ^a \pm 59	9.2 ^c \pm 1.3	3.9 ^b \pm 0.7	1.8 ^d \pm 0.3	37.3 ^b \pm 6.2	3.6 ^a \pm 0.8
12	3.3 ^a \pm 0.1	0.58 ^a \pm 0.26	139 ^b \pm 32	8.1 ^c \pm 2.5	4.9 ^c \pm 0.8	2.5 ^{abd} \pm 0.4	39.2 ^b \pm 6.9	2.1 ^b \pm 1.2

ap = antepartum, pp = postpartum

^{a-e} within a column, different superscripts indicate significant differences ($P < 0.05$) between the weeks investigated

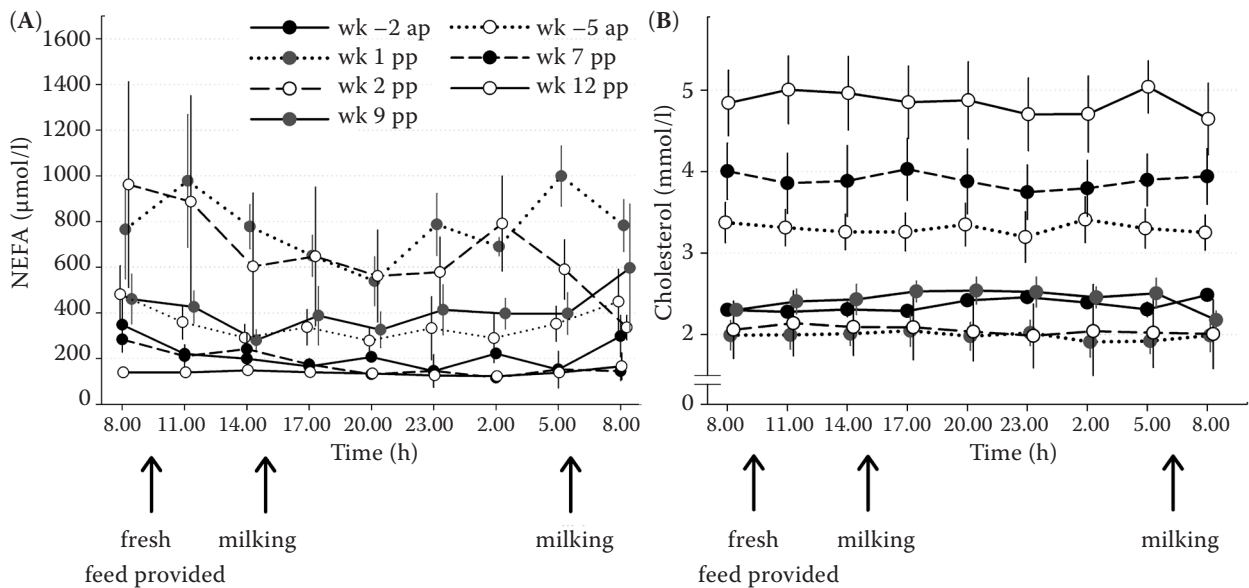


Figure 1. Serum concentrations of non-esterified fatty acids (NEFA) (A) and serum cholesterol concentrations (B) of four cows during a 24-h period assessed in different weeks before and after calving (means \pm SE)

wk = week, ap = antepartum, pp = postpartum

CV peaked at week 5 pp ($37.9 \pm 13.9\%$) and was the lowest at week 12 pp ($12.5 \pm 5.8\%$). During the remaining time points CVs differed between 25.9 ± 12.2 and $35.6 \pm 2.3\%$ (Table 2). However, no significant influence of specific time points on results during the day could be measured due to the marked variation in NEFAs particularly during the first two weeks of lactation ($P = 0.44$) (Figure 1A).

Mean insulin concentrations were the lowest at week 1 pp (1.7 ± 0.4 mU/l) as compared to values of the remaining time points reaching values of 9.2 ± 1.3 and 8.1 ± 2.5 mU/l at weeks 7 and 12 pp, respectively (Table 1). Each animal showed individual insulin concentration patterns resulting in inconsistent interindividual patterns. The interindividual CV varied between 29% (week 12 pp) and 74% (week -2 ap) (Table 2). No clear effect of sampling time point was evident ($P = 0.30$).

The dependency of cholesterol values on week relative to calving is reflected by lower values before parturition and during the first three weeks of lactation (2.0 ± 0.3 – 2.5 ± 0.3 mmol/l) as compared to the subsequent increase during the following weeks (3.3 ± 0.5 – 4.9 ± 0.8) (Table 1, Figure 1B). However, the interindividual CV varied at a low level between 3 and 7%. No significant fluctuation of results was found in regard with time of day ($P = 1.0$) (Figure 1B).

Sampling time relative to calving had a significant influence on total bilirubin serum concentra-

tions as the highest values were measured during weeks 1 and 2 pp (7.1 ± 3.9 and 6.1 ± 3.1 μ mol/l, respectively) as compared to values obtained during the remaining time points (1.8 ± 0.3 – 3.1 ± 0.2 μ mol/l) (Table 1). Interindividual values ranged between $17.4 \pm 3.1\%$ (week -2) and $31.5 \pm 4.9\%$ (week 7 pp) (Table 2) displaying no effect of sampling time point relative to calving. Also, values did not exhibit significant variation between the respective time points ($P = 0.43$).

Serum activity of AST varied almost 2-fold between 27.1 ± 2.5 and 50.6 ± 1.7 U/l during week -2 ap and week 2 pp, respectively (Table 1) demonstrating a significant effect of sampling time point relative to calving as the lowest concentrations were measured before parturition. The variation within the 24 h intervals was low with CV values between 3 and 10% (Table 2). The sampling time of the day had no effect on measured AST levels ($P = 0.98$).

The concentrations of urea varied up to 2-fold between 2.1 ± 1.2 and 4.1 ± 1.1 mmol/l at week 12 pp and during late pregnancy (week 2 ap), respectively (Table 1). The variation between the animals throughout the trial periods was reflected in CVs ranging between 12.2 and 48.9%. Variability within the animals was found to vary between 10.9 ± 1.8 and 24.0 ± 11.9 , but was not influenced by sampling time point relative to calving. Concentrations did not depend on sampling time of the day, too ($P = 0.84$).

Table 2. Means (\pm SD) of individual coefficients of variations ($n = 4$) of serum concentrations of metabolic key parameters (glucose, β -hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), insulin, cholesterol, total bilirubin (TB), aspartate aminotransferase (AST), urea) calculated from nine serum samples collected in 3-h intervals within 24 h

Week ap/pp	Glucose	BHB	NEFA	Insulin	Cholesterol	TB	AST	Urea
-2	6.2 \pm 2.4	15.8 \pm 0.8 ^a	25.9 \pm 12.2 ^{ab}	73.8 \pm 5.1 ^a	3.8 \pm 0.8 ^a	17.4 \pm 3.1	4.7 \pm 1.3 ^{ab}	10.9 \pm 1.8
1	6.9 \pm 0.3	15.4 \pm 3.4 ^a	27.2 \pm 3.5 ^{ab}	38.9 \pm 2.9 ^{bc}	3.4 \pm 0.4 ^a	23.7 \pm 5.7	8.2 \pm 1.9 ^a	19.5 \pm 3.5
2	8.3 \pm 4.3	14.1 \pm 7.5 ^a	30.0 \pm 12.9 ^a	44.0 \pm 15.8 ^b	4.7 \pm 1.8 ^a	26.3 \pm 10.6	6.7 \pm 5.7 ^{ab}	18.1 \pm 6.3
3	7.6 \pm 1.4	16.8 \pm 4.2 ^{ab}	35.6 \pm 2.3 ^a	35.1 \pm 11.9 ^c	6.7 \pm 2.3 ^b	28.6 \pm 2.5	10.6 \pm 1.3 ^c	17.5 \pm 5.4
5	7.3 \pm 3.7	26.9 \pm 3.1 ^c	37.9 \pm 13.9 ^a	51.8 \pm 9.2 ^b	3.8 \pm 1.5 ^a	20.6 \pm 9.4	7.9 \pm 3.6 ^a	24.0 \pm 11.9
7	5.7 \pm 0.8	19.0 \pm 5.7 ^b	32.8 \pm 12.1 ^a	35.9 \pm 8.7 ^c	3.5 \pm 0.5 ^a	31.5 \pm 4.9	3.2 \pm 0.4 ^b	13.6 \pm 6.0
12	6.4 \pm 1.6	23.1 \pm 4.3 ^{bc}	12.5 \pm 5.8 ^b	29.1 \pm 10.3 ^c	3.7 \pm 0.8 ^a	23.6 \pm 3.8	2.8 \pm 0.3 ^b	11.5 \pm 2.9
Mean of all weeks	7	19	29	44	4	25	6	16

ap = antepartum, pp = postpartum

^{a-c} within a column, different superscripts indicate significant differences ($P < 0.05$) between the weeks investigated

DISCUSSION

The objective of the study was to characterize the significance of metabolic parameters analyzed from a single blood sample and to survey the influence of sampling time point relative to calving on intraday variation of metabolites. Clearly, the methodological approach chosen was not appropriate to investigate circadian, diurnal, or ultradian patterns of the metabolites; respective investigations need much shorter intervals between subsequent samples. However, the intervals of 3 h between the samples represent a compromise between minimal disturbance of the normal behaviour of the cows in the herd and a maximal number of samples per day. A further prerequisite was the customization of the cows to the sample collection procedure, i.e. that several samples could be collected from a lying, ruminating animal. Thereby, an effect of stress due to restraint and venipuncture could be prevented which is known to affect blood concentrations of corticosteroids, prolactin, and growth hormone (Trenkle, 1978). Accordingly, irrespective of a limited number of cows, the results of this study provide a reliable sketch about the validity of single samples under typical feeding and housing conditions of dairy producers.

Levels of metabolic key parameters throughout the transition period and early lactation indicated a substantial lipomobilization around calving and were comparable to those reported by others (Aeberhard et al., 2001a, b; Reist et al., 2002).

The interindividual variation of the metabolic key parameters was obviously not affected by the

sampling time point relative to calving. At any time before and after calving, the interindividual variation of the respective parameters was quite similar. However, between the parameters, considerable differences were obvious with consequences for their potential usefulness in herd health programs.

Concentrations of glucose and cholesterol as well as AST activity varied throughout day and night only to a small extent ($CV < 10\%$).

With respect to glucose, the minimal variation is a consequence of the effective regulation by many hormones. In contrast to our study, an increase of plasma glucose concentrations was demonstrated in dairy cows during the night (Andersson and Lundstrom, 1984; Blum et al., 2000); a postprandial decrease was found after feeding in the morning as a consequence of enhanced insulin secretion and insulin-stimulated tissue uptake of plasma glucose (Frohli and Blum, 1988). However, no significant differences of glucose blood concentrations were found when comparing high-yielding and low-producing dairy cows at the same sampling time point relative to calving (Aeberhard et al., 2001b). Obviously, the adaptive capacity of high-producing cows is not significantly impaired compared to low-producing cows (Beerda et al., 2004). Thus, highly effective homeostatic mechanisms allow keeping blood glucose concentration at a normal concentration even during nutritional imbalance (Farin and Slenning, 2001) and metabolic challenges. Metabolic profiling has been largely unrewarding using glucose. Accordingly, the parameter has limited value for herd health monitoring because the animals' basic homeostatic mechanisms try

to hold blood parameters at normal even during severe nutritional imbalance (Farin and Slenning, 2001). A severe hypoglycemia is often found in ketotic cows, however, most of these patients exhibit clinical symptoms which can be easily recognized even without analyzing blood glucose concentration (Kaske et al., 2001a).

Cholesterol represents the second parameter with minimal intraday variation. Its homeostasis in dairy cows is almost unknown. Feed of ruminants contains negligible amounts of cholesterol. Despite of that, based on a mean cholesterol content of 200–300 mg/100 g milk fat (Precht, 2001), as much as 5 g cholesterol are released by the bovine mammary gland per day. Comparable to Guretzky et al. (2006), blood cholesterol concentration increased from 1–3 mM during the first weeks of lactation up to 6–7 mM reached roughly 8 weeks pp (Table 1). Interestingly, hepatic production seems to be rather unaffected by a discontinuous availability of precursors. In agreement with our results, even in more detailed studies a circadian pattern could not be demonstrated (Bitman et al., 1990). In cows suffering from production diseases, on the other hand, very low cholesterol concentrations are found. From this point of view, cholesterol is a very useful parameter for herd health monitoring.

The AST activity was the third parameter with only little intraday variation. This may be due to the relatively long half-life of this cytosolic enzyme (in humans 12–24 h) (Friedman, 2012). In the case of hepatocellular injury or necrosis, AST leaks out of the hepatocytes into the serum. However, AST is found in a wide variety of tissues, i.e. in contrast to glutamate dehydrogenase (GLDH) it is not a liver-specific enzyme.

Urea, NEFA, BHB, and total bilirubin are widely used parameters in herd medicine with a considerable higher intraday variation (CV 10–30%) compared to the latter parameters. Due to the results presented here, no significant effect of the time of the day was found, which is in contrast to other studies: gold standard for diagnosis of ketosis is blood BHB (Oetzel, 2004). Blood BHB has been found to increase after feeding due to ruminal production of butyric acid (Blum et al., 2000). In lactating cows, BHB concentrations more than doubled after feeding in the morning between 7.00 and 11.00 h (Manston et al., 1981).

The NEFAs are considered as indicators of lipomobilization and thereby a negative energy balance predominantly before calving. The concentration

has been described to rise during the night due to reduced energy intake and decrease after the morning feeding indicating reduced lipolysis and enhanced tissue NEFA uptake under the influence of increased circulating insulin levels (Blum et al., 2000). Thereby, NEFA concentrations should reach their nadir 4–5 h after feeding and peak just before the next feeding. Results presented here indicate that such changes do not occur when a mixed ration is fed for *ad libitum* intake once per day.

Plasma urea concentrations are influenced by the dietary protein and energy intake (Clement et al., 1991; Aeberhard et al., 2001b) or, more precisely, by the protein-energy relation (Oltner and Wiktorsson, 1983). In contrast to our study, a rise of urea concentration was found in the morning in cows fed a total mixed ration *ad libitum* (Lefcourt et al., 1999; Blum et al., 2000). The intraday variation was, however, comparable to data of the present study.

Interestingly, intraday variation of total bilirubin was unexpectedly high. In healthy cows, the concentration is rather low and increases only to a small extent even in severe liver disease. However, as NEFA and bilirubin compete with the transporter across the sinusoidal cell membrane into the hepatocyte, bilirubin increases during lipomobilization. As the NEFA concentrations varied within the day considerably, this explains in part the intraday variation of total bilirubin.

Insulin was found to vary to the largest extent during the day as indicated by a CV of > 40%. No consistent circadian patterns have been found for the secretion of insulin (Trenkle, 1978), but ultradian oscillations have been reported to occur in ranges of 2, 10–15, and 100–150 min intervals (Sturis et al., 1991). Insulin levels vary in direct relation to feeding in most of the studies with cattle (Hayirli, 2006). Two peaks in insulin concentrations were found in cows associated with twice daily feeding (Hove and Blom, 1973). Blum et al. (1985) also described peaks in insulin concentration 2–3 h after twice daily feedings.

The previously reported circadian patterns for some of the metabolites investigated were not detected in the present study. This may be partly due to the relatively long intervals between consecutive samples. In addition, the feeding regime has considerable impact. On the one hand, even if a mixed ration is fed *ad libitum*, dairy cows exhibit typical patterns of feed intake characterized by a high intake directly after providing the new feed and another peak roughly 12 h later

(Bitman et al., 1990). This should be reflected by typical concomitant changes of at least BHB, NEFA, and insulin. However, due of the metabolic demands of lactation, high-yielding cows usually eat more constantly than non-lactating animals as long as feed is available (Journet and Remond, 1976; Grummer et al., 2004). Furthermore, environmental conditions, social rank of individual cows in the herd, bunk space available per cow, and milking regime interact with patterns of feed intake. This may explain that significant intraday differences are relatively small. Thus, collection of blood samples for herd health programs may take place at any time of the day.

In herd medicine, the proportion of cows above or below a biologic threshold value (cut point) is required to assess the significance of a specific production disease for the herd of the producer (Oetzel, 2004). For example, a BHB serum concentration of 1.4 mmol/l is considered as an appropriate cut point for subclinical ketosis (Duffield, 2000). For practical monitoring programs, for each key parameter a respective alarm level is implemented. If, for example, more than 10% of the cows tested exceed a blood BHB concentration of 1.4 mmol/l, subclinical ketosis (SCK) is recognized as a herd problem requiring counteractions. The mean BHB concentration of the tested animals, on the other hand, is biologically almost irrelevant. The high intraday variation of BHB, NEFA, and urea represents a clear disadvantage for the monitoring process as a cow with a BHB concentration of, for example, 1.0 mmol/l is not necessarily fine but may be above the alarm level at another time of the day. To minimize the risk of blood monitoring results misinterpretation, the number of tested cows has to be rather large. Twelve cows have been proposed as the minimum sample size for herd-based tests with proportional outcomes (Oetzel, 2004). Furthermore, cows to be chosen for the assessment of the herd situation have to belong to the appropriate “at risk” group. Having in mind that SCK is a major problem of fresh cows, the testing of cows in the middle or late lactation for BHB does not provide relevant information and means wasting money and labour.

CONCLUSION

Monitoring of the metabolic status of a dairy herd has to take into account the considerable intraday

variation of generally accepted key parameters like BHB and NEFA. As the interpretation of test results is based on proportional outcomes, the risk of errors in herd-based testing is considerably higher for parameters with a high intraday variation. By contrast, cholesterol concentrations and AST activity are more reliable.

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Corresponding Author

Jun. Prof. Dr. Steffi Wiedemann, Institute of Animal Breeding and Husbandry, Christian-Albrechts-University, Olshausenstr. 40, 24098 Kiel, Germany
Tel. +494 318 804 533, fax +494 318 802 588, e-mail: swiedemann@tierzucht.uni-kiel.de
