Influence of Concentrate Feeding Frequency and Intrinsic Factors on Diurnal Variations of Blood Metabolites in Dairy Cows

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Introduction

The health of dairy cows can be monitored by their ability to maintain normal blood concentrations of numerous metabolites. Therefore, clinical biochemistry analyzes, using metabolic profile tests, were introduced in the early seventies.36 Since then, many researchers have been using biochemical analyzes as a diagnostic aid in subclinical conditions such as reproduction disorders 24,40 and in the assessment of nutritional status or feeding deficiencies.24,40,45

Blood glucose (GLC) is not a direct indicator of nutritional input, but concentrations are measured for detection of breakdown of its homeostasis.37 Blood glucose increases when rumen propionate concentration increases.1,11,34 Glucose concentrations give an indication of the activity of the rumen biomass51 and of the efficiency of gluconeogenesis in the liver. Ketone bodies15 and especially β-hydroxybutyrate (BHB) have also been used as indicators of the energy deficiency.22,23,27,31,37 Urea nitrogen (BUN) is a useful indicator of the nutritional energy-protein balance.26,41,42 Plasma urea nitrogen is typically elevated in the energy-deficient cows compared to those fed relatively high amounts of concentrates.20

Non-esterified fatty acids (NEFA) appear in blood as a consequence of the mobilization of body fat. During early lactation, glucose oxidation is reduced as a sparing mechanism for lactose synthesis. Therefore, fat oxidation is enhanced to provide the cow with energy4 and NEFA concentrations in blood are elevated. Cholesterol (CHOL) was found to be associated with improved fertility in cows.43,44 Furthermore, cholesterol concentrations are elevated with high dietary fat13,46,47 and with propylene glycol administration.17 It is also reported as an indicator of good fibre supply.25 The concentration of total serum protein (PROT) gives an indication concerning the long term energy and protein supply.

Diurnal fluctuations are known to be an important source of variations of many blood metabolites. Diurnal variations of glucose have been considered negligible by some authors,8,30,37 whereas Blum et al. showed a significant 24-hour pattern, depending on energy supply.7 Also β-hydroxybutyrate showed diurnal variations which appeared to be of major importance,7,9,30,49 with a typical increase after feeding. Conflicting information exists about the diurnal variations of BUN concentrations.20 Some authors found higher,21,39 some others lower32,35 concentrations of urea nitrogen in the morning milk compared to the afternoon. During the day, NEFA concentrations decreased after feeding.10,14,37 The diurnal pattern of cholesterol was considered negligible,5,7,48,49 whereas total protein showed either significant49 or not significant7 diurnal variations.

The results of some of these studies are contradictory. An explanation for the contradictions could be the relatively small number of cows used in many of these studies, leading to an overexpression of individual cow effects. Another hypothesis for this discrepancy is that the feeding dynamics (solubility of the components, feeding strategy) have an important effect on the daily pattern.

The purpose of this study was to determine the influence of the concentrate feeding frequency and some intrinsic factors on the diurnal variations of the blood metabolites glucose, β-hydroxybutyrate, urea nitrogen, non-esterified fatty acids, cholesterol and total protein.
in two herds with different concentrate feeding frequencies. These informations should yield knowledge for a better standardization of the sampling procedure when biochemical analyzes are to be used as diagnostic aids.

**Materials and Methods**

Two herds with similar production levels and demographic characteristics, but with different feeding strategies were chosen. The first herd had a traditional feeding system (TRAD) with two meals of roughage and concentrate per day, whereas in the second herd, two meals of roughage and up to six concentrate feedings per day with an automatic computerized distribution of concentrate (ACD) were given. Time of first feeding was 06h30 in herd TRAD and 06h00 in herd ACD, both beginning with grass silage.

In each herd, 20 cows divided in two groups of 10 (group 1: 40 to 110 days post partum (DIM); group 2: 150 to 230 DIM) were randomly selected. Blood samples for serum and plasma were taken through venipunction of the caudal vein four times daily (08h30, 11h00, 13h30 and 16h00).

Glucose (GLC), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) were analyzed in fluoride plasma, whereas urea nitrogen (BUN), cholesterol (CHOL) and total protein (PROT) were analyzed in serum.

To characterize the diurnal pattern of each metabolite, a multivariate repeated measures analysis of variance model was fitted to test the interactions of time (TIME) with the factors feeding strategy (TYPE), lactation group (GROUP) and the intrinsic factors parity (LN), days post partum (DIM) and daily milk yield (KDMI). Furthermore, the differences between each sequential sample and between the 8h30 and the 16h00 samples were tested with a Wilcoxon rank sum test for paired samples.

**Results and Discussion**

**Glucose, β-hydroxybutyrate and Urea Nitrogen**

For those three parameters, a rapid (within hours) reaction to the feeding of concentrate could be observed (Figures 1-3). The diurnal variations showed a significant difference between the two herds, which is most probably related to the frequency of concentrate feeding. Frequent distribution of small amounts resulted in a constant level of the parameters, whereas the patterns for the herd with only two larger concentrate meals showed marked differences between morning and afternoon.

These three parameters remain useful diagnostic tools in the evaluation of the protein and energy metabolism, but the influence of diurnal variations and type of feeding has to be taken into account.

**Non-esterified Fatty Acids, Cholesterol and Total Protein**

In contrast, the diurnal patterns of NEFA (Figure 4) and CHOL were similar for both herds, but more marked differences (although statistically not significant) in the shape of the curves were observed between the two production groups.

Time of sampling did not seem to be important for cholesterol because of the absence of significant diurnal variations. However, the number of days in lactation have an important influence on the value of this parameter. This has to be taken into account to determine sampling strategy or for analyzing and comparing CHOL concentrations in dairy cows.
Influence of production group

Only slight differences were observed between the two production groups within herds. For all the metabolites except for BHB, the stage of lactation (DIM) and the level of production (KGMI) did not have a major impact on diurnal variations. However, some effects were shown through the shapes of the NEFA curves which were different between both productions groups.

Conclusions

Under practical conditions, the strategy of sampling should focus on cows in the group at risk according to the clinical diagnosis or suspicion. In several studies, a relationship has been found between feeding and the metabolic situation on one hand and fertility on the other hand. Due to the marked diurnal variations, sampling strategy must be planned according to the problem that will be investigated. In a situation of insemination only once a day for example, biochemical analyses should be performed at the same time as inseminations occur.

In order to obtain consistent and comparable results of biochemical analyses, time after feeding has to be standardized for sampling. Under normal conditions, 4 to 5 hours after the beginning of feeding, the metabolites are most sensitive to changes potentially caused by metabolic disorders. At this time, concentrations of GLC and NEFA should then be at their nadir and BHB at its peak. If at this time NEFA are elevated, it can be concluded that the cow has not been able to compensate the energy deficiency and had to mobilize increased amounts of body fat. At the same time, the cow is at highest risk to show a breakdown of GLC homeostasis, which could be falsely normal at another time of the day. Blum\(^\text{7}\) showed that BHB increased for a longer period after feeding in energy deficient cows than in cows fed high energy diets. Therefore, this time of sampling should allow the detection of truly ketotic cows in minimizing the impact of a dietary induced increase of BHB in healthy cows.

These findings emphasize the need of a standardization of the time of sampling during the day and are a possible explanation why non-standardized samples will provide uninterpretable results. The necessity of a standardization of the collection of samples in order to allow an appropriate interpretation of the analyzes has already been reported.\(^{32,37}\)

As the dynamic changes during the day can be different between herds, a double sampling in the morning and in the afternoon could be a further use of biochemical analyzes in order to better understand the metabolic situation of a herd, especially if the morning and evening feeding strategies are different. For economic reasons, pooling of the individual samples has been proposed.\(^{55,51}\)

The present study shows different diurnal patterns in herds with different feeding practices, indicating that the feeding strategy, or the com-
position of the food, or both of them, have a major impact on blood metabolites.

References

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Edited by T Garland, Department of Veterinary Physiology and Pharmacology, Texas A & M University and A C Barr, Department of Toxicology, Texas Veterinary Medical Diagnostic Laboratory, USA

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