Predicting Risk of Ketosis in Dairy Cows Using In-Line Measurements of β-Hydroxybutyrate: A Biological Model

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ABSTRACT

Automated monitoring of individual cows to determine health status is a potentially valuable management tool, especially in large dairy herds. Herein is described the rationale, structure, and functionality of a biological model to predict risk of ketosis in individual cows using in-line measurements of the ketone body β-hydroxybutyrate (BHBA) in milk. The model also uses acceleration in milk yield, body fatness at calving, diseases in current lactation, and incidences of ketosis in earlier lactations as additional risk factors for ketosis. However, the model is designed to function merely on the basis of milk BHBA in the absence of other data. Values of milk BHBA are smoothed using a state space model before these are used in calculations in the biological part of the model. The model is designed to be updated each time a new BHBA measurement or a disease occurrence is available and then uses previous and current data. Outputs of the model are the risk of ketosis (value between 0 and 1, where 0 = no risk and 1 = clinical ketosis) and how many days until the next milk sample should be taken and analyzed for BHBA. At higher risks for ketosis, more frequent milk sampling is the recommended output. Test examples from cows for which BHBA has been measured extensively were used to show the functionality of the model. The model performed equally well when reductions in sampling frequency were applied, and it was also relatively robust to the addition of up to ±2 residual SD of random noise in the BHBA values. This model has the potential to provide the basis for a useful disease monitoring and management tool. However, thorough validation awaits a much larger dataset and testing of the model under a variety of on-farm situations.

(Key words: dairy cow, ketosis, β-hydroxybutyrate, disease monitoring)

Abbreviation key: ARF = additional risk factor, CLDHRisk = risk of ketosis because of current lactation disease history, DNS = days to next sample, IBR = indicator-based risk, MYAcc = acceleration in milk yield, OutRisk = output risk of ketosis, RLBHBA = risk caused by level of BHBA, RRCBHBA = risk caused by the rate of change in BHBA, SLBHBA = smoothed level of BHBA, SSBHBA = smoothed slope of BHBA.

INTRODUCTION

Automated disease monitoring has become more relevant because of a rapid structural development in dairy production, resulting in bigger herds and more cows being managed per person. Newer and bigger dairy farm systems often include new technical possibilities relative to the feeding, milking, and monitoring of dairy cows. Monitoring of individual cows for health status in such big dairy herds is of special interest, and future health management systems should help farmers with earlier identification of disease risks.

Ketosis is a metabolic disorder that primarily occurs 2 to 7 wk after calving (Halse, 1978; Gillund et al., 2001), and lactational incidence rates have been reported to be between 1.1 and 9.2% (Erb and Gröhn, 1988; Rasmussen et al., 1999; Østergaard and Gröhn, 2000). Clinical ketosis causes economic losses to the dairy farmer because of treatment costs, decreased milk production, impaired reproduction efficiency, and increased involuntary culling (Gustafsson and Emanuelsen, 1996; Fourichon et al., 1999; Rajala-Schultz and Gröhn, 1999; Østergaard and Gröhn, 1999; Reist et al., 2000). Ketosis is a disease that develops over several days or even weeks. Consequently, it should be possible to detect the disease in its subclinical stage before the cow develops strong clinical symptoms and, thereby, limit the farmer’s economic losses and the cow’s malaise.

Cow health management systems for early identification of diseases have been suggested on the basis of measurements of milk constituents (Dirksen, 1994; Hamann and Krömker, 1997; Mottram et al., 2002), and milk is easier to sample than blood or urine. Furthermore, within recent years, techniques have been developed for automated sampling and measurement of components in milk (Delwiche et al., 2001a; Pember-
ton et al., 2001; Godden et al., 2002), which makes milk a suitable medium for in-line measurements. The ketone bodies acetone, acetoacetate, and BHBA can all be measured in milk and are useful as direct indicators of physiological imbalance and clinical ketosis (Horber et al., 1980; Andersson, 1984; Geishauser et al., 2000; Enjalbert et al., 2001; Nielsen et al., 2003). However, for analytical reasons, BHBA is the most robust and applicable one, because acetone is an extremely volatile compound (Kaneko, 1989) and acetoacetate is a rather unstable compound that forms acetone spontaneously (Bergman, 1971; Bruss, 1989). Furthermore, when cows are fed a TMR, BHBA in milk is hardly sensitive to diurnal variation (Nielsen et al., 2003).

Thus, the technical and analytical possibilities are available for performing in-line measurements of milk BHBA as an indicator of ketosis. However, to extract useful management information from a time series of BHBA measurements, a biological model is required. Work relating to this issue has been presented for mastitis and detection of estrus (de Moll and Ouweltjes, 2001; Delwiche et al., 2001b; Friggens and Chagunda, accepted). It is well established that a number of risk factors other than BHBA exist for ketosis (Rajala and Gröhn, 1998; Rasmussen et al., 1999). Some of these, such as energy balance, are expected to be reflected in changes in BHBA, but others, such as prior disease, are not expected to be reflected in BHBA. The objective of this paper was to present a biological model that can estimate the risk of ketosis in individual cows by using in-line measurements of milk BHBA along with additional readily available information.

MATERIALS AND METHODS

Model Overview

The overall structure of the ketosis model is shown in Figure 1. The model has 2 kinds of inputs: the on-line measurements of BHBA used to generate an indicator-based risk (IBR) and a number of additional inputs used to generate an additional risk factor (ARF). To avoid double counting, one requirement for including an ARF is that it is not reflected in BHBA levels. The following ARF have been included in the model: disease recordings, body fatness at calving, milk yield, and DIM. However, the model is designed to be able to function in the absence of these additional inputs. Together, IBR and ARF are used to generate 2 outputs, namely an output risk of ketosis (OutRisk) and days until next sampling (DNS). The interval until next sampling is designed to vary the frequency of milk sampling for analysis of BHBA for a particular cow according to the OutRisk for that cow.

Inputs

The model is based on the following inputs: cow identification, DIM, parity, BHBA measurements, milk yield, body fatness at calving, and disease recordings. Cow identification, DIM, and parity are self-evident inputs and are not discussed further. The BHBA measurements are the main input for IBR, whereas milk yield and disease recordings are the main inputs for ARF. The elements of the 2 overall inputs, IBR and ARF, are described in detail in the following sections.

**Elements of the IBR.** The IBR is based on measurements of BHBA in milk (mmol/L). From these, a risk caused by the level of BHBA (RLBHBA) and a risk caused by the rate of change of BHBA (RRCBHBA) are calculated and combined to give IBR. The rate of change in BHBA is considered an important element of the model when identifying ketosis at an early stage.

The raw BHBA concentrations are smoothed before they are used in the calculation of IBR. The smoothing is done via an extended Kalman filter, using a local linear growth model with outliers (Smith and West, 1983). The assumptions made in the local linear growth model, estimation of parameters and implementation of the extended Kalman filter, are described by Korsgaard and Løvendahl (2002). In the following, this statistical procedure is referred to as a state space model and has the aim of eliminating or minimizing variation in BHBA caused by non-biological factors. In brief, the state space model assumes that any given milk BHBA value will belong to 1 of 4 populations of observations: normal, outlier, slope change, or level change. Thus, each time a new BHBA measurement is available, the state space model is triggered, and a set of 4 probabilities (between 0 and 1) will be calculated (probability of a level change in BHBA, probability of a slope change in BHBA, probability of a normal BHBA measurement, and probability of a BHBA measurement is an outlier) together with a smoothed level of BHBA (SLBHBA) and a smoothed slope of BHBA (SSBHBA). Values of the SSBHBA were calculated from the difference between consecutive level values. The structure of the model, where IBR is produced, is given in Figure 2. The numbers in circles in Figure 2 refer to equations, which are described in the following section.

**Baseline of BHBA.** A prerequisite for calculating the IBR is to establish a baseline for BHBA, i.e., a level that is considered normal for the individual cow at a given time in lactation. The reason for this is that BHBA is produced in the rumen epithelium as a normal occurring process when butyrate is absorbed from the rumen (Heitmann and Fernandez, 1986). Therefore, BHBA is a natural metabolite that may vary in concentration depending on diet. Thus, in the ketosis model,
Figure 1. Overall structure of the ketosis model.

Figure 2. The structure of the part of the model where the indicator-based risk is produced, i.e., the part using BHBA measurements in milk. The numbers in circles refer to equations in the text. MaxLevel = a scaling constant that reflects the level of BHBA assumed to be associated with clinical ketosis; MaxSlope = a scaling constant that reflects the increase in BHBA per day assumed to be associated with clinical ketosis; RiskSlope = risk of ketosis due to the rate of change in BHBA; RiskLevel = risk of ketosis due to the level of BHBA; pNormal = probability of a BHBA measurement being within the normal time series; pSlope = probability of a change in the slope; pLevel = probability of a change in the level; pOutlier = probability of a BHBA measurement being an outlier from the normal time series.
it is necessary to estimate the baseline of BHBA and subtract that from the actual measured BHBA concentration.

For a given cow at the start of lactation, the baseline is initially set to the SLBHBA (Level) provided that the baseline is ≤0.15 mmol/L. The use of a baseline with a maximum value of 0.15 mmol/L assumes that concentrations >0.15 mmol/L are associated with physiological imbalance that mediate subclinical or clinical ketosis. This is implemented by using a default baseline, which assumes a value of 0.15 mmol/L. Further, it is assumed that the baseline cannot increase during lactation; therefore, the baseline can be adjusted down to lower values by subsequent smoothed BHBA values but cannot be adjusted up to higher values:

If Level > default baseline, then baseline = default baseline. 
If Level ≤ default baseline, then baseline = Level. 
If Level > baseline then baseline = baseline.

As soon as BHBA is measured after calving, the baseline will adjust itself according to the individual cow (unless the SLBHBA is >0.15 mmol/L). Because the baseline is based on smoothed values (Level), it is unlikely that a single low BHBA value of, e.g., 0.01 mmol/L will cause a low baseline. We expect that 0.15 mmol/L is a relatively high maximum baseline, which is useful in most herds and countries. However, our knowledge about the variation in BHBA among herds and countries is limited, and the limits might need adjusting as more data are collected.

Under some conditions, it might have been reasonable that the baseline could change upward, because studies have indicated that ketogenic feedstuffs can increase the level of ketone bodies in milk and blood (Andersson and Lundström, 1985; Murphy, 1999). However, to ensure that the baseline does not increase because of BHBA produced in the liver, we prefer the restriction that the baseline is not allowed to increase and accept the resulting minor inaccuracy in ketosis detection.

**Risk caused by slope of BHBA.** As mentioned previously, IBR consist of 2 risks: RLBHBA and RRCBHBA. It is assumed that the greater the positive rate of change of BHBA (Slope), the greater the risk is of ketosis. The basic equation for this is

\[ RRCBHBA = \text{Slope} \times (1 + \text{signSlopeChan} \times \text{pSlope})/\text{MaxSlope} \]

where slope is the smoothed slope from the state space model, and pSlope is the probability for a slope change calculated by the state space model. MaxSlope is a scaling constant, which reflects the increase in BHBA per day that is assumed to be associated with clinical ketosis. It has been shown that milk BHBA can increase from 0.10 to 0.29 mmol/L during 2 d of feed restriction (65% of ad libitum intake) (Nielsen et al., 2003). Further, milk BHBA, in some severe clinical cases of ketosis or displaced abomasum, can increase from 0.05 to 0.15 mmol/L to 0.5 to 0.8 mmol/L during a 2-d period (n = 7 naturally occurring, veterinary diagnosed cases; N. I. Nielsen, 2005, unpublished). Therefore, the suggested value of MaxSlope is 0.3 mmol/L, which means that a cow with a Slope of 0.3 mmol/L will return a RRCBHBA of 1 (pSlope is here assumed to be 0). The RRCBHBA can assume negative values when there is a decreasing, i.e., negative slope.

The RRCBHBA is further increased if there is a high probability that it is a deviation from the normal time series, i.e., a slope change (pSlope increases). However, in the previous equation, this effect of pSlope does not differentiate between a positive and a negative deviation in BHBA. It follows from the assumption of increasing risk with increasing slope that negative deviations should reduce the risk (relative to no deviation), and positive deviations should increase the risk. This way, the farmer has the possibility of monitoring the risk decreasing after a cow has been treated for ketosis. The sign (+ or −) of the slope change (signSlopeChan) is, therefore, included.

**RLBHBA.** It is assumed that the higher the level of BHBA compared with the baseline level of the individual cow (baseline), the greater the risk of ketosis. The equation for RLBHBA is

\[ \text{RLBHBA} = [(\text{Level} - \text{baseline})/\text{MaxLevel}] \times (1 + \text{signLevelChan} \times \text{pLevel})/\text{MaxLevel} \]

where Level is the smoothed level from the state space model, and pLevel is the probability for a level change calculated in the state space model. MaxLevel is a scaling constant, i.e., a concentration of BHBA, where we assume that a cow is certain to have ketosis, i.e., RLBHBA = 1. The suggested value of MaxLevel is 0.8 mmol/L because milk BHBA levels of 0.5 to 0.8 mmol/L have been measured in clinical cases of ketosis (n = 11 naturally occurring, veterinary diagnosed cases; N. I. Nielsen, 2005, unpublished). This result is in accordance with studies using semi-quantitative determinations (cowside tests) of milk BHBA, where the highest values were reported to be >0.5 mmol/L (Geishauser et al., 1997; Enjalbert et al., 2001) or >1000 mmol/L (Geishauser et al., 2000; Carrier et al., 2004). The RLBHBA cannot assume negative values because Level is always higher or equal to the baseline and because pLevel is always between 0 and 1. To account for pLevel...
Figure 3. The structure of the part of the model where the additional risk factor (ARF) is produced, i.e., the part using milk yield, diseases, body fatness at calving, and BCS. The numbers in circles refer to equations in the text. dMY = change in milk yield; dt = change in time; DFC = days from calving.

Values associated with decreases in BHBA during recovery from ketosis, the sign (+ or −) on the level change (signLevelChan) is incorporated.

**Calculation of IBR.** Indicator based risk is calculated as an addition of RRCBHBA and RLBHBA. Although, IBR can assume values <0 or >1, given that the values of MaxSlope and MaxLevel are appropriate, the majority of values of IBR will be between 0 and 1.

\[
\text{IBR} = \text{RRCBHBA} + \text{RLBHBA} \quad [4]
\]

**Elements of the ARF.** Factors that are not directly reflected in milk BHBA but are known or assumed to impose a risk for ketosis are included in the model as an ARF. The intention with the inclusion of ARF is to supplement the IBR and, thereby, enable an identification of a risk cow as early as possible. However, ARF are given less weight than the IBR in calculating ketosis risk (see eq. [10]). The assumption that these factors do not directly cause changes in BHBA is an important one, including ARF that directly affect BHBA that would result in the model over-estimating ketosis risk because of double counting. The choice of ARF included and the manner of their inclusion was made accordingly. The elements making up ARF are described subsequently and include acceleration in milk yield, diseases in current lactation, lifetime number of ketosis incidences, and body condition at calving. The structure of the part of the model where ARF is produced is shown in Figure 3. The numbers in circles in Figure 3 refer to equations, which are described in the following.

**Acceleration in milk yield.** Acceleration in milk yield (MYAcc), i.e., slope of the milk yield curve (kg/d per d), is regarded as an overall index for the physiological challenge for the cow in early lactation to support milk production (Ingvartsen et al., 2003). It seems reasonable that a high MYAcc increases the risk of an imbalance in the cow’s fat and carbohydrate metabolism, i.e., the higher MYAcc, the higher the risk of ketosis. All other things being equal, the greater the potential to produce milk, the greater is the risk of ketosis. An illustration of how MYAcc depends on the milk yield profile during lactation is given in Ingvartsen et al. (2003). In the situation of missing BHBA measurements in early lactation, e.g., the colostrum period, the model predicts, as a result of MYAcc, a higher risk of ketosis in early lactation compared with, e.g., mid or late lactation. The risk of ketosis caused by an acceleration in milk yield (AccRisk) is calculated as follows:

\[
\text{AccRisk} = \frac{\text{MYAcc}}{\text{MaxAcc}} \quad [5]
\]

where MYAcc is the slope of the milk lactation curve for the individual cow and is a 5-d smoothed slope out-
put from the state space model; MaxAcc is a scaling constant, which defines the level of MYAcc that will return a AccRisk of 1. The current value for this constant is 3 kg milk/d. AccRisk can assume values <0, i.e., when milk yield decreases, so does the risk of ketosis. This may seem counter-intuitive as it is commonly observed that milk yield is decreased in association with ketosis. However, MYAcc is included here as an ARF of the overall physiological challenge to the cow and not as a direct indicator of ketosis. In this context, cows producing less milk are less physiologically challenged. If the milk yield decline, directly associated with ketosis, had been included as an ARF, then “double counting” would occur, as these declines in yield are correlated with changes in ketone bodies (Gustafsson and Emanuelson, 1996) and clinical incidences of ketosis (Østergaard and Gröhn, 1999) and are, thus, reflected in the IBR.

Current lactation disease history. Other diseases, especially those that disrupt voluntary feed intake, increase the risk of a subsequent ketosis. Lowered feed intake can lead to an increased fat mobilization, and if the liver fails to fully oxidize these fatty acids, the cow is often referred to as suffering from a secondary ketosis (Kronfeld, 1982). Therefore, we have included certain diseases in the calculation of an ARF called current lactation disease history (CLDHRisk). It is assumed that such disease occurrences will precede a rise in BHBA and, thereby, contribute to an early identification of a cow at risk.

It is further assumed that the effect of a disease is greatest on the day of occurrence and that this effect subsequently declines as a function of days since occurrence. The following function is used to calculate the risk of ketosis caused by a given disease (DisRisk) as a function of days since occurrence of the disease (DisDays), an expected risk period (DisT), an expected maximum risk from the disease (MaxDis), a rate coefficient (DisRat), and the severity of the disease (DisSev):

$$\text{DisRisk} = \text{DisSev} \times \text{MaxDis} \times \exp(-\exp(\text{DisRat} \times (\text{DisDays} - \text{DisT}))) \leq 0.37$$  

This is a sigmoid (Gompertz) function, which decreases from MaxDis to 0 as DisDays increases. The severity of the effect of each disease on the risk of ketosis is known to vary between diseases. Therefore, different diseases, i.e., right displaced abomasum, left displaced abomasum, milk fever, metritis, retained placenta, and mastitis have different values of MaxDis: 1, 0.9, 0.6, 0.5, 0.3, and 0.2, respectively. The DisDays variable is calculated as the difference between the date for the occurrence of the disease and the current date. The expected risk period (DisT) gives the number of days from occurrence until DisRisk has been reduced to ≤0.37 of MaxDis. The rate coefficient (DisRat) determines the shape of the curve and, given positive values, results in decreasing values of DisRisk from the day of occurrence. The severity of a given disease (DisSev) can assume values of 0.3 (mild), 0.6 (moderate), or 0.9 (severe).

Diseases cannot be considered totally independent. Therefore, it could lead to overestimation of the ARF if the risks of each disease are added together in a cow that experiences several diseases at the same time. Therefore, it is assumed that only the disease with the highest risk (DisRisk) on the given day is used as the current lactation disease history risk. This also ensures that CLDHRisk will not be overestimated for a cow receiving repeated treatments of the same disease within few days. Risk associated with current lactation disease history can assume values between 0 and 0.9, dependent on the type and severity of the disease.

The inclusion of the previously mentioned diseases is supported by the available epidemiological literature. Thus, the risk of ketosis was increased in cows having milk fever (Correa et al., 1993; Klerx and Smolders, 1997; Rajala and Gröhn, 1998), retained placenta (Gröhn et al., 1989; Correa et al., 1993; Klerx and Smolders, 1997), and metritis (Gröhn et al., 1989, 1990; Rajala and Gröhn, 1998), and, therefore, these diseases are included in CLDHRisk. Although, some studies have not identified mastitis to be a risk factor for ketosis (Rasmussen et al., 1999; Rajala and Gröhn, 1998), other studies indicate that, depending on the definition of mastitis, cows with acute or chronic mastitis had a higher risk of getting ketosis (Gröhn et al., 1989, 1990). Therefore, mastitis is included in CLDHRisk and, similar to the rest of the diseases, is included with the possibility of adding a severity depending on the veterinarian’s judgment of the cow’s symptoms. The development of left displaced abomasum is clearly associated with high ketone levels (Geishauser et al., 1997; Itoh et al., 1998). It is envisaged that it may be necessary to adjust the constants of MaxDis and possibly also inclusion of diseases as further evidence accrues.

Lifetime number of ketosis. The background for including a cow’s history of ketosis is that several studies have found that ketosis in a previous lactation increases risk of ketosis in the following lactation. Mäntysaari et al. (1991) estimated that cows treated for ketosis in the first lactation had a 17% risk of ketosis in the second lactation, whereas those that were not treated in the first lactation had a 4% risk of ketosis in the second lactation. The equivalent values in a study by Rasmussen et al. (1999) were 8 and 3%. Bendixen et al. (1987) reported that depending on parity and breed, cows with ketosis in a previous lactation had a 4 to 12 times higher risk of getting ketosis again.
The ARF caused by earlier incidences of ketosis is assumed constant during lactation because it expresses a constant susceptibility or different sensitivity between cows, i.e., it is considered a pseudo-genetic factor (ketosis is heritable; $h^2 = 0.02$ to 0.16; Gröhn et al., 1986; Mäntysaari et al. 1991; Uribe et al., 1995). The risk of ketosis caused by previous incidences of ketosis (HistRisk) is calculated on the basis of earlier incidences of ketosis (HistCon) and the severity of those (HistSev):

$$\text{HistRisk} = \text{HistCon} \times \text{HistSev} \quad [7]$$

Earlier incidence of ketosis (HistCon) assumes different values depending on whether the cow had ketosis in her first lactation (0.8), a previous lactation (0.6), or in >1 lactation (1.0). The highest risk is given for cows with >1 incidence of ketosis, whereas cows with ketosis in their first lactation have the second highest risk because first parity cows seldom get ketosis (Rasmussen et al., 1999; Østergaard and Gröhn, 2000). The inclusion of the severity of earlier incidences of ketosis (HistSev) allows differentiation between cows with mild (0.3) or moderate (0.6) cases of ketosis (subclinical; i.e., no obvious reduction in feed intake and milk yield but elevated ketone bodies) and more severe (0.9) cases (clinical; i.e., an obvious reduction in feed intake and milk yield and highly increased ketone bodies).

**Body fatness at calving.** The risk of ketosis caused by body fatness at calving is another ARF that can provide information in the very first days after calving, where the IBR is not in place yet because of lack of BHBA measurements. Reasons for missing BHBA measurements immediately after calving could be that colostrum is not suitable for in-line transportation as long as it contains lumps and cows might be in a calving pen the first few days after calving. Body fatness at calving can be assessed by BCS, which has been identified as a risk factor of subclinical and clinical ketosis (Markusfeld et al., 1997; Rasmussen et al., 1999; Duffield, 2000). The study by Rasmussen et al. (1999), cows had an average BCS at calving of 2.8, and cows with a BCS of 3.5 doubled their risk of getting ketosis compared with cows with a BCS of 2.0. Duffield et al. (2000) reported an incidence of clinical ketosis of 1% in cows with a BCS of ≤3 at calving, whereas cows with a BCS of ≥4 had an incidence of 6%; for subclinical ketosis, the incidence increased from 27 to 78%.

Given a scale from 0 to 5 and based on the previously mentioned results, it is assumed that a BCS at calving of <2.75 has no effect on the risk of ketosis, whereas the risk of ketosis increases from a BCS of 2.75 to 5.0. The same type of function is used as for the risk caused by a given disease (DisRisk), i.e., the risk of ketosis depending on the BCS at calving (FatRisk) is calculated as a function of days since calving (FatDays), an expected risk period (FatT), a rate coefficient (FatRat), and the difference between the actual BCS at calving (BCSCalv) and the optimal BCS (OptBCS, which is assumed to be 2.75):

$$\text{FatRisk} = ((\text{BCSCalv} - \text{OptBCS})/(5 - \text{OptBCS})) \times \exp(-\exp(\text{FatRat} \times (\text{FatDays} - \text{FatT}))). \quad [8]$$

All values of FatRisk <0 are converted to 0, i.e., cows with BCSCalv of <2.75 get a FatRisk of 0. Thus, FatRisk can assume values between 0 and 1. The FatDays variable is calculated as the difference between the calving date and the current date. The risk period (FatT) has a value of 7, which is the number of DIM until FatRisk has been reduced to ≤0.37 of the difference between BCSCalv and OptBCS (assuming this difference is positive). The rate coefficient (FatRat) has a value of 1.2 and determines the shape of the curve between the day of calving until the day when FatRisk reaches 0. Figure 4 shows how FatRisk develops for a cow with a BCSCalv of 3 and a cow with a BCSCalv of 4. The FatRisk variable only has an effect on the ketosis risk the first 8 d after calving, thereby ensuring that fat cows will get an increased risk just after calving when the IBR is not quite in place because of colostrum and few BHBA measurements.

**Calculation of ARF.** Additional risk factor is calculated as an addition of 4 risks: risk due to MYAcc (AcRisk), CLDHRisk, risk caused by previous incidences of ketosis (HistRisk), and risk caused by BCS at calving (FatRisk). There are no weighting factors in this equation because the relative importance of each of these factors is already controlled by the constants within the component equations (see eq. [5], [6], [7], and [8]).
$ARF = AccRisk + CLDHRisk \quad (9)$

$+ HistRisk + FatRisk.$

**Outputs**

There are 2 outputs from the model, which are available to the user, namely an ORK that combines IBR and ARF and an output that gives DNS (Figure 2). OutRisk is calculated as follows:

$$OutRisk = IBR + (ARF/ARFW). \quad (10)$$

The ARF weight (ARFW) is a constant used to weigh the importance of IBR against ARF. Intuitively, this should be a declining function of DIM, as, just after calving, the reliability of the IBR is at its lowest. However, DIM is already an inherent component of the ARF via MYAcc and FatRisk, which has the effect of placing greater weight on the ARF immediately post-calving. The value of ARFW is set to 4, reflecting the priority given to the indicator, BHBA, in this model. If the resulting OutRisk value is $>1$ or $<0$, then OutRisk is set equal to 1 or 0, respectively.

As mentioned earlier, DNS is designed to feedback to the sampling system so that the frequency of milk sampling (i.e., next analysis of BHBA for that particular cow) can be varied according to the calculated risks of ketosis. Thus, the higher risk a cow has for ketosis, the more frequently samples are taken. This parameter is designed to make best use of the opportunities afforded by automated, real-time, in-line sampling technology. The equation is

$$DNS = DNS_{def} \times (1 - IBR) \times (1 - ARF) \times (1 - signLevelChan \times pOutlier). \quad (11)$$

The DNS has a default value (DNSdef) of 4 d, and IBR and ARF are included separately in the equation rather than the combined OutRisk value, so that if either IBR or ARF is high, DNS decreases, regardless of their combined value. Further, if the latest BHBA value has a high probability that it is a positive deviation from the normal time series of a cow (signLevelChan is 1 and pOutlier is high), then a new sample is desired quickly to establish whether the cow is acquiring ketosis. If there is a negative deviation from the normal time series (signLevelChan is -1 and pOutlier is high), the cow is recovering from ketosis; therefore, it is less necessary with a new sample. If DNS is close to or equal to 0, this means that a milk sample should be taken from this cow at its next milking.

**RESULTS**

To illustrate the functionality, structure, and logic of the model, the following presents test examples from 3 cows in a research herd, in which milk BHBA was measured once daily for each cow. Those 3 cows were chosen because they represented cows with a constant low level, medium fluctuations, or higher fluctuations of BHBA.

The research herd consisted of 55 Danish Holstein, 55 Red Danish, and 40 Danish Jersey cows in a loose-housing system fed TMR for ad libitum intake. Various TMR consisted of grass silage, maize silage, rapeseed cake, barley, sugar beet cobs, minerals, and vitamins in varying proportions based on concurrent nutritional studies in the research herd. In the 2.5 yr during which milk samples were collected for BHBA analysis, there were 11 cases of veterinary-diagnosed clinical ketosis.

Cows were milked in automatic milking systems, and composite (metered) milk samples were collected automatically from every milking in 10-mL tubes. The automatic milk samplers were emptied in the morning and the afternoon, and milk samples were kept at 4°C until analysis. Milk samples were generally analyzed in the laboratory the following day, except for weekends. The method of Larsen and Nielsen (2005) was used to analyze BHBA in milk. The assay had an intra-assay coefficient of variation of 5.6 and 3.0% for low (0.08 mmol/L) and high (0.40 mmol/L) controls, respectively. The corresponding interassay coefficient of variation was 13.1 and 6.7% for low and high controls, respectively. The low and high controls had an accuracy (percentage bias) of +10.4 and -2.4%, respectively.

Figure 5 shows raw BHBA values from a Jersey cow for the first 50 d after calving and how those values are smoothed via the state space model. The baseline of BHBA indicates the level of BHBA, which is assumed normal for this cow.
Figure 6. The calculated risk of ketosis during the first 50 d of lactation (ORK) and the number of days to next sample (DNS), i.e., next measurement of BHBA, based on the daily smoothed values (A) or the actual model outputs of DNS values presented for the cow in Figure 5. The model output calls for sampling less frequent than daily, in this case, but more frequent than the default period of 4 d.

The cow was in first parity and had an average milk production of 24.2 kg/d (5.9% fat and 3.8% protein) and DMI of 15.8 kg/d during the first 50 DIM. The cow had no apparent drop in milk yield nor DMI, but fluctuations of BHBA suggest that this cow might have had subclinical ketosis.

Figure 6A shows how the outputs (OutRisk and DNS) of the model change according to the smoothed BHBA values shown in Figure 5. Sampling interval (DNS) decreases to nearly 2 d when the risk of ketosis for that cow is highest, i.e., an OutRisk of 0.4 around d 30 after calving. There were no reported incidences of any disease for this particular cow, and milk yield and BCS at calving were not included in the model run. Therefore, changes in OutRisk and DNS during the first 50 DIM are purely due to changes in the IBR. From this particular cow, there was one BHBA value input/d, and DNS was calculated each day during this 50-d period. However, if milk sampling were initiated by the DNS function of the model, a new BHBA value would only have been available to the model every 2nd to 4th d (Figure 6B), thereby reducing the number of samples substantially for this cow compared with daily samples as collected. The difference in OutRisk and DNS between using a BHBA input every day and BHBA inputs according to the DNS feature of the model are shown in Figure 6.

To illustrate the effect of other diseases on the risk of ketosis, Figure 7 shows a cow that was treated for metritis and mastitis on d 6 and 22 after calving, respectively. The metritis was reported with a moderate severity, i.e., DisSev = 0.6; the case of mastitis was severe, i.e., DisSev = 0.9. From d 6 of lactation, the risk of ketosis increased because of the occurrence of metritis and continued to be higher after the incidence of mastitis at d 22 compared with the OutRisk calculated only on the basis of BHBA measurements. At d 22, the risk caused by metritis was a bit higher than the risk caused by mastitis, i.e., CLDHRisk at d 22 is based on the metritis and not on the mastitis. At d 25 this shifts, i.e., mastitis causes a higher risk than metritis (not shown in Figure 7). The relatively small increase in ketosis risk caused by mastitis is expected because MaxDis for mastitis is low (0.2). Despite these diseases, this cow is calculated to have a low risk of ketosis (≤0.1) during the first 50 d of lactation (Figure 7). The reason for this is the low values of BHBA (smoothed ≤0.05).
Figure 8. The calculated risk of ketosis during the first 50 d of lactation for a cow that was treated for ketosis and left displaced abomasum (LDA) on d 22 and 37 after calving, respectively. The dotted line shows that if a cut-off value of 0.6 is assumed to reflect the transition from subclinical to clinical ketosis, then clinical ketosis would have been identified at d 17 and LDA at d 32 after calving.

DISCUSSION

This study has developed a model for early detection of ketosis based on measurements of BHBA in milk. Using few, but relevant, test examples, it has been shown that the model adequately represents the way in which the risk of ketosis changes in cows in relation to their values of daily BHBA measurements, DIM, and disease occurrence. Further, the model was robust to random noise in the BHBA values well beyond the level of variation associated with the assay used in this study.

Even though the risk of ketosis changes as per expectations and in accordance with available literature, this does not constitute an independent test of the model. Clearly, such a test would be of great value, but would be difficult to achieve. There are a number of reasons for this, including the need for a large dataset, given the expected frequencies of ketosis occurrence, with the necessary model input data available. We are currently establishing such a dataset, but it will be several years before sufficient numbers of clinical cases are available for a full-scale validation of the model. A further consideration relates to the need for an independent measure of ketosis in such a data set. The “gold standard” for identifying ketosis is an increase in ketone bodies, such as BHBA; this is especially the case with respect to subclinical ketosis (Geishauser et al., 2000; Carrier et al., 2004). Veterinarians usually use semiquantitative sticks, which measure 1 or more ketone bodies in milk or urine, in their diagnosis of ketotic cows (Geishauser et al., 2000; G. Berg, 2004, personal communication). It is likely that different veterinarians use different criteria on the semiquantitative scale when it comes to diagnosing a cow with ketosis. Any test of the model
against such data would ultimately be comparing one set of ketone body measurements with another, i.e., not an independent test. It is difficult to see how to assemble a test data set that is both independent and where ketosis is measured on a sufficiently nuanced scale to allow full validation of the model by controlled test. An alternative form of testing is through usage of the model across a range of conditions in herds, where the criterion for validation are user satisfaction and documented decreases in incidence of clinical cases. We are currently exploring these options, which are beyond the scope of the present paper and consequently not discussed further here.

Testing of systems to predict disease, such as the current model, usually involves considerations of specificity and sensitivity. These measures are dependent of the use of a threshold to distinguish among different categories, e.g., healthy, subclinical disease, and clinical disease. Although this approach is of major importance to testing, it is not necessarily so valuable in practice. The use of a threshold to generate lists of “alarm” cows may reduce the quality of information presented to the farmer. The skilled farmer often categorizes animals on a more nuanced scale, i.e., a degree of illness rather than healthy vs. sick. By presenting a risk of ketosis as output, this model would allow skilled users to respond to more detailed information and define their own action “thresholds.” In situations where this level of detail is too great, it would be easy to present risk of ketosis in categories by applying thresholds to the model outputs.

The additional risk factors for this biological model include information about daily milk yield, DIM, BCS at calving, and diseases. However, if this additional information is not available or only partly available, default values have been created so the model will run and an OutRisk will be produced. For example, if a disease is reported but no severity given, the calculation of CLDHRisk can be done anyway using a default value of 0.6, i.e. it is categorized with moderate severity. The model cannot, of course, consider diseases or milk yield information that are not included, but absence of such data does not mean that the output of the model is meaningless. This is because ARF is weighted by ¼ against IBR, which means that the measured BHBA is the most important factor for the size of the output risk. However, despite this model being fairly robust, it is important to state that the predictions of ketosis risks will be more valid if additional risk factors are included. This has to do with the fact that ARF influences DNS just as much as IBR.

Projecting DNS is an important feature of the biological model, because it takes advantage of the opportunities afforded by automated on-line sampling technology. Thus, sampling frequency can be increased at times when there is a high risk of ketosis and decreased when there is little risk of ketosis. Distribution of samples in this way increases the efficiency of early detec-
tion of ketosis as well as having economic advantages. It should be stated that if the model was used for cows in mid or late lactation, it would collect samples every 4th day because of a default value of 4 (assuming the cow has no ARF or IBR). However, the intention with the model is that it primarily should be used only in early lactation cows, e.g., the first 8 to 10 wk after calving, when most incidences of ketosis occur.

During the work of creating this biological model, many variables were considered as risk factors, but not all were included. Parity is an example of a factor that is clearly a risk factor for ketosis when looking in epidemiological literature (Rasmussen et al., 1999; Østergaard and Gröhn, 2000; Hardeng and Edge, 2001). Therefore, one might think of parity as an ARF. However, a biological explanation for older cows that get ketosis is their higher incidences of other diseases (e.g., milk fever, mastitis, and displaced abomasum) and their higher acceleration in milk yield compared with 1st parity cows. These are factors that are included in the model as ARF. Therefore, including parity separately as an ARF could potentially be including the same risk twice, i.e., double counting. Similar arguments apply for BW loss and feed intake.

CONCLUSIONS

The risk of ketosis can be potentially predicted using measures of BHBA in milk and other biological risk factors in a mathematical model. Test examples from cows where BHBA has been measured extensively shows the functionality of the model and suggests that this model, which incorporates other relevant biological effects on the risk of ketosis, has the potential to provide the basis for a useful disease monitoring and management tool. Further testing and thorough validation using a large dataset from cows from differing management systems is needed.

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