Novel model of monitoring of subclinical ketosis in dairy herds in Poland based on monthly milk recording and estimation of ketone bodies in milk by FTIR spectroscopy technology

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Abstract

The aim of this paper is to present the new system of monitoring of subclinical ketosis (SCK) in Poland, based on monthly milk recording. The preliminary results of such a monitoring are presented as well as the main risk factors for SCK in Poland. To our best knowledge, the system presented here is the only one all over the world, by which the whole nationally recorded population of cows is being systematically monitored for SCK.

SCK is an excess of circulating ketone bodies in the blood without clinical signs of ketosis, such as decreased appetite and weight loss. The lack of clinical signs makes SCK difficult to detect. However, using blood β-hydroxybutyrate acid (BHBA) testing to measure the incidence or prevalence of SCK in a herd is a powerful and useful tool. In Poland we use milk content of BHBA (BHBAM) and acetone (ACEM) to detect cows and herds in risk of SCK. BHBAM and ACEM are determined by MilkoScans with FTIR, placed in four labs of Polish Federation of Cattle Breeders and Dairy Farmers. The system was introduced into the practice in April 1, 2013 and about 720 000 cows are being monitored annually. The cows between 6 and 60 days in milk (DIM) are not diagnosed but identified as "in risk". These cows are pointed as "K!" A special statistical method was implemented to calculate the probable frequency of SCK (so called PFSK). If it is higher than 10 or 20%, the herd is recognized as "in risk" or "in high risk", respectively. The results of such a monitoring are presented to the farmers in monthly reports delivered by the internet.

A preliminary survey (after 24 months) of the results shows that about 10% of cows at 6-60 DIM are in a risk of SCK. Surprisingly, more ketotic cows have been found in lower productive herds than in higher productive ones. So, the high milk yield is not a risk factor for SCK in Poland. Other factors are shown and discussed in the paper.

Keywords: subclinical ketosis, monitoring, Fourier Transform InfraRed (FTIR).

Introduction

Excessive negative energy balance in the early lactation of dairy cows results in metabolic disorders, such as ketosis, fatty liver and displaced abomasum (LeBlanc, 2010). They are highly responsible for substantial financial losses due to decreased milk yield, poor reproduction, increased susceptibility to immunosupression and culling rate (Ingvartsen, 2006; McArt *et al.*, 2012).

Ketosis, defined as clinical (CK; blood β-hydroxybutyrate acid (BHBA) > 3 mmol/L) or subclinical (SCK; blood BHBA > 1.2 mmol/L) (Oetzel, 2007), is considered the most frequent metabolic disorder of dairy cows. In the study of Suthar *et al.* (2013) the average prevalence of SCK in 528 dairy herds in 10 European countries (5 884 cows) was 21.8%, ranging from 11.2 to 36.6%. For each ketotic cow the total costs of SCK have been calculated to be $960 (Esslemont, 2012) or $340 (Gohary, 2013). The lack of clinical signs makes SCK difficult to detect, especially on the herd basis.

Since the prevalence of SCK is so high, and economic losses so obvious, there is an unquestionable need for monitoring of SCK in dairy herds. Cow-side tests, using glucometers, strips, or nitroprusside powder to detect ketone bodies are commonly available but their sensitivity and specificity are highly variable (Oetzel, 2007). Moreover, a routine monitoring of SCK by these means is costly and difficult organizationally, especially in such countries like Poland where the average herd size is less than 10.

The method of milk chemical composition analysis based on Fourier Transform Infrared (FTIR) spectroscopy allows for the determination of ketone bodies (BHBA and acetone (ACE) in milk (Hansen, 1999), using calibrations proposed by de Ross *et al.* (2007). The first diagnostic model for detection of hyperketonemia in early lactation dairy cows at test days, using FTIR technique and test-day information, was published by Dutch group of van der Drift *et al.* (2012).

In Poland we use milk content of BHBA (BHBAM) and acetone (ACEM) to detect cows and herds in risk of SCK. BHBAM and ACEM are determined by MilkoScans with FTIR, placed in four labs of Polish Federation of Cattle Breeders and Dairy Farmers (PFCBDF). The system (Kowalski *et al.*, 2015) of SCK monitoring was introduced into the practice in April 1, 2013. To our best knowledge, the system presented here is the only one all over the world, by which **the whole nationally recorded population of cows is being systematically monitored for SCK**.

The objectives of this paper are to overview the model and system of SCK monitoring used in Poland by PFCBDF as well as to present the main statistical data on prevalence of SCK in Poland, based on the two-year monitoring.

A model of SCK monitoring in Poland

In the developing of the model our aim was to create a cheap, easy and massive monitoring method of dairy herds for SCK, based on monthly milk recording, to give the information on risks of individual cow and herd.

A model was developed based on the dataset which contained data collected in January - March 2012, from about 1100 dairy cows, from randomly selected Polish farms enrolled in the system of milk recording provided by PFCBDF. The cows were between 6 and 60 days in milk (DIM). A sample of milk from each cow was taken during the morning milking, and analyzed for chemical composition, including BHBAM and ACEM. Milk composition was determined in the laboratory of PFCBDF, using MilkoScan FT6000 (Foss Analytical A/S, Hillerød, Denmark). The FTIR instrument was calibrated for BHBAM and ACEM (de Roos *et al.*, 2007)

At the same test-day, between 1100 and 1400 h the blood samples were drown from the jugular or coccygal veins, and the concentration of blood BHBA (BHBABG) was determined using the Optium Xido glucometer (Abbott Laboratories Poland). The BHBABG was transformed to the laboratory value (BHBAB) using our own linear regression equation.

The model was developed using above dataset and logistic regression method. It was assumed that a cow was ketotic (depended variable) when BHBAB was 1.4 mmol/l The logistic regression equations were determined using stepwise and manual backward selection procedures (P-value for retention <=0.15). The factors (potentially predictive variables) tested were milk components, such as milk fat, protein, lactose, urea, BHBAM and ACEM contents, and milk fat-to-protein ratio (F / P). Sensitivity and specificity of the model was tested using receiver operator characteristic (ROC) analysis. The optimal cutoff value for each test variable or variables was defined as that cut point where sensitivity plus specificity were at a maximum (van der Drift *et al*., 2012).

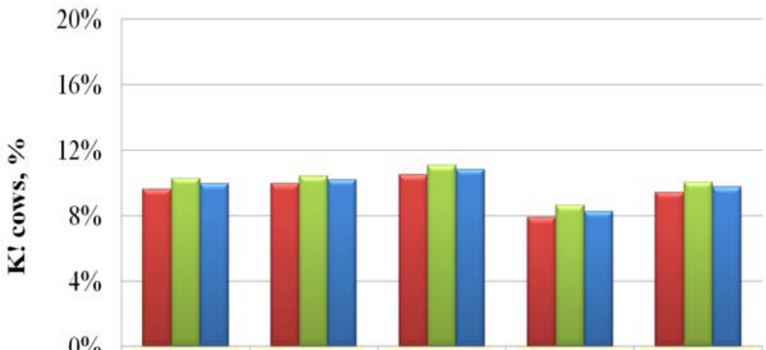
Finally, a logistic regression model which was introduced into the practice, consists of 3 independent variables: BHBAM ($P<0.001$), ACEM ($P<0.001$) and F / P ($P<0.035$). According to this model a cow is marked by the "K!" if she is considered as being in th e SCK risk. By indexing the cow as K! the farmer is informed that there is a considerable (> 70%) probability, that on the test-day this cow suffered from SCK. The estimation of K! is not equal with the diagnosis of SCK.

Since in Poland still a substantial part of recorded cows are located in small herds (less than 50 cows), a simply calculation of herd risk of SCK based on the percentage of K! cows is not valid. Instead, we developed the system in which the degree of SCK risk in the herd is determined by the statistical model. It considers the frequency of K! cows in the group of cows at 5-60 DIM, number of cows and sensitivity and specificity of the method. Finally, the farmers are informed about the risk of herd by so-called "estimated frequency of subclinical ketosis" (PFSK). The herd is considered with 90% probability as being "at risk" or "at high risk" if PFSK is higher than 10 or 20%, respectively.

Both parameters, i.e. K! (defining risk of individual cow) and PFSK (risk of herd) are provided to the farmer by PFCBDF either in the electronic (internet) or printed form.

What have we learnt about ketosis in Poland ?

As mentioned above, the system was introduced into the practice on April 1st, 2015. Since it, a huge number of data, exceeding 1.8 million, has been collected. A preliminary survey of the results shows that 9.73% of cows are in a risk of SCK (Figure 1). Moreover, the prevalence varies due to the area of Poland. The highest is found in area of Minikowo laboratory, where small farms are in majority, whereas the lowest prevalence is observed in the area of Krotoszyn, where bigger farms are much common.



	Parzniew	Jeżewo	Minikowo	Krotoszyn	Poland
2013/2014	9,60%	9,95%	10,50%	7,88%	9,42%
2014/2015	10,29%	10,40%	11,07%	8,61%	10,03%
2 years	9,94%	10,19%	10,80%	8,26%	9,73%
N of milk samples	548283	343228	448171	507194	1846876

Figure 1. Prevalence of SCK in Poland (2 year monitoring - 2013/2014 and 2014/2015), and in the areas of four laboratories of PFCBDF.

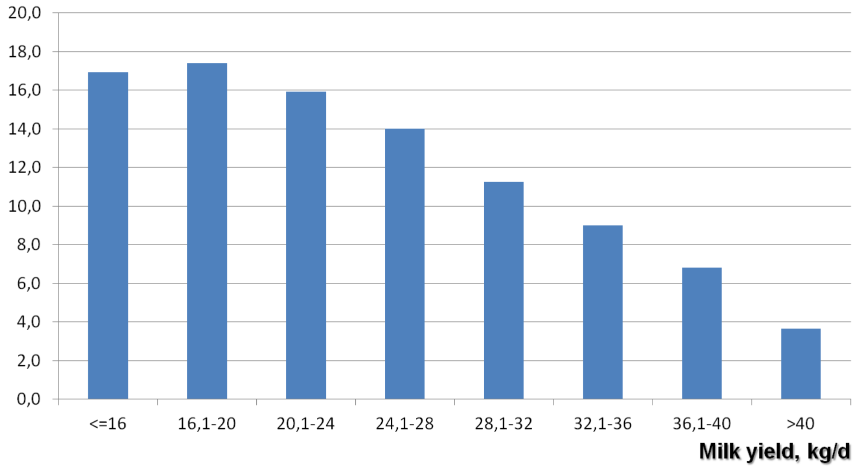


Figure 2. Prevalence of SCK in Poland depending on daily milk yield of monitored cows on the test-day.

Surprisingly, more ketotic cows were found in lower productive herds than in higher productive ones (Figure 2). Among cows producing daily more than 36 litres of milk only less than 6.5% were considered as ketotic, whereas the cows producing 16.1-20.0 litres of milk more than 17.5% were at risk of SCK. Similar tendency was found when the prevalence was analyzed in relation to the average daily milk yield of the herd. So, the high milk yield seems not to be a risk factor for SCK in Poland.

According to Oetzel (2007), there are two types of ketosis in the early lactation period of dairy cows. The "type 1" ketosis is defined as spontaneous and it is related to underfeeding, especially in the peak of lactation. In our system of monitoring we assume that this type of ketosis occurs from 22 till 60 DIM. On the other hand, a cow is in the "type 2" ketosis when she is in a negative energy balance and begins mobilizing body fat prior to or at calving (Oetzel, 2007). Fat cows are at the highest risk for this problem because they are

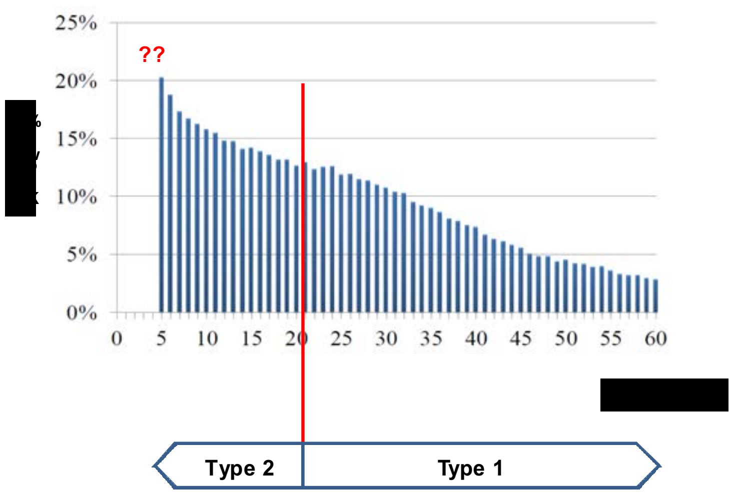


Figure 3. Prevalence of two types of ketosis in Poland depending on days in milk (DIM): type 1 (22-60 DIM) and type 2 (5-21 DIM).

prone to dry matter intake depression around calving, but thinner cows are also at risk if nutritional management during the pre-fresh and/or maternity period is poor. In our system of monitoring we assume that this type of ketosis occurs from 5 till 21 DIM. The prevalence of SCK in Poland based on such a categorization of ketosis types is presented in Figure 3. The average prevalence of type 2 ketosis was much higher than type 1 (15.2 vs. 7.2%, respectively). Unfortunately, the system of monitoring cannot consider the cows being in 2-4 DIM, but we assume that on these days the prevalence might be even higher.

The prevalence of above types of ketosis depends not only on the DIM, but also on the parity (Figure 4). The primiparous cows in Poland are much more prone to the type 2 ketosis than the older cows. In our opinion, among factors responsible for higher risk of type 2 ketosis the overfeeding (excessive body condition) is the most important. On the other hand, older cows are more than primiparous ones suspected to the type 1 ketosis, due to the higher requirements for energy which is related to the higher milk yield in the peak of lactation period. The differences among parities should be considered in the educational programme provided by the PFCBDF, including feeding and welfare.

Based on the results of one year (2014/2014) monitoring, among 20 371 dairy farms in the system, 7.3% of them have been classified as "in risk" and 11.4% as "in high risk", which means that almost 18.7% farms are considered "with ketosis". They should be included in the educational programme provided by the PFCBDF.

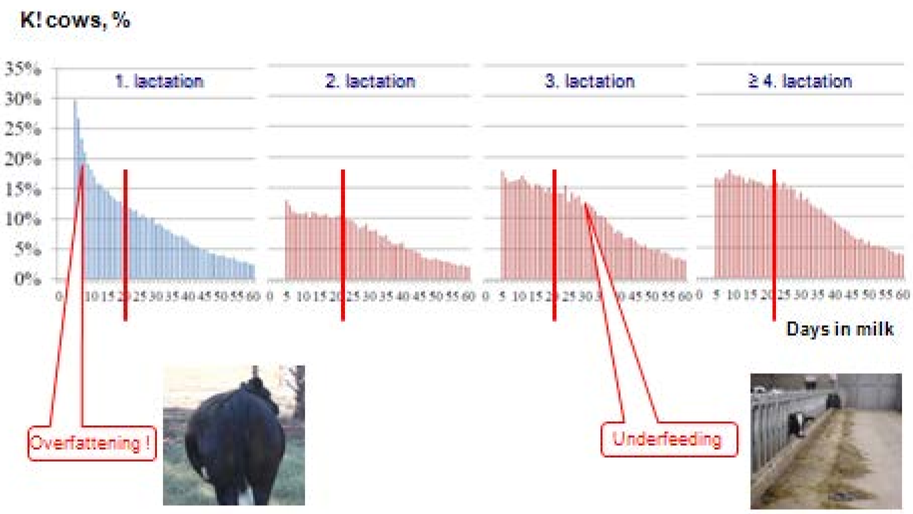


Figure 4. Prevalence of two types of ketosis in Poland depending on days in milk (DIM) and parity: type 1 (22-60 DIM) and type 2 (5-21 DIM).

Conclusions

The experience from two years of ketosis monitoring in Poland based on milk recording and milk analysis for ketone bodies using FTIR technology clearly shows that a system has been well accepted by the farmers. The prevalence of subclinical ketosis in Poland is lower than in other countries due to the differences in methodology of monitoring. However, it is still too high both on the cow and herd level for a profit of farmers. From the two year monitoring we have found that milk yield is not a risk factor of SCK in Poland. We have also found that in the categorization of types of ketosis the parity should be considered.

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