Routine testing for milk beta-hydroxybutyrate for the detection of subclinical ketosis in dairy cows

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Abstract

The milk BHB concentrations were determined semiquantitatively by means of Ketolac BHB[R] in 284 Israeli-Holstein cows (3 herds) on day 5, 6 or 7 postpartum. The prevalence of ketolactia indicating subclinical or clinical ketosis differed markedly between herds as did the incidence in cows calving in summer or in autumn. Cows with milk BHB concentrations in the range ≥ 100 - 199 μmol/l, in particular those with ≥ 200 μmol/l were at markedly higher risk to develop clinical ketosis during early lactation than cows having a lower milk BHB content at the time of testing. Relationships between increased milk BHB concentrations at the time of testing and the incidence of genital disorders as well as to impaired reproduction in the following period could be shown. Milk testing for BHB therefore, though performed only at the end of the first week postpartum, conveyed valuable informations. It seems that routine testing for ketolactia is particularly important in cows under heat stress.

Key words: milk beta-hydroxybutyrate - routine testing for milk BHB - subclinical ketosis - herd monitoring - metabolic imbalances

Introduction

Several investigations have shown that routine testing for milk ketones during early lactation of the high producing cow can indicate subclinical ketosis or predict subsequent clinical ketosis.[8,9,10,11,12] In these studies usually the nitroprusside based Rothera powder (or a similar one) has been used. It reacts mainly to acetoacetate (AcAc) and to a (markedly) less degree to acetone (Ac). However, the sensitivity of the test is relatively low, when the blood ketone body level fluctuates in the borderline range.[11] Recently a dry test for the semiquantitative determination of beta-hydroxybutyric acid (BHB) in bovine milk became available (Ketolac BHB[R] - Hoechst Roussell Vet).[5] Though the new dipstick test measures semiquantitatively, even milk BHB concentrations below the upper physiological limit, that is, below 100 μmol/l according to the current assessment, can be determined. The milk BHB test proved to be useful both for monitoring high lactating dairy cows routinely for subclinical ketosis as well as for the diagnosis of clinical ketosis in the individual cow.[3,4,6,7] The purpose of this study was to determine, whether one milk BHB determination only, performed at the end of the first week postpartum, would give an insight into the prevalence of ketolactia in the herd and would indicate cows at risk of clinical ketosis.

Materials and Methods

The milk BHB determinations by means of Ketolac BHB[R] were performed in 284 Israeli-Holstein cows, 201 multiparous, 83 primiparous, in 3 herds: herd 1 (n = 106), herd 2 (n = 84), herd 3 (n = 94). The cows were tested on day 5, 6 or 7 after calving. The milk examination was carried out according to the instructions of the producer. As proposed by the producer, the results should be assessed as follows:

0 - 49 μmol/l	normal	(-)
50 - 99 μmol/l	normal	(-)
100 - 199 μmol/l	uncertain	(±)
200 - 499 μmol/l	positive	(+)
500 - 999 μmol/l	highly positive	(++)
≥ 1000 μmol/l	highly positive	(+++)

The BHB ranges are indicated on a color chart attached to the pack. In the present study BHB concentrations of 100 μmol/l and higher were assessed as positive.

Results

Table 1 summarizes the results of the milk examination for BHB in the three herds. There turned out a marked difference in the proportion of cows with milk BHB concentrations ≥ 100 μmol/l between herd 1 and the herds 2 and 3: 26% in herd 1 against 16% and 19% in herd 2 and 3 respectively. The difference was probably caused by an oversupply of energy during dry period

Table 1. Prevalence of ketolactia (BHB) in Israeli-Holstein cows tested on day 5, 6 or 7 postpartum in 3 herds.

BHB µmol/l	< 50	50-99	100-199	≥ 200
Herd 1 (n=106)	47 (44%)	31 (29%)	17 (16%)	11 (10%)
Herd 2 (n= 84)	43 (51%)	28 (33%)	9 (11%)	4 (5%)
Herd 3 (n= 94)	49 (52%)	28 (30%)	8 (9%)	9 (10%)
Σ (n=284)	139 (50%)	87 (31%)	34 (12%)	24 (8%)

and a higher lipid mobilisation postpartum resulting therefrom in the cows of herd 1.

As shown in table 2, in the primiparous cows the proportion of cows with milk BHB concentration ≥ 100 µmol/l was numerically greater than in the multiparous (25% : 18%).

Table 2. Prevalence of ketolactia (BHB) in Israeli-Holstein cows tested on day 5, 6 or 7 postpartum: primiparous versus multiparous; 3 herds

BHB µmol/l	< 50	50-99	≥ 100
Primiparous (n = 83)	31 (37%)	31 (37%)	21 (25%)
Multiparous (n = 201)	108 (54%)	56 (28%)	37 (18%)

A marked difference in the incidence of ketolactia can be stated as well when the results obtained during the summer calving period are compared with those observed in the autumn, as shown in table 3. In the autumn, 15% of the cows had milk BHB values ≥ 100 µmol/l whereas the proportion was twice as much during the summer. The difference was most pronounced in multiparous cows (30% : 12%). The seasonal difference was probably caused by a higher energy deficit postpartum (the so-called 'energy gap') in summer due to the lowered feed intake under heat stress.

The great influence of the herd factor can be demonstrated most clearly by the incidence of clinical ketosis

Table 3. Prevalence of ketolactia (BHB) in Israeli-Holstein cows tested on day 5, 6 or 7 postpartum: summer calvings (July - September) versus autumn calvings (October - January); 3 herds.

BHB µmol/l	< 50	50-99	≥ 100
Summer calvings (n = 102)	36 (35%)[a]	36 (35%)	30 (30%)[c]
Autumn calvings (n = 182)	103 (57%)[b]	51 (28%)	28 (15%)[d]

a, b $X^2 = 11.87$ $p < 0.001$ c, d $X^2 = 7.91$ $p < 0.01$

in the three herds (table 4). As already mentioned the high incidence in herd 1 probably reflects the higher lipolysis rate in these cows due to overfeeding during the dry period.

As can be concluded from the data in table 5, cows having a milk BHB concentration ≥ 100 µmol/l on day 5, 6 or 7 postpartum are at a markedly higher risk to develop a clinical ketosis than cows with a lower milk BHB level. **The risk of subsequent clinical ketosis increases drastically at milk BHB levels ≥ 200 µmol/l.**

The proportion of cows not conceiving during 150 days postpartum as well as the proportion of cows not conceiving until 210 days postpartum or culled were significantly higher in animals having milk BHB levels ≥ 100 µmol/l on the day of testing (table 6). In this group, there was also a higher (nonsignificant) incidence of retained placenta and endometritis (table 7).

Discussion and Conclusions

When evaluating the results of the present study there has to be considered that the assessment of the milk BHB values as given by the producer, in particular the assessment 'uncertain' for 100 - 199 µmol/l, refer

Table 4. Incidence of clinical ketosis during high lactation in Israeli-Holstein cows tested for ketolactia (BHB) on day 5, 6 or 7 postpartum: variation between herds.

	Cows examined	Clinical ketosis n	%
Herd 1	106	20 ≙	18.8
Herd 2	84	2 ≙	2.3
Herd 3	94	3 ≙	3.2
Σ	284	25 ≙	8.8

Table 5. Incidence of clinical ketosis during high lactation in Israeli-Holstein cows tested for ketolactia (BHB) on day 5, 6 or 7 postpartum: relation to the milk BHB concentration on the day of testing.

BHB µmol/l	Cows examined	Clinical ketosis n		%
0 - 49	139	2	≙	1
50 - 99	87	5	≙	6
100 - 199	34	6	≙	18[a]
≥ 200	24	12	≙	50[b]

a, b X^2+ = 6.88 $p < 0.01$

Table 6. Incidence of cows not conceiving until 150/210 days postpartum or culled in relation to the milk BHB concentration on day 5, 6 or 7 postpartum in 3 Israeli-Holstein herds.

BHB µmol/l	< 50	50-99	≥100
Cows submitted to first insemination (n = 236)	114	75	47
Not conceiving until 150 days p.p.	37 (≙ 32%)	20 (≙ 27%)	23 (≙ 49%)
	57 (≙ 30%)[a]		23 (≙ 49%)[b]
Cows tested for milk BHB (n = 284)	139	87	58
Cows not conceiving until 210 days p.p. or culled	43 (≙ 31%)	28 (≙ 32%)	31 (≙ 53%)
	71 (≙ 31%)[c]		31 (≙ 53%)[d]

a, b X^2 = 5.92 $p < 0.05$ c, d X^2 = 9.73 $p < 0.01$

Table 7. Incidence of reproductive disorders in Israeli-Holstein cows tested for ketolactia (BHB) on day 5, 6 or 7 postpartum, 3 herds.

BHB µmol/l	< 50	50-99	100-199	≥ 200
Cows tested for milk BHB (n = 284)	139	87	34	24
Cows with endometritis/retained placenta	50 (36%)	30 (34%)	15 (44%)	11(46%)
	80 (≙ 35%)		26 (≙ 45%)	

to the diagnosis of clinical ketosis. In other investigations including milk Ac, urine AcAc or/and blood BHB concentrations as reference parameters there could be shown that in early lactation (3 - 4 weeks postpartum) milk BHB concentrations in the range of 100 µmol/l to 199 µmol/l as a rule indicate subclinical ketosis.[4,6,7] In the same studies at least 20 percent of the milk BHB values in the BHB range between 50 and 99 µmol/l indicated subclinical ketosis as well when the milk Ac concentration was taken as reference parameter.

It can be concluded therefore, that at least 26% of the freshly calved cows in herd 1, 16% in herd 2 and 18% in herd 3 were suffering from subclinical ketosis at the time of the milk testing. These numbers are in the range as observed in other high producing dairy herds in the first week of lactation. In a preceding study in an Israeli-Holstein herd, e.g. 20% of 94 cows had a milk Ac concentration above the threshold level of 250 µmol/l in the first week postpartum. The milk testing for BHB revealed a marked higher prevalence of subclinical ketosis in herd 1 and these data indicated a higher risk of clinical ketosis and of other postparturient problems to be expected in this herd.

Furthermore, the milk testing for BHB revealed a very much higher prevalence of subclinical ketosis in cows calving in the summer period than in those calving in autumn. As already mentioned, it is possible that the higher milk BHB levels are connected with the more pronounced negative energy balance postpartum evident in the summer.

The influence of the herd situation becomes evident too when comparing the incidences of clinical ketosis (table 4). As can be concluded from the data in table 5, cows with a milk BHB level ≥ 200 µmol/l are at high risk to develop subsequently a clinical ketosis; these cows, therefore, should be treated prophylactically. Though it is true that the majority of cows with subclinical ketosis recover spontaneously, it has been proved that many of these patients reduce their milk production during this period or do not achieve their nutritional and/or genetical production potential. Several studies revealed reduction of the daily milk yield by 5 to 30%. Beyond that, a relationship between elevated ketone body levels in the body fluids of the cow and impaired fertility has been demonstrated. There is also evidence for such an interrelationship in the data presented in this communication (table 6). Cows with milk BHB concentrations between 100 and 199 µmol/l therefore should be fed a glucoplastic substance (e.g. propylene glycol) for several days and the ration should be adjusted to the energy demand of the animal as far as possible. Concurrent diseases, if present, should be treated as well and the milk test should be repeated not later than after one week.

Finally the questions arise, whether one milk test solely can give sufficient information, what would be the best time for milk testing and in which way the herd monitoring could be optimized (in regard to the cost-benefit relation). As far as can be concluded from the few comparable data available up to now, the highest incidence of ketolactia can be observed during the first three weeks of lactation. The ketolactia profile however - the incidence per week during that period - appears to be not uniform. In a preceding study in an Israeli-Holstein herd comprising 94 freshly calved cows, the incidence of cows with increased milk Ac concentrations peaked in the first week.[7] In a German Holstein-Friesian herd (n = 49), monitored by milk Ac (quantitatively) and BHB (Ketolac BHB) determination during eight weeks postpartum, the highest incidence was observed in the second week.[7] For that reason it is advisable to evaluate the ketolactia profile first before restricting the milk testing, if necessary, to the week(s) of the highest incidence.

There is no doubt that more ketosis prone cows are filtered out when the milk BHB test is performed twice (in weeks 1 + 2 or 2 + 3) or several times; however, the present study shows that even one milk test solely performed at the end of the first week postpartum, can convey valuable information. The monitoring of all calving cows for milk BHB did not only show the prevalence of ketolactia in the herd and the risk of ketosis in the individual animal but also indicated the risk of impaired reproduction in the following period. **It seems that routine testing for ketolactia is particularly important in cows under heat stress when the incidence of positive cows is high. By prophylactic antiketogenic treatment of positive cows the economical losses can be reduced.**

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