DIAGNOSIS OF SUBCLINICAL KETOSIS IN DAIRY COWS

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Abstract: Ketosis is a common disease in high producing dairy cows during the early lactation period. Subclinical ketosis (SCK) and periparturient diseases considerably account for economic and welfare losses in dairy cows. Subclinical ketosis poses an increased risk of production-related diseases such as clinical ketosis, displaced abomasum, retained placenta, lameness, mastitis and metritis. Production efficiency decreases (lower milk production, poor fertility, and increased culling rates), which results in economic losses. Increased concentrations of circulating ketone bodies, predominantly β-hydroxybutyrate (BHB), without the presence of clinical signs of ketosis are considered as SCK. It is characterized by increased levels of ketone bodies in the blood, urine and milk. The gold standard test for ketosis is blood BHB. This ketone body is more stable in blood than acetone or acetoacetate. The most commonly used cut-points for subclinical ketosis are 1.2 mmol/L or 1.4 mmol/L for BHB in the blood. Clinical ketosis generally involves much higher levels of BHB, about 3.0 mmol/L or more. Usually, detection of SCK is carried out by testing ketone body concentrations in blood, urine and milk. A variety of laboratory and cowside tests are available for monitoring ketosis in dairy herds. But no cowside test has perfect sensitivity and specificity compared to blood BHB as the gold standard test. The aim of this review is to overview diagnostic tests for SCK in dairy cows, including laboratory and cowside tests.

Keywords: dairy cows, subclinical ketosis, laboratory tests, cowside tests
Introduction

Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows (Ospina et al., 2010; Brunner et al., 2019). Subclinical ketosis (SCK) is an important production disease of dairy cows and continues to cause significant economic losses to the dairy industry. Ketone body levels in blood, urine and milk can be monitored to detect SCK in cows, and to increase their chances of successful lactation (Geishauser et al., 2001; Seifi et al., 2011; Zhang et al., 2012). Ketosis results in decreased milk production, impaired fertility and increased frequency of other diseases. Most cases occur in the first 6 weeks to 2 months after calving. As the course of the disease is often subclinical, early detection is very important. SCK causes greater losses than clinical ketosis because it occurs more frequently and often cannot be detected by farmers (Duffield, 2000; Geishauser et al., 2000; Oetzel, 2004, 2007; Brunner et al., 2019).

Herds with ketosis problems in early lactation cows also tend to have increased incidence of displaced abomasum (>8%) and increased herd removals in the first 60 days in milk (>8%). The costs associated with SCK in affected cows are substantial, and include the loss of milk yield (up to about 7%). An additional major cost associated with high incidence of subclinical and clinical ketosis is the increased risk for numerous other health disorders, such as displaced abomasum, mastitis, metritis, lameness and reduced reproductive efficiency (Cook et al., 2001; Oetzel, 2007; Duffield et al., 2003; Seifi et al., 2011; Brunner et al., 2019).

Across the world, SCK prevalence was 24.1%, ranging from 8.3% to 40.1% (Brunner et al., 2019). The prevalence of subclinical ketosis in ten European countries was on average 21.8% (ranging from 11.2 to 36.6%), and clinical ketosis was 3.7% (0.4 to 11.1%). The prevalence of subclinical ketosis in Serbia was up to 19.5% in 42 herds (Suthar et al., 2013).

On average, 40% of cows have SCK at least once during lactation, while clinical ketosis affects on average 5% of cows (Oetzel, 2004; Suthar et al., 2013). However, some reports have indicated that the incidence of SCK may affect 40% of cows, with the incidence rate varying widely among farms, and may be as high as 80% on individual farms (Jenkins et al., 2015; Brunner et al., 2019).

The gold standard test for ketosis is blood BHB. This ketone body is more stable in blood than acetone or acetoacetate (Tyopponen and Kauppinen, 1980). The most commonly used cut-point for SCK is ≥ 1.2mmol/l of blood BHB (Geishauser et al., 1998; Duffield, 2000; Duffield and Bagg, 2002; Zhang et al., 2012; Djokovic et al., 2013). Early lactation cows with blood BHB concentrations above this cut-point are at threefold greater risk to develop displaced abomasum or clinical ketosis, and cows with blood BHB concentrations above 2.0 mmol/L are at risk for reduced milk yield (Duffield, 2000). Some studies use a slightly higher cut-point 1.4mmol/L of blood BHB for defining SCK (Geishauser et al., 2000; Carrier
Diagnosis of subclinical ketosis in dairy cows

et al., 2004; Oetzel, 2004, Iwersen et al., 2009). The exact cut-point chosen usually has a minor effect on the interpretation of herd-based results. Clinical ketosis generally involves much higher levels of BHB, about 3.0 mmol/L or more (Oetzel, 2007).

A variety of laboratory and cowside tests are available for monitoring ketosis in dairy herds. Diagnosing ketosis in a herd requires a completely different diagnostic approach than diagnosing ketosis in an individual cow. Comparing blood BHB results from a small number of cows to normal ranges is not appropriate. Herd-based testing is performed by subsampling 12 or more dairy cows, representative of the animals at risk for ketosis (about 5 to 50 days in milk), followed by the evaluation of the proportion of cows above the cut-point of 1.4 mmol/L. The alarm level for the proportion of cows above this cut-point shows an average ketosis prevalence of about 15% (Duffield and Bagg, 2002). The other author suggests using 10% as the alarm level for herd-based ketosis testing (Oetzel, 2004).

Milk is a very suitable sample for the determination of ketosis as it can easily be collected by farm personnel compared to blood and urine samples. In cases of SCK, the content of BHB in milk is increased but concentrations are lower than in the blood (Samiei et al., 2010). BHB concentration in milk can be measured in the field by using the semi-quantitative colorimetric dipstick test. The cut-off value is 100 to 200 μmol/L and higher values indicate ketosis (Dirksen and Breitner, 1993; Eicher, 2004; Oetzel, 2007).

This review aims to overview diagnostic tests for SCK in dairy cows, including laboratory and cowside tests.

**Types of ketosis in dairy herds**

Herd ketosis problems can be categorized into three general types of ketosis (Table 1). Each type is of different etiology and therefore requires a different prevention and diagnosis strategy. There is an overlap between the categories, and herds may have a combination of the types. These classification types are largely adapted from Swedish authors (Holtenius and Holtenius, 1996) and have been described in detail (Herdt, 2000).
Table 1. Summary of types of ketosis observed in dairy herds (modified from OETZEL, 2007)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III. Butyric Acid Silage Ketosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest risk period</td>
<td>3 to 6 weeks after calving</td>
<td>1 to 2 weeks after calving</td>
<td>Very high or high</td>
</tr>
<tr>
<td>Description</td>
<td>Spontaneous, underfeeding</td>
<td>Fat cows, fatty liver</td>
<td>Normal or High</td>
</tr>
<tr>
<td>Blood BHB</td>
<td>Very high</td>
<td>High</td>
<td>Variable</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>Low</td>
<td>Low (may be high initially)</td>
<td>Variable</td>
</tr>
<tr>
<td>Blood insulin</td>
<td>Low</td>
<td>Low (may be high initially)</td>
<td>Variable</td>
</tr>
<tr>
<td>Body condition</td>
<td>Probably thin</td>
<td>Often fat (or lost fat)</td>
<td>Variable</td>
</tr>
<tr>
<td>Fate of NEFA</td>
<td>Ketone bodies</td>
<td>Liver triglycerides initially, then ketone bodies</td>
<td>Variable</td>
</tr>
<tr>
<td>Liver gluconeogenesis</td>
<td>High</td>
<td>Low</td>
<td>Variable</td>
</tr>
<tr>
<td>Liver pathology</td>
<td>None</td>
<td>Fatty liver</td>
<td>Variable</td>
</tr>
<tr>
<td>Prognosis</td>
<td>None</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Key diagnostic test</td>
<td>Post-fresh BHB</td>
<td>Pre-fresh NEFA</td>
<td>Silage analysis</td>
</tr>
<tr>
<td>Key intervention</td>
<td>Post-fresh management and nutrition</td>
<td>Pre-fresh management and nutrition</td>
<td>Destroy, dilute or divert the silage</td>
</tr>
</tbody>
</table>

Diagnosis of ketosis

Laboratory tests

Enzyme catalysis method: The enzyme catalysis method is a traditional test for blood serum BHB determination in cows. The test kit requires the use of an ultraviolet spectrophotometer or biochemistry analyzer and can be used to determine blood serum BHB values in humans and animals (Zhang et al., 2012). This is the gold standard for the detection of ketosis in dairy cows. The most commonly used blood BHB cut-points for SCK are ≥1.2 mmol/L (Geishauser et al., 1998; Duffield, 2000; Duffield and Bagg 2002; Zhang et al., 2012; Djokovic et al., 2013) or ≥1.4 mmol/L (Geishauser et al., 2000; Carrier et al., 2004; Oetzel, 2004; Iwersen et al., 2009) or only ≥ 1.0 mmol/L (Whitaker, 1997; Kinoshita et al., 2010; Ospina et al., 2010; Jozek et al., 2017; Djokovic et al., 2018a). BHB concentrations in blood and milk sera can be measured using a biochemical analyzer (Samiei et al., 2010). A statistically significant correlation (r=0.705, p<0.01) was determined between BHB concentrations in blood and milk sera of cows. The best sensitivity (94%) and specificity (74%) were observed for BHB measurements in milk, with the optimal cut-point for BHB in milk of ≥ 80 µmol/L,
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Fourier transform infrared (FTIR) spectrometry method: As a diagnostic method for screening dairy cows for SCK by determining milk acetone concentration using FTIR spectrometry (Hansen, 1999; Heurer et al., 2001), FTIR spectrometry is fast, inexpensive and easy to implement on a large scale. FTIR spectrometry was used to measure the concentration of BHB and acetone in milk to detect SCK. The sensitivity and specificity of this method is 70% and 95%, respectively, with 25 to 27% false positives and 6 to 7% false negatives, when using cut-points of 0.15mmol/L for acetone and 0.1mmol/L for BHB (De Roos et al., 2007). When testing BHB and acetone concentrations in milk by FTIR, higher sensitivity (80%) and specificity (71%) were obtained in the detection of SCK compared to blood BHB, with the cut-point of ≥1.2 mmol/L (Van Kogne et al., 2010). According to previous research, the FTIR test based on milk acetone and BHB values is valuable in the detection of dairy cows for SCK (Zhang et al., 2012).

Fluorometric determination of BHB levels: The fluorometric determination of BHB values in milk and blood plasma based on an enzymatic method has been described (Williamson et al., 1962, 1974; Larsen and Nielsen, 2005). This fluorometric method correlated closely with results obtained by the traditional spectrophotometric method (r=0.987, p<0.001) (Larsen and Nielsen, 2005). The advantages of the fluorometric determination of BHB are that detection results are not affected by the hemolysis of blood samples, and that whole milk samples do not need pre-treatment. This method is excellent for large numbers of samples, especially for large-scale in-line sampling of milk (Larsen and Nielsen, 2005; Zhang et al., 2012).

Gas liquid chromatography (GLC) to test acetone levels in blood and milk: GLC method using N-propanol as an internal standard is valuable for the determination of acetone and BHB in milk and blood serums (Enjalbert et al., 2001; Zhang et al., 2012). The best cut-point for blood acetone was 175 μmol/l, with the sensitivity of 91.7% and specificity of 68.3%, and for milk acetone 160 μmol/L, with 90% sensitivity and 57.4% specificity, compared to the gold standard ketosis test (blood BHB), cut-point >1.2 mmol/L. The cut-point level for acetone in milk was 400 μmol/L or higher for ketotic cows (Cook et al., 2001), and the same cut-point level was used in detecting acetone in milk by the qualitative and quantitative salicylaldehyde test for SCK in dairy cows (Venkateswarlu and Choudhuri, 2001).

Nuclear magnetic resonance (NMR) spectroscopy and gas chromatography-mass spectrometry (GC-MS) to test acetone and BHB values: High-resolution NMR spectroscopy and GC-MS were used to determine acetone and BHB values in the blood (Klein et al., 2010; Zhang et al., 2012).
experiment, acetone and BHB were only used as indicators of energy metabolism during the transitional period in dairy cows, and the diagnosis effect of SCK was not investigated (Klein et al., 2010).

**Cowside tests for ketosis**

Different types of cowside tests are available for monitoring ketosis in dairy herds. However, none of the cowside tests have perfect sensitivity and specificity compared to blood BHB. Sensitivity is true positive results compared to the gold standard ketosis test (blood BHB) and specificity is true negative results compared to the gold standard ketosis test (blood BHB). Therefore, the gold standard ketosis test (blood BHB) is the most accurate for herd monitoring, and is particularly suitable for investigating herds with ketosis. Cowside ketosis tests have the advantages of lower cost, less labor and immediate results, when compared to blood BHB testing. This makes them particularly useful for making (or excluding) a clinical diagnosis of ketosis in individual sick cows. However, testing herds for ketosis requires a very different testing strategy compared to diagnostic decision-making for individual sick cows (Oetzel, 2007).

**Cowside urine tests for ketosis:** Urine can be evaluated for cowside ketosis testing. However, it is much more difficult to collect a urine sample than a cowside milk sample. This is an important practical limitation on farms, which greatly increases labor costs for testing. Urine acetoacetate can be evaluated quantitatively by nitroprusside tablets (Acetest, Bayer Corp. Diagnostics Division, Elkhart, IN). This test has excellent sensitivity but poor specificity (Nielen et al., 1994; Carrier et al., 2004; Oetzel, 2004). This makes it a useful test for evaluating individual sick cows, but not very useful for herd-based monitoring (Osborne et al., 2002; Oetzel, 2007).

**Cowside milk (Nitroprusside powder) tests for ketosis:** Cowside milk tests have huge advantages over urine cowside tests for ease of collection and for certainty that all eligible cows can be tested. However, milk tests are generally not as sensitive as urine tests in detecting ketosis. Nitroprusside powders (Utrecht powder, Keto Check powder) can be used to qualitatively test milk acetoacetate. However, these tests generally have very poor sensitivity (Table 2) for ketosis compared to blood BHB and cannot be recommended as tests for herd-based monitoring. They have some, but very limited value as cowside tests for diagnostic decisions for individual cows (Osborne et al., 2002; Eicher, 2004; Oetzel, 2007).
Table 2. Sensitivity and specificity of cowside milk nitroprusside powders compared to blood BHB (cut-point of ≥1.2 mmol/L or ≥1.4 mmol/L)(modified from OETZEL, 2007)

<table>
<thead>
<tr>
<th>Test type/study</th>
<th>BHB Cut-point</th>
<th>Herds tested</th>
<th>% ketosis</th>
<th>Total samples</th>
<th>TP</th>
<th>FN</th>
<th>FP</th>
<th>TN</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utrecht powder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nielen et al., (1994)</td>
<td>≥1.4mmol/L</td>
<td>18</td>
<td>10.3%</td>
<td>185</td>
<td>17</td>
<td>2€</td>
<td>7</td>
<td>159</td>
<td>89%</td>
<td>96%</td>
</tr>
<tr>
<td>Geishauser et al., 1998</td>
<td>≥1.2mmol/L</td>
<td>25</td>
<td>16.4%</td>
<td>529</td>
<td>37</td>
<td>50</td>
<td>0</td>
<td>442</td>
<td>43%</td>
<td>100%</td>
</tr>
<tr>
<td>KetoCheck powder (≥trace)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geishauser et al., (1998)</td>
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<td>25</td>
<td>16.4%</td>
<td>529</td>
<td>24</td>
<td>63</td>
<td>0</td>
<td>442</td>
<td>28%</td>
<td>100%</td>
</tr>
<tr>
<td>Carrier et al., (2004)</td>
<td>≥1.4mmol/L</td>
<td>1</td>
<td>7.5%</td>
<td>878</td>
<td>28</td>
<td>38</td>
<td>9</td>
<td>803</td>
<td>42%</td>
<td>99%</td>
</tr>
<tr>
<td>Bioketone powder (≥trace)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>529</td>
<td>24</td>
<td>63</td>
<td>0</td>
<td>442</td>
<td>28%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Legend: BHB – beta-hydroxybutyric acid, mmol/L; Ketosis – blood BHB ≥1.2 mmol/L or ≥1.4mmol/L; TP – true positives, FN –false negatives; FP – false positives; TN – true negatives.

Cowside milk (BHB) tests for ketosis: The most promising cowside milk ketone test used is a semi-quantitative milk BHB test strip manufactured by Sanwa Kagaku Kenkyusho Co., Ltd. (Nagoya, Japan). This test strip is marketed under various names (KetoTest, Ketolac BHB, and Sanketo paper) in different parts of the world. Results of numerous studies evaluating the sensitivity and specificity of the milk BHB test strip compared to blood BHB results are presented in Table 3. When used at the cut-point of ≥100 μmol/L, this test is about 83% sensitive and 82% specific. For individual cow testing, the cut-point of ≥50 μmol/L provides better sensitivity (89%) but has a false positive rate of 69%. Increasing the cut-point to ≥200 μmol/L reduces test sensitivity to 54% (Table 3). At this higher cut-point, the test is of little value for diagnosing ketosis in individual sick cows but has potential use for herd-based evaluations. The best cut-point for herd monitoring when using the milk BHB strip appears to be ≥200 μmol/L. At this cut-point, the prevalence of positive test results is similar to true prevalence, allowing the same alarm level for ketosis prevalence (10%) to be used for both tests. Unfortunately, milk BHB test strip prevalence changes little as true prevalence increases, making the test practically useful only for identifying herds with a very high prevalence of ketosis (Oetzel, 2007).
Table 3. Sensitivity and specificity of cowside milk BHB test compared to blood BHB (cut-point of ≥1.4 mmol/L) (modified from OETZEL, 2007)

<table>
<thead>
<tr>
<th>Test type/Study</th>
<th>Herds Tested</th>
<th>% Ketosis</th>
<th>Total Samples</th>
<th>TP</th>
<th>FN</th>
<th>FP</th>
<th>TN</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk BHBA strip (≥ 50 µmol/L)</td>
<td>21</td>
<td>11.9%</td>
<td>469</td>
<td>51</td>
<td>5</td>
<td>182</td>
<td>231</td>
<td>91%</td>
<td>56%</td>
</tr>
<tr>
<td>Geishauser et al. (2000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Carrier et al. (2004)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oetzel, 2004</td>
<td>17</td>
<td>17.2%</td>
<td>221</td>
<td>34</td>
<td>4</td>
<td>36</td>
<td>147</td>
<td>89%</td>
<td>80%</td>
</tr>
<tr>
<td>Pooled data (by cow)</td>
<td>39</td>
<td>10.2%</td>
<td>1573</td>
<td>144</td>
<td>318</td>
<td>318</td>
<td>1094</td>
<td>89%</td>
<td>77%</td>
</tr>
<tr>
<td>Milk BHB strip (≥100 µmol/L)</td>
<td>Jorritsma et al. (1998)</td>
<td>8</td>
<td>8.4%</td>
<td>190</td>
<td>14</td>
<td>2</td>
<td>31</td>
<td>143</td>
<td>88</td>
</tr>
<tr>
<td>Geishauser et al. (2000)</td>
<td>21</td>
<td>11.9%</td>
<td>469</td>
<td>45</td>
<td>11</td>
<td>99</td>
<td>314</td>
<td>80</td>
<td>76%</td>
</tr>
<tr>
<td>Carrier et al. (2004)</td>
<td>1</td>
<td>16.5%</td>
<td>248</td>
<td>39</td>
<td>2</td>
<td>65</td>
<td>142</td>
<td>95</td>
<td>69%</td>
</tr>
<tr>
<td>Duffield et al. (2003)</td>
<td>5</td>
<td>27.2%</td>
<td>235</td>
<td>52</td>
<td>12</td>
<td>64</td>
<td>107</td>
<td>81</td>
<td>63%</td>
</tr>
<tr>
<td>Carrier et al. (2004)</td>
<td>1</td>
<td>7.6%</td>
<td>883</td>
<td>50</td>
<td>17</td>
<td>54</td>
<td>762</td>
<td>75</td>
<td>93%</td>
</tr>
<tr>
<td>Oetzel, (2004)</td>
<td>17</td>
<td>17.12%</td>
<td>221</td>
<td>33</td>
<td>5</td>
<td>32</td>
<td>151</td>
<td>87</td>
<td>83%</td>
</tr>
<tr>
<td>Pooled data (by cow)</td>
<td>53</td>
<td>12.6%</td>
<td>2246</td>
<td>233</td>
<td>49</td>
<td>345</td>
<td>1619</td>
<td>83</td>
<td>82%</td>
</tr>
<tr>
<td>Milk BHB strip (≥200 µmol/L)</td>
<td>Jorritsma et al., (1998)</td>
<td>8</td>
<td>8.4%</td>
<td>190</td>
<td>14</td>
<td>2</td>
<td>31</td>
<td>143</td>
<td>88</td>
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<td>99</td>
<td>314</td>
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<td>17.2%</td>
<td>221</td>
<td>17</td>
<td>21</td>
<td>5</td>
<td>178</td>
<td>45%</td>
<td>97%</td>
</tr>
<tr>
<td>Pooled data (by cow)</td>
<td>52</td>
<td>12.1%</td>
<td>1998</td>
<td>129</td>
<td>112</td>
<td>100</td>
<td>1657</td>
<td>54%</td>
<td>94%</td>
</tr>
</tbody>
</table>

Legend: BHB – beta-hydroxybutyric acid, mmol/L; Ketosis – blood BHB ≥1.4 mmol/L; TP – true positives, FN – false negatives; FP – false positives; TN – true negatives.
**Cowside blood tests for ketosis:** A cowside blood BHB test system using a human instrument marketed for diabetic patients (Precision Xtra™ blood glucose and ketone monitoring system, Abbott Laboratories, Abbott Park, IL). The glucometer/ketometer system is very easy to use cowside. A strip is inserted in the meter, less than a drop of blood is added to the end of the strip, and results are displayed in about 15 seconds. The strips do not require calibration prior to use. It is necessary to collect a small amount of blood from the tail vein using a small needle (25 gauge) and a small syringe (1 mL) to use with the glucometer/ketometer. The preliminary results with the glucometer/ketometer system are very encouraging. The system is more accurate as a ketometer (for BHB) than as a glucometer (glucose). Sensitivity and specificity for BHB appear to be outstanding (over 95%), (Heuwieser et al., 2007; Oetzel, 2007; Konkol et al., 2009; Voyvoda and Erdogan, 2010).

**Other tests for the detection of SCK in dairy cows**

**Fat and protein in milk:** Dairy cows suffer from negative energy balance (NEB) during the first two weeks of lactation, a high mobilization of lipids from body fat reserves, ketogenesis and hypoglycaemia (Elitok et al., 2010; Djokovic et al., 2014, 2015). A portion of the free fatty acids that are mobilized are directly incorporated into milk fat, resulting in an increase in milk fat percentage. By contrast, milk protein percentage will slightly decrease in these cows due to a reduction in energy supply. Fat to protein ratio (FPR) in milk is used to monitor the prevalence of SCK in a herd (Eicher, 2004; Richard, 2004; Gantner et al., 2009; Jenkins et al., 2015). AFPR greater than 1.5 indicates SCK, whereas a FPR lower than 1.1 indicates rumen acidosis (Cejna and Chladek, 2005). Using a blood BHB level of 1.2 mmol/L or higher as a cut-point concentration for SCK, both the test-day fat percentage and the test-day protein percentage were significantly associated with SCK (Duffield et al., 1997). The specificity of FPR for the detection of SCK was lower (77-81%) than cowside milk (BHB) tests (KetoLac BHB test with a cut-point of 200 µmol/L of BHB in milk) and cowside urine tests (KetoStix test), (97 - 99%) (Krogh et al., 2011). Jenkins et al. (2015) reported high sensitivity of FPR > 1.42 or lower (> 1.35 or > 1.25) for SCK, and found that these cut-points could be used as a screening test. FPR is a good measure of SCK at the whole herd level, but it is not sensitive enough for the diagnosis of SCK in individual cows (Zhang et al., 2012).

**Nonesterified fatty acid (NEFA):** The optimal cut-point for NEFA in blood serum for SCK, by the ROC A, was >0.26 mmol/L, with 82.54% sensitivity and 91.89% specificity, compared to BHB in the blood with a cut-point of >1.2 mmol/L as the gold standard test (Aslet et al., 2011).
**Blood biochemical indicators of ketosis:** Based on blood biochemical indicators, SCK in cows may be diagnosed in the following values of these indicators (BHB >1.2 mmol/L, glucose <2.5 mmol/L, NEFA>0.26 mmol/L and TG <0.12 mmol/L) and blood values of NEFA >0.7 mmol/L and AST activity above 100 IU/L, which is indicative of hepatic lipidosis (Oetzel, 2004; Gonzalez et al., 2011; Djokovic et al., 2013, 2018a).

**Conclusions**

Measurement of blood BHB values in serum or plasma is the gold standard test for the diagnosis of SCK. The most commonly used cut-points for SCK are ≥ 1.2 mmol/L or ≥1.4 mmol/L for BHB in the blood. The cowside blood BHB test using a hand-held meter (ketometer) has higher levels (above 95%) of sensitivity and specificity than other cowside tests, and can replace laboratory blood BHB testing. The cowside milk (BHB) test (a Ketolac BHB test strip with a cut-point of ≥200 µmol/L of BHB in milk) is a potentially useful tool for routine herd monitoring for SCK in early lactation dairy cows.

**Dijagnoza subkliničke ketoze kod mlečnih krava**

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**Rezime**

Standardnizlatni test za dijagnozu subkliničke ketoze kod mlečnih krava jeste merenje koncentracije beta-hidoksibuterne kiseline (BHB) u krvnom serumu ili krvnoj plazmi. Najčešće korišćene granične vrednosti za dijagnozu subkliničke ketoze jesu vrednosti za BHB u krvi veće od 1.2 mmol/L ili veće od 1.4 mmol/L. Cowside blood test zaodređivanje koncentracije BHB u krvi jeste jednostavni ručni uređaj (ketometar) i može se upotrebljavati na farmi, a ovaj test ima visoki procenat (više od 95%) senzitivnosti i specifičnosti u odnosu na druge cowside testove i može zameniti laboratorijska ispitivanja za testiranje BHB u krvi u dijagnozi subkliničke ketoze. Cowside milk BHB test, odnosno ketolac BHB test trakice sa graničnim vrednostima većim od 200 µmol/L za BHB u mleku je supotencijalno korisno sredstvo za rutinski pregled stada mlečnih krava na početku laktacije za dijagnozu subkliničke ketoze.

**Ključne reči:** mlečne krave, subklinička ketoza, laboratorijski testovi, cowside testovi.
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