Milk acetone determination by the photometrical method after microdiffusion and via FT infra-red spectroscopy

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Abstract
Milk acetone (AC) and betahydroxybutyrate (BHB) are important indicators of the energy metabolism of cows (ketosis occurrence) and an effective method for their determination, with reliable results, is of great importance. The goal of this work was to investigate the infrared method MIR-FT in terms of its calibration for milk AC and to develop a usable procedure. The microdiffusion photometric (485 nm; Spekol 11) method was used with salicylaldehyde as a reference (Re) and mid infrared spectroscopy FT (MIR-FT: Lactoscope FT-IR, Delta; MilkoScan FT 6000, M-Sc) as an indirect method. The acetone addition to milk had no recovery using MIR-FT (Delta). The reference AC set must have acceptable statistics for good MIR-FT calibration (M-Sc) and they were: 10.1 ± 9.74 at a geometric mean of 7.26 mg l–1, and a variation range from 1.98 to 33.66 mg l–1. The AC correlation between Re and MIR-FT (M-Sc) was markedly better at 0.80 (P<0.01). The conversion of >10 mg l–1 as an AC subclinical ketosis limit could be > –0.80 (feedback 0.158 mmol l–1 = 9.25 mg l–1) and > –1.66. This could be important for ketosis monitoring (using M-Sc).

Key words: cow; raw milk; ketosis; acetone; betahydroxybutyrate; reference sample; spectrophotometry; infrared spectroscopy; calibration; validation

Abbreviations:
AC acetone
BCS body condition score
MIR-FT mid infrared spectroscopy with Fourier's transformations
M-Sc Milko Scan FT 6000
MSs milk samples
NEB negative energy balance
NRL-RM National reference laboratory for raw milk
Re reference

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INTRODUCTION

Milk ketones (acetone) importance

Ketone (acetone; AC) concentration in milk is a suitable indicator of energy metabolism in terms of the terminal stage of body fat reserve catabolism (metabolic destruction) and ketosis expansion in dairy cows (Gravert et al. 1986, Gustafsson and Emanuelson 1993, Geishauser et al. 1997, Hanuš et al. 1999, Khaled et al. 1999, Mottram and Masson 2001, Mottram et al. 2002, Wood et al. 2004). Therefore AC is a metabolic milk indicator for the successful control of the negative energy balance (NEB) of cows, which is a hazardous period in lactation often beginning with a higher AC level in the milk.

We investigated the significant negative correlation (r = –0.47 and –0.42) to feedstuff energy and also to milk yield (r = –0.30; Gravert et al. 1991) in dairy cows with an increased AC content (>0.25 mmol l–1) in early lactation. The heritability for milk AC content was 0.30 (Gravert et al. 1991) and was similar to the milk yield coefficient. Therefore the implementation of milk AC evaluation as an indicator of energy balance in milk recording, including the determination of breeding value, is recommended. The genetic (breed) aspects of subclinical and clinical ketosis occurrence and milk AC were also studied (Mäntysaari et al. 1991, Wood et al. 2004), and the use of milk AC during lactation as an indicator of the energy state for genetic improvement of feed intake and energy utilization by dairy cows is recommended (Gravert et al. 1986, 1991). Positive correlations (r = 0.30 and more; P<0.001) were found between the logarithm of AC and the fat/protein ratio (Hanuš et al. 2011c, d) in cow milk which is another indicator of cow energy metabolism (Geishauser and Ziebell 1995, van Knegsel et al. 2010). This coefficient confirmed the predictive ability of both indicators; in particular, AC in blood and milk is highly correlated (r = 0.96; Enjalbert et al. 2001).

Prevention is especially important (Miettinen 1995, Gasteiner 2000, 2003) for lowering the economic losses caused by ketosis. Effective diagnoses and monitoring are necessary for good prevention of subclinical ketosis (Hanuš et al. 1999, 2011c, d), but the diagnostic level (relevant discrimination ketone or acetone limit) is still not accepted in a uniform way. It varies from 2 to 41 mg l–1 (from 0.03 to 0.7 mmol l–1; Gustafsson and Emanuelson 1993) for milk AC, but mostly from 7 to 23 mg l–1 (from 0.12 to 0.4 mmol l–1; Gravert et al. 1986, Miettinen 1995, Hanuš et al. 1999, Gasteiner 2000). The determination of the discrimination (diagnostic threshold) limit depends on the definition of subclinical ketosis. It is sometimes seen as defined daily milk yield decrease (Gustafsson and Emanuelson 1993, Miettinen 1994, Heuer et al. 2001, Janů et al. 2007) during early lactation and at other times as body condition score (BCS) loss (Hanuš et al. 1999). The BCS method was also evaluated in terms of the prediction of dairy cow NEB (Heuer et al. 1999, Gasteiner 2003) in ketosis prevention. Other authors (Miettinen 1995, Green et al. 1999, Gasteiner 2003) have tried to modify early lactation metabolism and the corresponding malnutrition, using supplements of various glucoplastic and hepatoprotective substances (such as propylene glycol, monensine, Silybum marianum seeds) into the feed rations of dairy cows.

Some methods of milk acetone determination

A method of determining photometrical milk AC by microdiffusion into an alkaline solution of salicylaldehyde has already been described (O’Moore 1949, Vojtíšek 1986). Recently, MIR-FT (Fourier transform mid infrared spectroscopy) milk AC determination was introduced as a suitable possibility for the determination of milk ketone (Hansen 1999, Heuer et al. 2000, de Roos et al. 2006, van Knegsel et al. 2010). The calibration of this procedure to measure milk AC and beta-hydroxybutyrate (BHB) was carried out according to the results of the chromatography method. Also, various stable tests for the semi-quantitative detection of ketones in urine and milk were investigated, compared and evaluated successfully as effective (Geishauser et al. 1997, Carrier et al. 2004). These were based mostly on various modifications of the nitropruside reaction. Hanuš et al. (1999) evaluated the milk stable Ketotest and found it efficient for ketosis monitoring. Enjalbert et al. (2001) mentioned the determination of milk beta-hydroxybutyrate by enzymatic analysis and using the stable strip test Ketolac. They found good diagnostic efficiency for both methods. Ketolac showed valuable results with a threshold concentration from 70 to 100 μmol l–1. They evaluated the practical simplicity of Ketolac very positively.

The aims of this paper were: to verify the possibilities of the modern MIR-FT method in terms of its calibration to milk AC (ketones) determination; to develop a practical method for the preparation of relevant reference (calibration)
standard samples; to describe and evaluate aspects of calibration and result reliability, and to evaluate the possibilities of proficiency testing.

MATERIAL AND METHODS

Reference and indirect milk acetone determination

In this work, AC was investigated by spectrophotometry measurement at a wavelength of 485 nm using a Spekol 11 apparatus (Carl Zeiss Jena, Germany). AC was absorbed into an alkali solution of KOH with salicylaldehyde (O’Moore 1949, Vojtíšek 1986) for 24 hours microdiffusion in special vessels which were hermetically closed and cultivated in darkness at a temperature of 25 ºC. The conditions were comparable both for MSs and for the scale of reference samples. The Spekol was calibrated by a reference sample scale with five points of AC increase from 1 to 20 mg l⁻¹. The method was used to determine reference (Re) values.

An MIR-FT – in technical design as Lactoscope FT-IR (Delft Instruments, The Netherlands) – and MilkoScan FT 6000 (M-Sc; Foss Electric, Denmark) were used as an indirect method which was calibrated or controlled under the conditions mentioned in the Czech National reference laboratory for raw milk (NRL-RM) at the Research Institute for Cattle Breeding in Rapotín and in the Laboratory for Milk Analysis (routine laboratory for milk quality and milk recording) in Buštěhrad, both under the umbrella of the Czech Moravia Breeders Association in Prague. The instruments were operated according to the relevant producer instruction manuals.

Milk samples for ketone and acetone investigation method comparison

Various sample milk data sets were used for experimental AC calibration evaluation:

I) Individual cow milk samples (MSs; n = 224) from milk recordings of a randomly selected dairy herd were analysed using M-Sc on log AC and log BHB, and their mutual relationship was characterized.

II and III) Individual MSs (n = 11; n = 37) selected in a dairy herd which was suspected of ketosis occurrence according to veterinary investigation with the goal of obtaining especially higher AC concentrations according to M-Sc preliminary results in the milk recording. MSs were analysed on AC content using the reference method (Re) and the results were compared with the MIR-FT method (M-Sc and Delta). Other composition and properties of milk samples were as follows: II for n = 11, fat 3.24±0.47, protein 3.58±0.18, lactose monohydrate 5.06±0.12%, urea 29.58±3.75 mg 100 ml⁻¹, somatic cell count 206±156 10⁴ ml⁻¹; III for n = 37, 3.91±1.21, 3.16±0.46, 4.92±0.35%, 26.06±7.26 mg 100 ml⁻¹, 104±183 10³ ml⁻¹.

IV) The milk sample was modified with the goal of increasing the AC content to reach the upper value of the calibration line. The AC was increased by the method of artificial addition using reference standards (water solutions) for the Spekol method calibration line. The increase was performed by 10 as minimal and 40 mg l⁻¹ as maximal AC addition into the milk. The original milk had approximately 5 mg l⁻¹ of basic AC content. The modified samples were measured using the Re and MIR-FT (Delta) method.

V) Individual MSs (n = 14) were collected in a high yield dairy herd of Holstein cows (10 000 kg of milk per standard lactation) where a high probability of ketosis occurrence existed according to previous investigations (Hanuš et al. 2011c, d) and the above mentioned literature overview. Animals in the first lactation month were sampled in the milking parlour during morning milking. The goal was to obtain all the higher AC values and an acceptable variation range in the calibration sample set to achieve an objective assessment of the relationships between the results of the methods for the determination of AC in milk. The MSs obtained were analysed for AC content using the reference method (Re) and the results were compared with the MIR-FT method (M-Sc and Delta).

Statistical evaluation of calibrations and result validation

Milk AC concentration values were used in their original form (mg l⁻¹) and also after logarithmic transformation (log₁₀) because they usually did not exhibit a normal frequency data distribution. Under such conditions the arithmetical mean may not be a suitable representative parameter of AC data files and the geometric mean and median use is better. The evaluation of the original AC (BHB) data or log data was used differently depending on the specific case, with the goal of obtaining a maximal determination value. Regression analysis was used to evaluate the calibration and validation results with a comparison of correlation coefficients. The evaluation was processed in the Microsoft Excel programme.
RESULTS AND DISCUSSION

The best way to support and improve milk quality in terms of the metabolic state for the processing of milk products and for good human (consumer) health, is to perform an efficient system of regular investigation (Hanuš et al. 1999, 2011c, d), monitoring (in early lactation) and interpretation of the results of milk ketones, and consequently a ketosis prevention (Miettinen 1995, Gasteiner 2000, 2003, Mottram and Masson 2001, Mottram et al. 2002; Table 1). This was our main aim.

Table 1. Possible practice interpretation scheme for the milk acetone – subclinical ketosis problem

<table>
<thead>
<tr>
<th>Diagnostical indication values of milk acetone (mg l⁻¹)</th>
<th>Diagnostic interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>preserved roughage feeding (winter, eventually also summer season) –</td>
<td>&lt;7</td>
</tr>
<tr>
<td>green zone of feeding (summer season) –</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

**Interpretation**

<table>
<thead>
<tr>
<th>diagnosis</th>
<th>healthy dairy cow</th>
<th>possible start of subclinical ketosis</th>
<th>less important subclinical ketosis</th>
<th>important subclinical ketosis</th>
<th>possibility of clinical ketosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>direct and indirect risks</td>
<td>–</td>
<td>–</td>
<td>ketosis start</td>
<td>reproduction aggravation</td>
<td>reproduction and milk yield</td>
</tr>
<tr>
<td>associate events</td>
<td>–</td>
<td>–</td>
<td>decrease of feed input, increase</td>
<td>decrease of feed input,</td>
<td>reproduction and quality</td>
</tr>
<tr>
<td>and symptoms</td>
<td>–</td>
<td>–</td>
<td>of milk fat, body condition loss</td>
<td>increase of milk fat, body</td>
<td>aggravation, abomasum</td>
</tr>
<tr>
<td>clinical symptoms</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>condition loss</td>
<td>displacement, acidosis, liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>steatosis, immunosupression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>and mastitis</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Framework appropriate prevention and treatment measures</th>
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<tbody>
<tr>
<td>measures</td>
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</table>
Measurement of log AC and log BHB on the original (delivered by Foss Electric) calibration level of M-Sc showed a good relationship between ketones. Correlation coefficient \( r = 0.89 \) \((P<0.001)\) was calculated. 79.8% of result variability in log BHB is explainable by variability in the log AC results (Fig. 1). This fact is in good accordance with the theoretical ratio of ketones in terms of the physiological and pathological rules in hypothetical chart (Fig. 2), which is logical and quite promising for MIR-FT technology in the modification we used. The relationship of MIR-FT results to the reference method was not evaluated. Milk quality from cows with metabolic problem is often worsened. In the case of the occurrence of subclinical ketosis it is not possible to routinely discard such milk from commercial delivery as in the case of subclinical mastitis where it can be done according to the value of the somatic cell count (Barbano et al. 2006). Milk is delivered into the dairy plant without the possibility of such problem elimination. This fact can threaten the quality of the running of technological processing procedures and also of the milk products.

The possibilities for determining MIR-FT milk ketone levels have been mentioned but their reliability is still open to discussion. Currently the relevant calibration procedures are tested in terms of result reliability. Van Knegsel et al. (2010) have mentioned the higher sensitivity (80%) of both milk BHB and AC, determined by MIR-FT (M-Sc), in detecting hyperketonemia compared with milk fat to protein ratio (66%). The specificity was similar for the 3 diagnostic tests (71, 70 and 71%). De Roos et al. (2006) evaluated the infrared MilkoScan FT 6000 (MIR-FT) method and its calibration on the basis of AC, acet-acetate and BHB determination in individual milk samples in terms of sensitivity (70%), specificity (95%) and percentage of false positive (27%) and false negative (7%) results in relation to ability of the method to identify cow subclinical ketosis. The usability of a herd control programe is mostly influenced by the sensitivity and positive prediction value of the tests as much as its costs. The application method has been confirmed by research as still suitable for the practical monitoring of disease.
The arithmetical mean of the reference set of MSs was 5.91 ± 1.76 mg l⁻¹ (variation coefficient, \(v_x = 29.7\%\)) by the Re method (Table 2). The variation range was from 3.13 to 8.54 mg l⁻¹ which is not enough for the efficient detection of subclinical ketosis. A linear regression analysis showed a slight relationship (0.21; \(P > 0.05\)) between AC by the Re method and log BHB by MIR-FT (M-Sc) and a negative relationship (–0.31; \(P > 0.05\)) between AC by Re method and log AC by Re method and log AC by MIR-FT (M-Sc). The relationship between log AC and log BHB by MIR-FT (M-Sc) was slight in this case (0.13; \(P > 0.05\)). Therefore these results were not interpretable from the point of view of a method. They could have been caused by the close variation range. The relationship between log AC by the Re method and log AC by MIR-FT (Delta) after calibration (validation) was 0.41 (\(P > 0.05\)), which was the method perspective, only slightly promising (Fig. 3).

**Fig. 2.** Hypothetical scheme of acetone (ketone) concentration increase in milk or other body liquids such as the blood or urine of ruminants along ketosis degree

**Fig. 3.** Relationship between log AC (mg l⁻¹) by reference (Re) method and log AC by MIR-FT (Delta) after calibration (validation), evaluation II
The arithmetical mean of reference set of MSs was $6.10 \pm 1.02$ mg l$^{-1}$ ($v_x = 16.7\%$) by the Re method (Table 2). The variation range was from 5.07 to 9.11 mg l$^{-1}$ which is also slight in terms of the indication of subclinical or clinical ketosis, as in set II. However, a linear regression analysis showed better results. The relationship between log AC and log BHB by MIR-FT (M-Sc) was again close; similar to the sample set I (0.87; $P<0.001$; Fig. 4) as it is logical in terms of ketosis development (Fig. 2). The correlation between AC content by the Re method and log AC by MIR-FT (M-Sc) was 0.59 ($P<0.001$; Fig. 5) and 34.7% of variability in log AC values is explainable by AC values using the Re method. The correlation between AC content by the Re method and log BHB by MIR-FT (M-Sc) was 0.50 ($P<0.01$; Fig. 6) and only 25.2% of variability in log BHB values is explainable by AC value variations using the Re method. These results were quite promising for the possibility of indirect (MIR-FT) ketone determination.

Table 2. Statistical results of acetone (AC) content in reference sets of milk samples along various experimental evaluations (II, III and V) using reference (Re) and MIR-FT method

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>II</th>
<th>III</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Re</td>
<td>MIR-FT*</td>
<td>Re</td>
</tr>
<tr>
<td>Acetone</td>
<td>AC</td>
<td>AC</td>
<td>AC</td>
</tr>
<tr>
<td>Unit</td>
<td>mg l$^{-1}$</td>
<td>mg l$^{-1}$</td>
<td>mg l$^{-1}$</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>37</td>
</tr>
<tr>
<td>x</td>
<td>5.91</td>
<td>5.92</td>
<td>6.10</td>
</tr>
<tr>
<td>sd</td>
<td>1.76</td>
<td>0.53</td>
<td>1.02</td>
</tr>
<tr>
<td>m</td>
<td>6.18</td>
<td>5.80</td>
<td>5.87</td>
</tr>
<tr>
<td>min.</td>
<td>3.13</td>
<td>5.00</td>
<td>5.07</td>
</tr>
<tr>
<td>max.</td>
<td>8.54</td>
<td>7.00</td>
<td>9.11</td>
</tr>
</tbody>
</table>

n = number of cases, x = arithmetic mean, sd = standard deviation, m = median, min. = minimum, max. = maximum, * = Delta after calibration, ** = M-Sc original level

$r = 0.871^{***}$  $n = 37$

Fig. 4. Relationship between log AC and log BHB by MIR-FT (M-Sc), evaluation III
The AC was artificially added into the milk sample in various ratios because of the fierce AC increase. MSs were measured using relevant methods (Re and MIR-FT /Delta, calibration accepted in step II). The AC addition was not registered using the MIR-FT method, but the use of the Re method naturally was. The Re method measured 4.91 and 5.23 mg l⁻¹ of AC content in milk without AC addition and from 10.23 to 45.22 mg l⁻¹ along a scale with AC addition. The MIR-FT method recorded values of 5.03 and 5.78 mg l⁻¹ of AC content in milk without AC addition and from 5.24 to 5.12 mg l⁻¹ along a scale with AC addition in the same order (Fig. 7). That is the reason why it is clear that the AC addition was not recorded by the MIR-FT method (Delta). Therefore the method of the artificial addition of AC into milk to achieve an upper values (points) of the calibration line at MIR-FT calibration was assessed as impracticable regarding zero recovery.
The reference AC sample set mean was 10.1 ± 9.74 (vx = 96.4%) at a geometric mean of 7.26 and median 6.42 mg l–1 (Table 2). The variation range of this reference set from 1.98 to 33.66 mg l–1 was satisfactory with regard to the principles and practice of subclinical ketosis diagnostics using an analysis of body liquids on increased content of ketones. In spite of this a disadvantage was the lower frequency of the higher values (>20 mg l–1, high subclinical ketosis suspicion) in the sample set (21.4%). A practically advantageous value could be 50% for the purpose mentioned, by the method of qualified estimation.

The AC correlation relationship between the reference method and MIR-FT Delta was 0.32 (P > 0.05; Fig. 8). This means that it was not marked and was aggravated after calibration acceptance (0.24). Therefore, this result of the calibration experiment was not persuasive. The log AC relationship between the reference method and MIR-FT M-Sc was markedly better – expressed by a correlation coefficient of 0.80 (P < 0.01; Fig. 9). It means that 64.5% of AC M-Sc variations were explainable by AC variability in reference values. The same relationship between the results of log AC by the reference method and log BHB by the instrumental method 0.58 (P < 0.05; Fig. 10) was also interesting. The only question is a recalculation and for a practically acceptable expression of the result in conventional units it means the interpretation of concentration units from an analytical point of view.

The correlation in the sample set between log AC and log BHB using the MIR-FT M-Sc instrument was a high 0.90 (P < 0.001; Fig. 11). It is logical, when the dynamics of ketosis in animals as in evaluation I (Fig. 1) and according to Fig. 2 are taken into account. It means that 81.2% of the variability of results in log BHB is explainable by variability in the log AC instrumental results. The conversion of >10 mg l–1 as the AC subclinical ketosis limit (Fig. 2; Table 1) according to the selected relationships of the AC results in milk by the reference method to log AC, and log BHB (r = 0.79 and 0.52; P < 0.01 and P > 0.05) using the MIR-FT M-Sc, could be > –0.80 (which is more confident), and > –1.66 (which is less confident because of the insignificance of the
Fig. 8. AC (mg l⁻¹) correlation relationship between reference (Re) method and MIR-FT Delta, evaluation V

\[ y = 0.534x + 8.3942 \]
\[ R^2 = 0.1038 \]

\[ r = 0.322 \, \text{ns} \quad n = 14 \]

Fig. 9. Log AC relationship between reference (Re; mg l⁻¹) method and MIR-FT M-Sc, evaluation V

\[ y = 0.6387x - 1.3461 \]
\[ R^2 = 0.6467 \]

\[ r = 0.804^{***} \quad n = 14 \]
Fig. 10. Relationship between the reference (Re; mg l⁻¹) method log AC and MIR-FT M-Sc log BHB, evaluation V

\[ y = 0.4945x - 2.0806 \]
\[ R^2 = 0.3372 \]

\[ r = 0.581^{***} \quad n = 14 \]

Fig. 11. Correlation in reference sample set between log AC and log BHB using MIR-FT M-Sc, evaluation V

\[ y = 0.9664x - 0.8855 \]
\[ R^2 = 0.8124 \]

\[ r = 0.901^{***} \quad n = 14 \]
correlation) for instrumental values (Fig. 12). It is important from the interpretation point of view for the discrimination of limit values to the ketosis problem (Fig. 2; Table 1) – suspicion of subclinical ketosis occurrence. It is interesting in terms of possible feedback (control back calculation). There was calibration of the Re method and original adjustment (Foss) of MIR-FT in the experimental focus. In the case of log AC conversion −0.80 this instrumental value (MIR-FT M-Sc) is equal to 0.158 mmol l⁻¹ (AC; 9.25 mg l⁻¹). This result is in good accordance with our previous results and recommendations. The accordance between the analytical result mentioned, and agreement and interpretation experience from previous papers were confirmed by this conversion. These
research results are also in good agreement with literature references already mentioned. In this connection van Knegsel et al. (2010) introduced discrimination limits for subclinical ketosis exclusion (98%) in case of AC < 0.07 and for BHB < 0.023 mmol l⁻¹. Heuer et al. (2001) mentioned a milk AC concentration threshold for subclinical ketosis in intervals from 0.4 to 0.7 mmol l⁻¹ with a positive prediction probability of 76%. Research work with milk AC and ketosis identification in animals using a calibrated infrared spectroscopy on the principle of the MIR-FT was performed also by Hansen (1999). He investigated the satisfactory reliability for classification of dairy cows into two groups – healthy and probably with ketosis. It was performed in samples which varied from 0 to 2.8 mM of AC, with a determination coefficient of 0.81 and a reliability of 0.27 mM.

In conclusion, usable procedures for MIR-FT calibration for the determination of milk AC and the monitoring of ketosis were developed and validated. Development of a practical table (Table 3) was completed using NRL-RM results in this paper. It was based on specific milk sample selection and modifications at the reference sample set preparation for MIR and MIR-FT calibration and proficiency testing purposes. The results mentioned can be marked as promising for ketosis monitoring in individual samples from milk recording. It is possible to reach practically applicable results in ketosis monitoring by the construction of a suitable calibration sample set and value transformations as well as by the efficient selection from the possible combinations of mentioned variants offered.

Table 3. Modification of raw cow milk samples in position of reference standards for calibration or control samples for proficiency testing to creation of necessary value scale (variation range) at MIR and especially MIR-FT method in National reference laboratory for raw milk in Rapotín

<table>
<thead>
<tr>
<th>Measured milk component</th>
<th>Reference samples (calibration)</th>
<th>Control samples (proficiency testing)</th>
<th>Way of modification and validated source of procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>Yes</td>
<td>Yes</td>
<td>Decrease by milk arising (lower volume) and dilution using water solution with specific composition. Increase by milk arising (upper volume). * 2010b and 2011b.</td>
</tr>
<tr>
<td>Crude protein</td>
<td>Yes</td>
<td>Yes</td>
<td>Decrease by milk dilution using water solution with specific composition. Increase by selection of specific dairy herd with good energy nutrition. Mild increase by milk arising (lower volume). * 2010b and 2011b.</td>
</tr>
<tr>
<td>Casein</td>
<td>Yes</td>
<td>Yes</td>
<td>Decrease by milk dilution using water solution with specific composition. Increase by selection of specific dairy herd with good energy nutrition. Mild increase by milk arising (lower volume). * 2010b, d and 2011b.</td>
</tr>
<tr>
<td>Solids non fat</td>
<td>Yes</td>
<td>Yes</td>
<td>Decrease by milk dilution using water solution with specific composition. Increase by selection of specific dairy herd with good energy nutrition. Mild increase by milk arising (lower volume). * 2010b and 2011b.</td>
</tr>
<tr>
<td>Urea</td>
<td>No</td>
<td>Yes</td>
<td>Increase by artificial addition (only MIR-FT). Mild increase by milk arising (lower volume). * 2008b, 2009a, 2011b; Hering et al. 2008.</td>
</tr>
<tr>
<td>Citric acid</td>
<td>Yes</td>
<td>No</td>
<td>Increase by artificial addition (only MIR-FT). * 2009b.</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>Yes</td>
<td>No</td>
<td>Increase by mechanical stress of milk and by selection of specific herd with poor energy nutrition of cows. * 2008a, c, 2009c; Genčurová et al. 2009.</td>
</tr>
<tr>
<td>Milk freezing point</td>
<td>No</td>
<td>Yes</td>
<td>Decrease by NaCl addition into water samples. Increase by water addition into milk samples. * 2009a, 2010a.</td>
</tr>
<tr>
<td>Acetone (Ketones)</td>
<td>Yes</td>
<td>No</td>
<td>Increase by selection of specific herd and cows with insufficient energy nutrition in lactation beginning. * 2010c, 2011a, c, d.</td>
</tr>
</tbody>
</table>

* Hanuš et. al., MIR (traditional technology of mid infrared spectroscopy with optical filters), MIR-FT (mid infrared spectroscopy with using of Fourier’s transformations)
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