Evaluation of a portable ketometer for on-site monitoring of blood β-hydroxybutyrate concentrations in dairy sheep and goats

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SUMMARY

On-site measurement of blood β-hydroxybutyrate (BHB) concentration is a valuable tool to monitor the energy status in dairy ruminants. The aim of the study was to assess the agreement of a portable ketometer with standard laboratory method for on-site measurement of BHB in dairy sheep and goats. Three hundred and forty-six animals from 12 farms were overall blood sampled: 237 dairy sheep (186 dry and 51 lactating) and 109 dairy goats (39 dry and 70 lactating). Agreement between the portable WellionVet BELUA device (WEL) and the laboratory method was evaluated for each group of animals with Bland-Altman plots and concordance correlation coefficients (CCC). Additionally, sensitivity, specificity and k-statistics for WEL at the BHB threshold of ≥0.8 mmol/L were calculated. BHB values with WEL were highly correlated to laboratory results. A systematic bias of ca. 0.2 mmol/L was observed in all groups of animals. With the addition of this bias as a correction factor, CCCs improved significantly (0.90-0.96 for sheep and 0.93-0.98 for goats). At the threshold of ≥0.8 mmol/L, sensitivity was 70.0%-94.1% for sheep and 87.5%-100.0% for goats, and specificity 94.1%-84.8% for sheep and 100.0% for goats. Moreover, dichotomized test agreement was moderate for dry sheep, substantial for all sheep and almost perfect for all other cases, when the correction factor was added. To conclude, WEL is a device with satisfactory agreement with laboratory values in small ruminants when a correction factor of 0.2 mmol/L is added on obtained BHB values.

Keywords : β-hydroxybutyrate; ketosis; pregnancy toxemia; rapid test; small ruminants

Introduction

Within the productive cycle of sheep and goat farming, the periparturient period is critical both for animal health and performance [20]. Prolific, high producing ewes and goats, especially under- and over-conditioned animals, are at higher risk for negative energy balance periparturiently and therefore, more susceptible to pregnancy toxemia during late pregnancy and to ketosis during lactation [30, 3].

Mortality in pregnancy toxemia is high and treatment is expensive and generally unsuccessful [30], making prevention essential. Early and accurate diagnosis of subclinical pregnancy toxemia and ketosis is important for the dairy sheep and goat industry, allowing timely application of preventive measures [3].

The energy status can be estimated by measuring blood β-hydroxybutyrate (BHB) concentrations [4], which is the predominant ketone body in blood [17] and is used to assess the adequacy of nutrition at late pregnancy or early lactation.

Sheep with serum BHB concentrations ≥0.8 mmol/L are considered at risk for developing pregnancy toxemia [30, 31, 23]. A research-based threshold for ketosis risk assessment for lactating sheep and goats is lacking; therefore, the aforementioned one is used for the latter animals also.
Measurement of blood BHB concentration in the laboratory is still the gold standard for the diagnosis of pregnancy toxemia and ketosis. However, on-site BHB measurement with hand-held portable ketometers is gaining increased popularity in both cattle and small ruminants’ clinical practice and research, mainly due to the immediately available results. Of course, it is important for portable ketometers to correlate well with laboratory BHB values.

There are several portable ketometers in the market. Most of them have been tested and were considered accurate in dairy cows [26, 33]. For sheep and goats, similar evaluations are much less. WellionVet BELUA (WEL) portable ketometer is developed for dairy cows; however, it has not been evaluated for sheep and goats. Therefore, the aim of the present study was to assess the accuracy of a hand-held ketometer for on-site rapid measurement of blood BHB concentrations in dairy sheep and goats, compared to the laboratory method.

Materials and Methods

FARMS, ANIMALS AND STUDY DESIGN

The study was conducted on 12 dairy farms (6 sheep and 6 goat farms), located at Macedonia, Epirus and Thessaly regions, Greece. Farms were selected to represent typical small ruminant rearing conditions in Greece [6, 7]. The farmers gave informed consent for the animals to be included in the study and the testing procedures. The study was conducted in compliance with institutional and ethics guidelines and approved by the Research Committee of the Aristotle University of Thessaloniki (protocol number 93791).

The study started in October 2016 and finished in March 2017. Three hundred and forty six (346) animals were overall blood sampled: 237 dairy sheep (186 dry and 51 lactating) and 109 dairy goats (39 dry and 70 lactating). The sampled dry animals were approximately 5-20 days before parturition and the lactating ones 5-20 days after parturition.

SAMPLE COLLECTION AND MEASUREMENTS

One blood specimen was drawn once from the jugular vein of each animal into 10 mL plain glass tube without anticoagulant (BD Vacutainer®, Plymouth, United Kingdom) for serum BHB measurement. Samples were immediately placed in a cooler, transported to the Clinic of Farm Animals of the Faculty of Veterinary Medicine, and centrifuged (3000 g for 15 min) immediately upon arrival (within 3 hours from sampling). Serum was transferred into polyethylene tubes and stored at -80 °C until assay (within 7 days from storage).

Blood BHB concentrations were measured both on-site and at the laboratory. On-site BHB was measured with WellionVet BELUA (MED TRUST Handelsges.m.b.H., Marz, Austria) done just after each individual blood sampling, following manufacturer’s instructions.

The enzymatic Ranbut assay according to Randox (Randox Laboratories Ltd., Crumlin, UK) was used to measure BHB in the laboratory, in an Abbott Architect c8000 analyzer (Abbott Laboratories, Abbott Park, Illinois, USA). The control solutions used were the Randox Human Assayed Multiseras levels 2 (HN1530) and 3 (HN1532). Biases were -2.76% and -1.78% for level 2 (target concentration: 0.290 mmol/L) and 3 controls (target concentration: 1.180 mmol/L), respectively. The intra- and inter-assay coefficients of variation for the above analyses were less than 3%, according to CLSI protocol NCCLS EP5-A [13].

STATISTICAL ANALYSIS

For statistical analysis, data were entered into a computerized database and analyzed with the MedCalc Statistical Software v.17.8.6 (MedCalc Software bvba, Ostend, Belgium). Descriptive statistics were carried out for the variables under study.

The agreement between WEL and the laboratory reference method for dairy sheep and goats was depicted using the Bland-Altman graphical procedure [2]. In this graph, differences between two methods are plotted against the average values of both methods. Satisfactory agreement is obtained when most differences (95%) lie close to zero and within 95% limits of agreement, defined as mean difference ± 1.96 x standard deviations. Mean difference represents a systematic effect or bias.

Lin’s concordance correlation coefficient (CCC) [17] was also estimated as an index of agreement between the two methods. Strength of agreement is evaluated as the degree that measurements fall on a 45° line through the origin (correlation line). Calculation of CCC incorporates both measures of accuracy and precision. An interpretation of CCC for continuous variables has been proposed by McBride [21], as poor (CCC<0.90); moderate (0.90<CCC<0.95); substantial (0.95<CCC<0.99) and almost perfect agreement (CCC>0.99).

The threshold of 0.8 mmol/L was used to classify ewes and goats as healthy (<0.8 mmol/L) and at risk for developing pregnancy toxemia or ketosis (≥0.8 mmol/L). Sensitivity (SE), specificity (SP) and k-statistics for the hand-held meter at the cut-off point (BHB concentration ≥0.8 mmol/L) were also calculated. Cohen’s kappa coefficients were interpreted according to Landis and Koch’s [16] guidelines. (≤0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80 and 0.81–1.00 as indicating poor, fair, moderate, substantial, and almost perfect agreement for categorized variables, respectively).

Results

Descriptive statistics for measured BHB concentrations in dairy sheep and goats using the two different methods (laboratory and point-of-care test) are shown in Table I.
Bland-Altman plots for dairy ewes and goats are presented in Figure 1. The percentages of differences within limits of agreement were 97.3%, 94.1% and 96.2% for dry, lactating and all sheep, respectively, and 94.9%, 95.7% and 96.3% for dry, lactating and all goats, respectively. A consistent under-prediction of BHB concentration obtained with WEL was observed in all groups of animals. Systematic biases ranged from 0.18 to 0.22 mmol/L. A regression analysis between differences and average values of both methods revealed no proportional bias.

The CCCs are presented in Table II. Biases arisen from mean difference lines in Bland-Altman plots were considered as correction factors. They were added to WEL values, CCCs were calculated again (Table II; Figure 2) and were markedly improved.

Twenty-six out of 237 sheep and 10 out of 109 goats had BHB values ≥0.8 mmol/L as measured with the laboratory method. SE, SP and test agreement (k-statistics) for BHB measured with WEL at the cutoff of BHB ≥0.8 mmol/L, both before and after using the correction factors, are presented in Table III. Evidently, SE and test agreement were substantially improved. With the addition of the correction factor, about 3.3% of sheep with a WEL BHB value <0.8 mmol/L were falsely classified as negatives and 17.4% of sheep with a WEL BHB value ≥0.8 mmol/L were false positives. Regarding goats, only 1% of specimens with a WEL BHB value <0.8 mmol/L were false negatives.

**Discussion**

Hand-held ketometers are widely used for the detection of clinical and subclinical energy-related disorders (i.e. ketosis and pregnancy toxemia) in both cattle and small ruminants [24, 26, 27]. Diagnostic accuracy of a portable ketometer measuring whole blood BHB concentration proved superior to milk and urine strips [33].
Hand-held ketometers' agreement with standard laboratory methods has to be investigated. In order to use them as on-field decision-making tools for the implementation of preventive actions, accuracy and precision as well as sensitivity and specificity for an established threshold should be calculated. Several studies have evaluated the performance of portable meters for measuring blood BHB concentration in dairy cows [12, 15, 24, 14, 10, 11, 34, 26, 8, 32, 1, 22, 19]. These studies found high correlation and test agreement with the reference laboratory methods. In a systematic review and meta-analysis, the most tested portable device had a summary SE and SP of 94.8% and 97.5%, respectively, at a threshold of 1.2 mmol/L [33]. Other evaluated ketometers had SE of 74.4%-100.0% and SP of 73.5%-100.0% at the same threshold [1].

Few studies have evaluated the accuracy of portable ketometers in small ruminants [27, 5, 9, 28, 29]. Panousis et al. [27] found a high correlation (r=0.99) with laboratory results and high SE (98.6%) and SP (98.2%) in dairy ewes with BHB concentration ≥0.8 mmol/L. Similarly, Doré et al. [5] and Pichler et al. [28] reported a Pearson's correlation coefficient of 0.98 in dairy goats and 0.96 (jugular vein) in dairy ewes, respectively, using the same device.

Pearson's correlation coefficient (r) or the non-parametric Spearman correlation coefficient (r_s) is a measure of linear relationship between two continuous variables but is considered an inappropriate measure of agreement [2]. Hornig et al. [9] and Pichler et al. [29] used, additionally, the Bland-Altman graphical procedure for evaluating the agreement between hand-held ketometers and reference laboratory methods in sheep and goats, respectively. Hornig et al. [9] observed only slight systematic (+0.06 mmol/L) and proportional (from graphical observation) biases. On the contrary, Pichler et al. [29], comparing two different ketometers, found a positive systematic bias of +0.12 mmol/L (ear vein) and +0.21 mmol/L (jugular vein) together with significant proportional bias with ketometer 1, and a negative systematic bias of -0.21 mmol/L (ear vein) and -0.24 mmol/L (jugular vein) with ketometer 2, compared to standard laboratory analysis in dairy goats.

### Table I: Descriptive statistics of blood β-hydroxybutyrate (BHB) concentrations (mmol/L) in dairy sheep and goats measured with laboratory method and with the portable ketometer (WEL).

<table>
<thead>
<tr>
<th>Species</th>
<th>Method used</th>
<th>n</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>Laboratory</td>
<td>237</td>
<td>0.55</td>
<td>0.48</td>
<td>0.24</td>
<td>0.16-1.63</td>
</tr>
<tr>
<td></td>
<td>WEL</td>
<td></td>
<td>0.36</td>
<td>0.30</td>
<td>0.24</td>
<td>0.10-1.50</td>
</tr>
<tr>
<td>Goats</td>
<td>Laboratory</td>
<td>109</td>
<td>0.47</td>
<td>0.39</td>
<td>0.44</td>
<td>0.13-4.30</td>
</tr>
<tr>
<td></td>
<td>WEL</td>
<td></td>
<td>0.26</td>
<td>0.20</td>
<td>0.42</td>
<td>0.10-4.10</td>
</tr>
</tbody>
</table>

### Table II: Concordance correlation coefficients for blood β-hydroxybutyrate (BHB) concentrations with the laboratory method and the portable ketometer, before and after adding the appropriate “correction factor” for dairy ewes and goats.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>CCC (without CF)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>237</td>
<td>0.72</td>
<td>0.67-0.76</td>
</tr>
<tr>
<td></td>
<td>with CF</td>
<td>0.94</td>
<td>(0.92-0.95)</td>
</tr>
<tr>
<td>Goats</td>
<td>109</td>
<td>0.87</td>
<td>0.84-0.90</td>
</tr>
<tr>
<td></td>
<td>with CF</td>
<td>0.97</td>
<td>(0.96-0.98)</td>
</tr>
</tbody>
</table>

CCC: Concordance Correlation Coefficient
CI: Confidence Interval
CF: Correction Factor (+0.20 mmol/L)

### Table III: Sensitivity, specificity and test agreement (k) for blood β-hydroxybutyrate (BHB) ≥0.8 mmol/L measured with the portable ketometer (WEL) for dairy ewes and goats before and after addition of the appropriate “correction factor”.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>k-statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>237</td>
<td>53.8%</td>
<td>99.5%</td>
<td>0.655</td>
</tr>
<tr>
<td></td>
<td>with CF</td>
<td>88.5%</td>
<td>94.8%</td>
<td>0.734</td>
</tr>
<tr>
<td>Goats</td>
<td>109</td>
<td>50.0%</td>
<td>100.0%</td>
<td>0.645</td>
</tr>
<tr>
<td></td>
<td>with CF</td>
<td>90.0%</td>
<td>100.0%</td>
<td>0.942</td>
</tr>
</tbody>
</table>

CF: Correction Factor (+0.20 mmol/L)
In our study, mean BHB concentrations for both dairy sheep and goats measured with WEL were consistently lower by 0.18-0.22 mmol/L than those measured in the laboratory. However, 95% limits of agreement in our study were less wide than those previously reported. They were -0.46 to +0.34 [9], -0.48 to +0.23 (ear vein) and -0.59 to +0.18 (jugular vein) with the ketometer 1 and -0.26 to +0.68 (ear vein) and -0.10 to +0.58 (jugular vein) with the ketometer 2 [29]. In our study the wider limits of agreement were observed in dry goats (-0.04 to 0.41).

Blood BHB values obtained with WEL were highly correlated with laboratory results; moreover, the agreement with laboratory values was independent of BHB concentration. Measurements with WEL were precise, in means of reproducibility. However, WEL was moderately accurate as a constant systematic bias was observed in Bland-Altman plots. Accuracy and overall agreement were significantly improved with the addition of the correction factor.

WEL was highly specific but not sensitive, and had substantial test agreement (except for dry sheep, where it was moderate) for detection of animals at risk for developing pregnancy toxemia or ketosis (serum BHB concentrations ≥0.8 mmol/L). SE and test agreement of the device were markedly increased when the appropriate correction factors were considered for statistics; test agreement became almost perfect for lactating sheep and goats, dry goats and all goats. However, it should be noted that the number of animals with elevated BHB was relatively small (11% of sheep and 9.2% of goats), especially when subgroups of dry and lactating animals were analyzed, despite the adequate number of specimens. A wider range of BHB values would allow for more safe conclusions to be made about the detection of hyperketonemia in sheep and goats.

To conclude, the hand-held ketometer WellionVet BELUA has a significantly high agreement with laboratory blood BHB concentrations when a correction factor of 0.2 mmol/L is added on the obtained results. Therefore, it is considered a suitable on-farm energy status monitoring tool for the early diagnosis of sheep and goats at risk of developing pregnancy toxemia or ketosis.

Acknowledgement

Authors acknowledge the financial support by MED TRUST Handelsges.m.b.H. Samples were processed and results evaluated uninfluenced and independently by the sponsor.

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