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RESEARCH ARTICLE

Validation of A Portable Beta-Hydroxybutyric Acid Analyser for The Diagnosis of Subclinical Ketosis in Dairy Cows

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ABSTRACT

On-farm determination of beta-hydroxybutyric acid (BHBA) with an accurate analyser is important for rapid diagnosis of subclinical ketosis (SCK) or clinical ketosis in dairy cows. The accuracy of the Taidoc BHBA portable analyser has not yet been tested and validated. The objective was the validation of the Taidoc BHBA analyser with another device Wellion (already validated) in 84 dairy cows. Whole blood was collected from the coccygeal vein and immediately analysed with Taidoc and Wellion in one drop of whole venous blood of 84 dairy cows. The averages of BHBA concentration were 0.79±0.66 and 1.09±0.51 mmol.l^{-1} for Wellion and Taidoc, respectively. The correlation coefficient was r=0.64 ($p<0.001$). Method comparison by the Passing-Bablok regression equation (y=0.0664+0.06111x) showed no agreement between the two instruments. The intercept A and slope B did not include 0.0 and 1.0, respectively. The Bland-Altman plots of agreement indicated that the mean deviation was 0.30 mmol.l^{-1} and the total deviation was 2.02 mmol.l^{-1}, which was unacceptably high. The weighted kappa value was 0.26, indicating minimal agreement. AUC, sensitivity and specificity for the diagnosis of SCK at cut-off point ≥1.2 mmol.l^{-1} BHBA were 0.78, 64.7% and 91.0%, respectively. There was no agreement between the Taidoc and the Wellion BHBA portable analyser for dairy cows and they cannot be used interchangeably. Taidoc gave much higher BHBA values than Wellion and its sensitivity and specificity for SCK diagnosis was not comparable to Wellion.

Keywords: Beta-hydroxybutyric acid, Dairy cow, Subclinical ketosis, Taidoc, Wellion

Süt İneklerinde Subklinik Ketozis Teşhisi İçin Portatif Beta-Hidroksibütirik Asit Analiz Cihazının Validasyonu

ÖZ

Süt çiftliklerinde beta-hidroksibütirik asidin (BHBA) doğru bir cihazla ölçülmesi, subklinik ketozisin (SCK) veya klinik ketozisin hızlı teşhisi için önemlidir. Taidoc BHBA taşınabilir analiz cihazının doğruluğu henüz valide edilmemiştir. Bu çalışma, Taidoc BHBA analiz cihazını valide etmek için daha önceden validasyonu yapılmış Wellion cihazı ile 84 süt ineğinde karşılaştırılarak yapılmıştır. Süt ineklerinde V. coccygea'dan alınan bir damla kan Taidoc ve Wellion ile analiz edilmiştir. BHBA konsantrasyonlarının ortalamaları Wellion ve Taidoc için sırasıyla 0,79±0,66 ve 1,09±0,51 mmol.l^{-1} olarak bulunmuştur. İki cihaz arasında korelasyon katsayısı r=0,64 ($p<0,001$) çıkmıştır. Passing-Bablok regresyon denklemiyle (y=0,0664+0,06111x) yöntem karşılaştırması, iki cihaz arasında bir uyum olmadığını gösterdi. Intercept ve slope sırasıyla 0.0 ve 1.0'ı içermemiştir. Bland-Altman uyum grafikleri, ortalama sapmanın 0,30 mmol.l^{-1} ve toplam sapmanın 2,02 mmol.l^{-1} olduğunu gösterdi ki, bu kabul edilemeyecek kadar yüksek çıkmıştır. Ağırlıklı kappa değeri 0.26 çıkmış ve bu değer minimum derecede bir uyumu göstermiştir. SCK'nın teşhisi için BHBA≥1,2 mmol.l^{-1} kesme noktasında eğri altındaki alan, duyarlılık ve özgüllük sırasıyla 0,78, %64,7 ve %91,0 çıkmıştır. Süt inekleri için Taidoc ve Wellion BHBA taşınabilir analiz cihazları arasında bir uyum bulunmamış ve cihazlar birbirlerinin yerine kullanılamazlar. Taidoc, Wellion'dan çok daha yüksek BHBA değerleri vermiştir ve SCK teşhisi için duyarlılığı ve özgüllüğü Wellion ile karşılaştırılabilir oranda değildir.

Anahtar Kelimeler: Beta-hidroksibütirik asit, Subklinik ketozis, Süt ineği, Taidoc, Wellion

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INTRODUCTION

Clinical ketosis and subclinical ketosis (SCK) are economically important metabolic diseases in dairy cows, and they are observed more frequently in the second to fourth weeks after calving (Duffield 2000; Mostert et al. 2017; Deniz et al. 2020; Steeneveld et al. 2020). The indicator for the diagnosis of ketosis is an elevated beta-hydroxybutyric acid (BHBA) concentration in the blood or milk, which in dairy cows can be analysed directly with a cow-side analyser on the farm (Voyvoda and Erdogan 2010; Berge and Vertenten 2013; Suthar et al. 2013; Khol et al. 2019; Aksoy et al. 2022). SCK is a form of ketosis without clinical signs (Duffield 2000; Suthar et al. 2013; Deniz et al. 2020; Steeneveld et al. 2020). The diagnosis of ketosis or SCK in cows is an important point on farms for immediate action, as it has a large impact on farmers' profitability due to milk yield losses costing €130-150 per SCK case (Mostert et al. 2017; Steeneveld et al. 2020) and other associated metabolic diseases such as displaced abomasum, retained placenta, mastitis, metritis, reproduction problems and early culling (Duffield 2000; Suthar et al. 2013; Raboisson et al. 2015; Deniz et al. 2020; Aksoy et al. 2022). The cut-off point for BHBA in blood has been accepted as ≥ 1.20 mmol.l^{-1} for SCK diagnosis (Voyvoda and Erdogan 2010; Khol et al. 2019; Deniz et al. 2020; Aksoy et al. 2022). Cow-side analysis of blood BHBA facilitates immediate diagnosis of SCK compared to costly laboratory serum analysis (Iwersen et al. 2009). There are many commercial blood or milk BHBA analysers on the market worldwide, but the question has been whether or not their accuracy has been validated. Many bloods or milk BHBA analysers have been validated and their accuracy tested and published (Geishauser et al. 2000; Carrier et al. 2004; Iwersen et al. 2009; Voyvoda and Erdogan 2010; Iwersen et al. 2013; Pineda and Cardoso 2015; Tatone et al. 2016; Macmillan et al. 2017; Jansen et al. 2021), including the Wellion whole blood BHBA analyser (Khol et al. 2019; Jansen et al. 2021). There are no published data on the validation and accuracy of the Taidoc cow-side BHBA analyser in dairy cows, although it is already used on the market. The aim of the present study was to test the accuracy and validation of the Taidoc cow-side portable BHBA analyser with another already validated device, Wellion, in dairy cows' whole venous blood.

MATERIAL and METHODS

This study protocol was approved by the Ethics Committee of Muğla Sıtkı Koçman University with approval number MUDEM-HADYEK, 15.09.2020, 08/20.

Eight-four dairy Holstein cows were randomly included in the study from different dairy farms in the western cities in Turkey. Twenty-one of 84 dairy cows were primiparous, the rest (n=63) were multiparous. The study cows were within one month postpartum. The study was conducted between June 2020 and October 2021. Ambient temperatures varied between 13 and 40°C during sampling. According to the farmers and veterinarians, none of the cows showed any clinical signs of disease at the time of blood collection. Blood was gently collected from the coccygeal vein (Jansen et al. 2021) into non-anticoagulant containing injector and one drop of whole blood was immediately used for the analysis of BHBA in cow-side portable analysers Wellion (WellionVet Belua, Med Trust Handels GmbH, Marz, Austria) and Taidoc (Taidoc Technology Corporation, Wugu dist. 24888 New Taipei City, Taiwan). The cut-off point of BHBA for SCK definition was set at ≥1.20 mmol.l^{-1} BHBA (Voyvoda and Erdogan 2010; Suthar et al. 2013; Khol et al.

2019; Deniz et al. 2020; Aksoy et al. 2022). Taidoc is a real-time BHBA analyser and gives the result in 5 seconds. It uses 0.7 µl whole blood for the analysis of BHBA. The minimum and maximum measurement ranges of Taidoc are 0.1 and 8.0 mmol.l^{-1} BHBA. The accuracy of Taidoc was reported by the manufacturer to be ±0.5 mmol.l-1 when ≤2.00 mmol.l-1 BHBA. The device delivers 25% more when > 2.00 mmol.l^{-1} BHBA was measured. The chemical reaction for BHBA analysis in Taidoc is based on beta-hydroxybutyrate dehydrogenase activity (electrochemical). In the present study, the cut-off points of BHBA <2.00 mmol.l^{-1} and ≥2.00 mmol.l^{-1} were also used for observation. Wellion is a portable BHBA analyser and displays the result of the analysis in 8 seconds. It uses 0.8 µl of whole blood for the analysis of BHBA. Compared to the reference method (Khol et al. 2019), the average values of BHBA analysed with Wellion and the reference method (wet biochemistry, optical spectrometry) were 0.73±0.94 and 0.71±0.93 mmol.l^{-1}, respectively. The correlation, kappa value and AUC were satisfactory for good agreement (r=0.95, 0.89, 0.99) with the reference method. BHBA concentration is analysed by BHBA dehydrogenase enzyme (electrochemical) in Wellion in a range of 0.1 to 8.0 mmol.l^{-1}. As the Wellion showed good agreement with the standard reference laboratory method (Khol et al. 2019) and demonstrated good diagnostic performance for SCK diagnosis (Khol et al. 2019; Jansen et al. 2021), the Wellion was used as the reference device in the present study.

Statistical analysis

MedCalc software (MedCalc Software Ltd Acacialaan 22, 8400 Ostend, Belgium) version 2022 was used to perform the statistical analyses. The normality of the data was checked using the Shapiro-Wilk test. Descriptive statistics as x±sd (mean and standard deviation) and the percentage of sick cows based on cut-off points were presented when needed. The correlation coefficient was calculated between Taidoc and Wellion. The Passing-Bablok regression equation (Jensen and Kjelgaard 2006; Zulle 2011) was used between Taidoc and Wellion to compare the methods. Bland Altman's Plots of Agreement Test was used to test the agreement between Taidoc and Wellion (Bland and Altman 1999; Jensen and Kjelgaard 2006; Giavarina 2015). Receiver Operating Characteristic Analysis (ROC) (Simundic 2009; Trevethan 2017) was performed to determine the sensitivity (Se), specificity (Sp) and area under the curve (AUC) of Taidoc at the cut-off point ≥1.20 mmol.l^{-1} BHBA for Wellion. Cohen's kappa statistic (Cohen 1960; Macmillan et al. 2017) was used for inter-rater reliability of variables (≤0.00 no agreement, 0.01-0.20 still or slightly, 0.21-0.40 fairly, 0.41-0.60 moderately, 0.61-0.80 substantially, 0.81-1.00 perfect agreement). However, the kappa coefficient was also interpreted according to McHugh (2012) from a medical perspective as follows: 0-20 no agreement (0-4% reliability of data), 21-39 minimal agreement, 0.40-59 weak agreement, 0.60-0.79 moderate agreement, 0.80-0.90 strong agreement and > 0.90 perfect agreement (81-100% reliability of data).

RESULTS

The averages of BHBA concentration were 0.79±0.66 mmol.l^{-1} (min: 0.10, max: 2.70 mmol.l^{-1}) and 1.09±0.51 mmol.l^{-1} (min: 0.20, max: 3.10 mmol.l^{-1}) for Wellion and Taidoc, respectively. The mean difference between Taidoc and Wellion was 0.30 mmol.l^{-1} (38% higher). The averages of BHBA concentrations and the percentage of sick cases at defined cut-off points were presented in Table 1. The correlation between Wellion and Taidoc was significant but not very high, so the correlation coefficient was r=0.64 (p<0.001) (Fig. 1). Comparison with the Passing-Bablok regression

method (y=0.663+0.611x) yielded systematic and proportional errors (Fig. 1), as the 95% CI of intercept A did not include '0' (0.547 to 0.725) and slope B did not include '1' (0.500 to 0.789). The weighted kappa coefficient was calculated to be 0.26±0.068 (95% CI: 0.129 - 0.396) indicating a low agreement. The mean and total deviations between Taidoc and Wellion were 0.30 and 2.02 mmol.l^{-1} BHBA, respectively, according to Bland-Altman plots of agreement (Fig. 2). Analysis of ROC showed that the AUC, Se and Sp of Taidoc compared with Wellion at a cut-off point ≥1.20 mmol.l^{-1} BHBA were 0.78, 64.7% and 91.0%, respectively (Fig. 3).

Table 1. Descriptive statistics of Wellion and Taidoc for defined cut-off points of beta-hydroxybutyric acid (BHBA) in whole venous blood of dairy cows.

	Wellion (W)			Taidoc (T)			Difference of mean (T-W)
	No. Cows	%	x+sd	No. Cows	%	x+sd	in %
All cows	84	100	0.79±0.66	84	100	1.09±0.51	38.0
BHBA<1.20 mmol.l^{-1}	67	79.8	0.50±0.30	59	70.2	0.84±0.21	68.0
BHBA ≥1.20 mmol.l^{-1}	17	20.2	1.90±0.52	25	29.8	1.66±0.56	-12.6
BHBA <2.00 mmol.l^{-1}	76	90.5	0.63±0.43	77	91.7	0.96±0.30	52.4
BHBA ≥2.00 mmol.l^{-1}	8	9.5	2.32±0.32	7	8.3	2.48±0.45	6.9

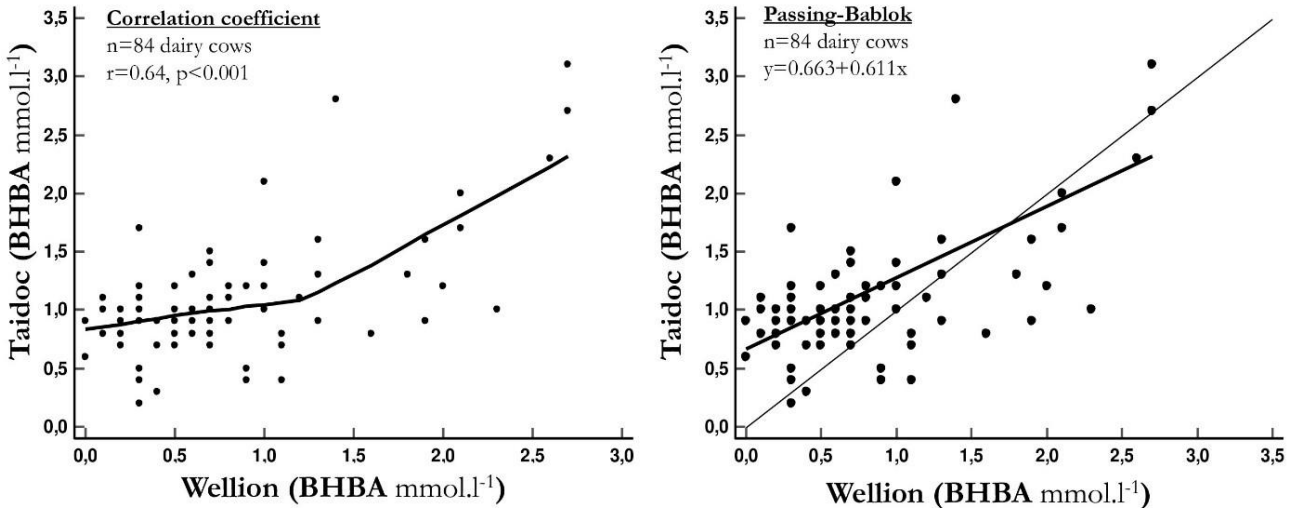

Figure 1. The correlation coefficient and Passing-Bablok regression equation between Taidoc and Wellion for the analysis of whole blood beta-hydroxybutyric acid (BHBA) concentration in dairy cows.

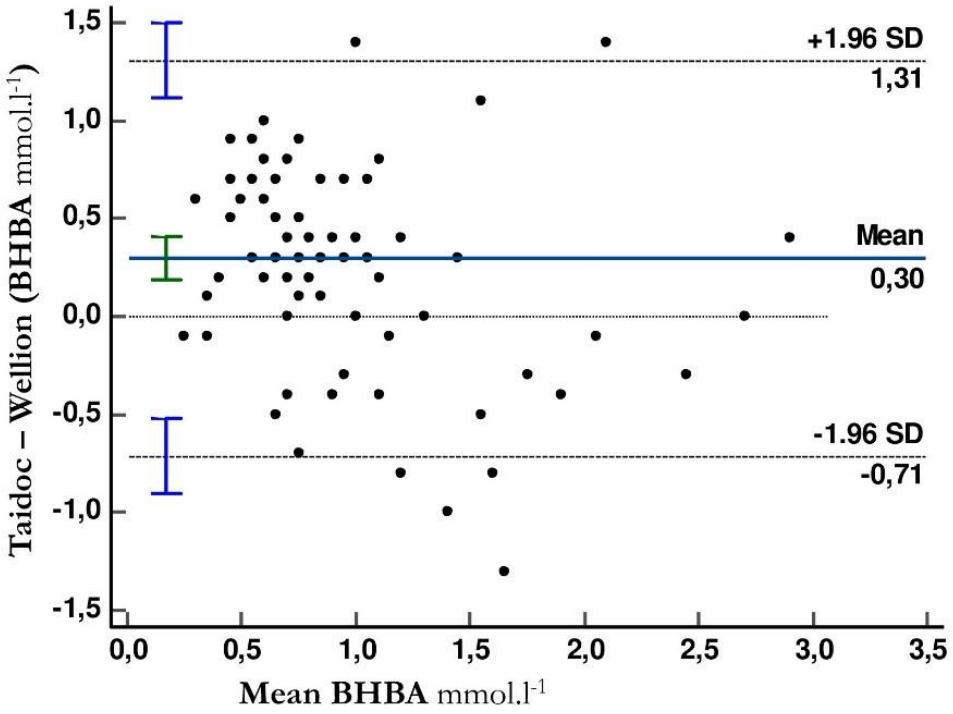

Figure 2. Bland-Altman plots of agreement between Taidoc and Wellion for the analysis of whole blood beta-hydroxybutyric acid concentration (BHBA) in dairy cows.

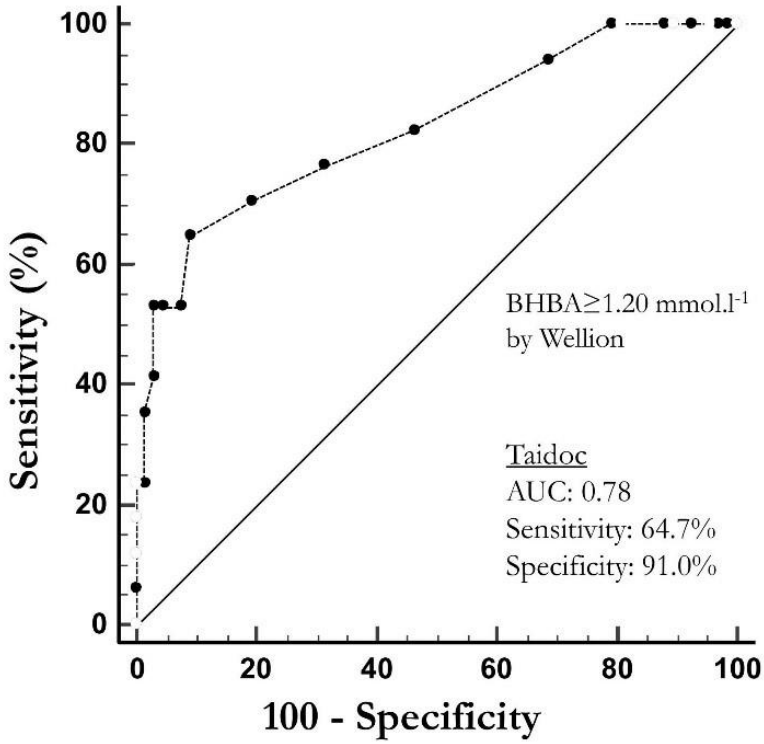

Figure 3. Receiver operating characteristics curve analysis for Taidoc based on cut-off point of beta-hydroxybutyric acid (BHBA) ≥ 1.20 mmol.l^{-1} in whole blood of dairy cows tested by Wellion. AUC: area under the curve

DISCUSSION

BHBA is an important parameter for the diagnosis of economically important SCK or clinical ketosis in dairy cows after calving (Suthar et al. 2013; Khol et al. 2019; Deniz et al. 2020). There are a number of devices that are portable and useful on the farm for the analysis of BHBA in dairy cows. However, the accuracy of BHBA analysers for the diagnosis of ketosis needs to be tested in cows using a previously validated test device or gold method (Iwersen et al. 2009; Voyvoda and Erdogan 2010; Khol et al. 2019; Jansen et al. 2021) to be sure that the disease is present or not. In the present study, Taidoc, a BHBA analyser for cows, was validated in the farms with another already validated BHBA analyser, Wellion. Both analysers were portable devices for use on farms. Wellion was previously compared to a reference method and proved consistent in both capillary and whole blood with satisfactory correlation coefficients, kappa values and sensitivity (Khol et al. 2019; Jansen et al. 2021). However, no validation study of Taidoc in dairy cows has been published to date, however a different brand of the same manufacturer (BHB-check) for BHBA analysis was validated by others (Jansen et al. 2021). The kappa coefficient showed fair agreement between Wellion and Taidoc. McHugh (2012), pointed out that the kappa value between 0.21 and 0.39 should represent minimal agreement in medical science and that the percentage agreement and the kappa value have strengths and limitations, so they should be interpreted cautiously. The kappa value of the present study was consistent with this statement. Furthermore, Khol et al. (2019), found a kappa value of 0.89 between Wellion and the reference method, indicating strong agreement. Kappa coefficient was not available in the validation study of Jansen et al. (2021). Moreover, Passing-Bablok, a powerful method agreement statistic (Jensen and Kjelgaard 2006; Zulle 2011), showed significant systematic and proportional errors in the regression equation between Wellion and Taidoc. The regression lines were incredibly far apart. This indicated no agreement between the two instruments. However, other studies did not provide Passing-Bablok regression equations when comparing two methods (Iwersen et al. 2009; Khol et al. 2019), but some others (Voyvoda and Erdogan 2010; Jansen et al. 2021) used this powerful statistic of method comparison (Jensen and Kjelgaard 2006; Zulle 2011). Many studies used the Pearson correlation coefficient to demonstrate linear correlation when comparing methods (Iwersen et al. 2009; Voyvoda and Erdogan 2010; Macmillan et al. 2017; Khol et al. 2019). Although the linear correlation coefficient and coefficient of determination were significantly high, it was not satisfactorily high between Wellion and Taidoc in the present study. The correlation coefficient between Wellion and the reference method was found to be r=0.94 and 0.95 for capillary and venous whole blood, respectively (Khol et al. 2019). Another study (Jansen et al. 2021) reported a correlation coefficient of r=0.76 for Wellion compared to the reference method; however, that was Spearman correlation coefficient. The correlation coefficient between Taidoc and Wellion was lower than reported in these studies. All samples were collected within a short time interval and analyses were performed by the same person in the present study. Therefore, factors such as temperature, handling and diurnal variations should have no influence on the results (Jansen et al. 2021). On the other hand, a significant and high correlation does not mean that the two instruments are in good agreement (Giavarina 2015; Macmillan et al. 2017), as linear regression is based on the random errors of the dependent variables. A very high correlation coefficient such as r=0.98 did not give

good agreement when analysing pO2 with two different blood gas analysers, so there were systematic and proportional errors in the Passing-Bablok regression equation (Metin et al. 2023). Therefore, Passing-Bablok or Deming regression equations (Jensen and Kjelgaard 2006; Zulle 2011) or Lin's concordance correlation coefficients (Macmillan et al. 2017) were used in the regression equation for the method comparison, as they provide information on proportional and systematic errors for both instruments. Thus, in the present study, both the linear correlation coefficient and the Passing-Bablok regression equation did not provide satisfactory results for the relationship between Wellion and Taidoc. The present study also found that the mean and total deviations between Wellion and Taidoc are very high according to Bland-Altman plots of agreement. Taidoc has systematically measured higher BHBA concentrations compared to Wellion. There was almost zero mean difference in the validation study of Wellion compared with reference method (Kohl et al. 2019), however other study (Jansen et al. 2021) found a mean of -0.269 mmol.l^{-1} difference for another BHBA analyser (BHB-check) manufactured by the same company. Taidoc's instructions for use indicate that the device measures 25% higher levels when BHBA is >2.0 mmol.l^{-1}. Since in the present study only 7 out of 84 cows had a BHBA concentration of >2.0 mmol.l^{-1}, this should not have a major impact on the mean value of the BHBA concentration. This means that Taidoc systematically gave unacceptably higher BHBA values than Wellion. Furthermore, the total deviation of 2.02 mmol.l^{-1} of confidence interval appeared unacceptable from a clinical perspective, as the BHBA cut-off point for SCK diagnosis is ≥1.20 mmol.l^{-1}, which has been accepted by many others (Voyvoda and Erdogan 2010; Suthar et al. 2013; Khol et al. 2019; Deniz et al. 2020; Aksoy et al. 2022). The BHBA value is an important indicator for the veterinarian to treat SCK or clinical ketosis, of course in accordance with the clinical manifestation. However, cows with SCK show no signs of ketosis (Duffield 2000; Suthar et al. 2013; Deniz et al. 2020), but it is still detrimental and has an economic impact on dairy farm profitability (Mostert et al. 2017; Steeneveld et al. 2020). Therefore, the disease needs to be properly identified in order to take measures for treatment and prophylaxis. Although the AUC (0.70 -0.80) in ROC analysis was sufficiently high for Taidoc indicating good diagnostic accuracy (Simundic 2009), the Se and Sp values were very low, suggesting that Taidoc did not sensitively identify the sick and healthy cows detected by Wellion. The Se value of Wellion was reported to be 89 - 98% in the capillary and venous blood of cows compared to the reference method (Khol et al. 2019). On the other side, others (Jansen et al. 2021) reported 0.97 AUC for Wellion indicating a perfect diagnostic performance. So compared to Wellion, Taidoc was not able to maintain the sufficient Se value. Thus, it provided 47% more SCK diagnoses, which could mean many false positives, and the differences in mean values of BHBA overall, at <2.00 mmol.l^{-1} and ≥2.00 mmol.l^{-1} cut-off points were 38.0%, 52.4% and 6.9% higher, respectively. This was not in accordance with the specifications of the device reported by the manufacturer. Furthermore, much higher Se (96%) (Iwersen et al. 2009), 85-90% (Voyvoda and Erdogan 2010), 98-100% (Iwersen et al. 2013; Pineda and Cardoso 2015), 94.8% (Tatone et al. 2016) have been reported for SCK diagnosis of a portable BHBA analyser depending on the cut-off points of the BHBA. In addition, a validation study found a concentration dependence of Se and Sp based on different cut-off points of the BHBA as well as the Passing-Bablok regression equation, which should be taken into account in further studies (Jansen et al. 2021). In summary, the present validation study showed that Taidoc and Wellion cannot be used interchangeably,

as Taidoc did not meet the requirements of the statistical evaluations. Taidoc provided systematically higher BHBA values than Wellion, which was unacceptably high for the correct diagnostic purpose of SCK. Although Wellion was validated with the reference standard method and showed good agreement and satisfactory diagnostic performance, it is recommended to compare both Wellion and Taidoc with a gold standard method using a wet biochemical assay. According to the results of the present study, the chips and test kits of the Taidoc device should be improved by the manufacturer for the measurement of BHBA in dairy cows.

Ethical Statement: This study protocol was approved by the Ethics Committee of Muğla Sıtkı Koçman University with approval number MUDEM-HADYEK, 15.09.2020, 08/20).

Declaration of Competing Interest: Authors declare no conflicts of interest.

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REFERENCES

Aksoy, K., Deniz, A., Demir S., Onmaz, A.C. (2022). Blood and Milk Beta- hydroxybutyric Acid Concentrations in Diff erent Dairy Cattle Breeds and Association of Subclinical Ketosis with Postpartum Health Disorders, Culling Rate, Body Condition Score, Parity and Milk Production in Holstein. Kafkas Universitesi Veteriner Fakültesi Dergisi, 28 (2): 235-246.

Berge, A.C., Vertenten, G. (2013). A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. Journal of Dairy Science, 97: 2145–2154.

Bland, J.M., Altman, D.G. (1999). Measuring agreement in method comparison studies. Statistical Methods in Medical Research, 8:135-160.

Carrier, J., Stewart, S., Godden, S., Fetrow, J., Rapnicki, P. (2004). Evaluation and Use of Three Cowside Tests for Detection of Subclinical Ketosis in Early Postpartum Cows. Journal of Dairy Science, 87:3725–3735.

Cohen, J. (1960). A coefficient of agreement for nominal scales. Educational and Psychological Measurement, 20: 37-46.

Deniz, A., Aksoy K., Metin, M. (2020). Transition period and subclinical ketosis in dairy cattle: association with milk production, metabolic and reproductive disorders and economic aspects. Medycyna Weterynaryjna, 76 (9): 495-502.

Duffield, T.F. (2000). Subclinical ketosis in lactating dairy cattle. The Veterinary Clinics of North America. Food Animal Practice, 16, 231-253

Jansen, H.M., Zschiesche, M., Albers, D., Wemheuer, W., Sharifi, A.R., Hummel, J. (2021). Accuracy of Subclinical Ketosis Detection with Rapid Test Methods for BHBA in Blood in Commercial Dairy Farms. Dairy, 2: 671-683.

Jensen, A.L., Kjelgaard-Hansen, M. (2006). Method comparison in the clinical laboratory. Veterinary Clinical Pathology, 35:276-286.

Geishauser, T., Leslie, K., Tenhag, J., Bashiri, A. (2000). Evaluation of Eight Cow-Side Ketone Tests in Milk for Detection of Subclinical Ketosis in Dairy Cows. Journal of Dairy Science, Feb;83(2):296-299.

Giavarina, D. (2015). Understanding Bland Altman analysis. Biochemia Medica, 25: 141–151.

Iwersen, M., Falkenberg, U., Voigtsberger, R., Forderung, D., Heuwieser, W. (2009). Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. Journal of Dairy Science, 92, 1–7.

Iwersen, M., Klein-Jöbstl, D., Pichler, M., Roland, L., Fidlschuster, B., Schwendenwein, I., Drillich, M. (2013). Comparison of 2 electronic cowside tests to detect sub¬clinical ketosis in dairy cows and the influence of the tem¬perature and type of blood sample on the test results. Journal of Dairy Science, 96: 7719-7730.

Khol, J.L., Freigassner, K., Stannitznig, A., Tichy, A., Wittek, T. (2019). Evaluation of a handheld device for the measurement of beta-hydroxybutyrate in capillary blood obtained by the puncture of the vulva as well as in venous whole blood in cattle. Polish Journal of Veterinary Sciences, 22: 557–564.

Macmillan, K., López, Helguera, I., Behrouzi, A., Gobikrushanth, M., Hoff, B., Colazo, M.G. (2017). Accuracy of a cow-side test for the diagnosis of hyperketonemia and hypoglycemia in lactating dairy cows. Research in Veterinary Science, Dec; 115:327-331.

McHugh, M.L. (2012). Interrater reliability: the kappa statistic. Biochemia Medica, 22(3): 276-282.

Metin, M., Aksoy, K., Pekmezci, A., Deniz, A. (2023). Comparison of the blood gas analyser Edan i15 Vet with the blood gas analyser Gem Premier 3000 for the analysis of blood gases, electrolytes, hemoglobin and hematocrit in lactating Holsteins. Medycyna Weterynaryjna, 79 (2), 83-90.

Mostert, P.F., Bokkers, E.A.M., van Middelaar, C.E., Hogeveen, H., de Boer, I.J.M. (2017). Estimating the economic impact of subclinical ketosis in dairy cattle using a dynamic stochastic simulation model. Animal, 1-10.

Pineda, A., Cardoso, F.C. (2015). Technical note: Validation of a handheld meter for measuring β-hydroxybutyrate concentrations in plasma and serum from dairy cows. Journal of Dairy Science, 98: 8818-8824.

Simundic, A.M. (2009). Measures of diagnostic accuracy: basic definitions. EJIFCC, 19:203-2011.

Steeneveld, W., Amuta, P., van Soest, F.J.S., Jorritsma, R., Hogeveen, H. (2020). Estimating the combined costs of clinical and subclinical ketosis in dairy cows. PLoSONE, 15(4):e0230448.

Suthar, V.S., Canelas-Raposo, J., Deniz, A., Heuwieser, W. (2013). Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. Journal of Dairy Science, 96: 2925-2938.

Tatone, E.H., Gordon, J.L., Hubbs, J., LeBlanc, S.J., DeVries, T.J., Duffield, T.F. (2016). A systematic

review and meta-analy¬sis of the diagnostic accuracy of point-of-care tests for the detection of hyperketonemia in dairy cows. Preventive Veterinary Medicine, 130: 18-32.

Trevethan, R. (2017). Sensitivity, specificity, and predictive values, foundations, pliabilities, and pitfalls in research and practice. Frontiers in Public Health, 5:1-7.

Voyvoda, H., Erdogan, H. (2010). Use of a hand-held meter for detecting subclinical ketosis in dairy cows. Research in Veterinary Science, Dec;89(3):344-351.

Zulle, L.B. (2011). Comparison of methods: Passing-Bablok regression. Biochemia Medica, 21: 49-52.