Early recognition of altered lactate levels is considered a useful prognostic indicator in disease detection for both human beings and animals. It is reasonable therefore to hypothesize that a portable, point of care (POC) spectrophotometric device for analysis of lactate levels, may have an application for field veterinarians across a range of conditions and diagnostic procedures. In this study, a total of 72 cattle in the transition period underwent POC spectrophotometric lactate measurement with a portable device (The Vet Photometer) in the field, with a small portion of blood used for comparative ELISA evaluation. Lactate measurements were compared using a of Passing-Bablok regression analysis and Bland-Altman plots. The Vet Photometer lactate measurement results were in agreement with those generated by the ELISA method. Values for the agreement were derived, in a 95% CI between -1.3 and 0.99, and a positive correlation (r=0.71) between the two measurements. The equation $y= 0.68x + 0.60$ was achieved using a Passing-Bablok regression analysis. There were no statistical differences in mean values between the measurement methods. In conclusion, a novel veterinary POC spectrophotometric device “Vet Photometer” is an accurate device for evaluation of lactate levels in healthy transition cows.

**Key words:** accuracy, bovine, portable lactate meter, transition period
Introduction

Lactate is one of the end products of anaerobic metabolism (such as that which results from intense exercising of the muscles), but levels of lactate (Lc) can also be elevated in various pathological disorders involving decreased perfusion of tissue together with peripheral circulatory alterations (Liao et al. 1995, Bakker et al. 1996, Bernardin et al. 1996, Wittek et al. 2004). It has been postulated that Lc is indicative of poor prognosis in displaced abomasum in dairy cows, as well as abdominal pain in horses (Moore et al. 1976, Wittek et al. 2004, Figueiredo et al. 2006, Johnston et al. 2007, Dele-salle et al. 2007). It has previously been suggested that, regarding cattle practice, Lc measurement could indicate congestion/necrosis related to uterine torsion (Murakami et al. 2017), or be a useful predictor for right-sided abomasal disorders (Figueiredo et al. 2006). In another study, plasma Lc readings of 3.6 - 4 mmol/L, calculated with the standard method and POCT re- spectively, emerged as a realistic prognostic biomarker regarding mortality (Coghe et al. 2000).

It should be taken into account that Lc concentration is a frontier parameter reflecting early adaptation mechanisms during the transition period, and the authors of this paper mentioned above produced transition period lactate analyses data in an unpublished preliminary study. In that study a total of 14 cows, beginning at -2 weeks prepartum through to parturition (day0) and +2 weeks postpartum, were analysed for Lc concentrations, with slight increases on days -7 and 0 being detected. The study’s authors concluded aerobic metabolism was unaffected.

The accuracy of, and comparisons between, cow-side lactate measurements in different disorders (e.g. pneumonia, displaced abomasum), using different amperometric POCT devices, have been previously investigated (Coghe et al. 2000, Karapınar et al. 2013).

The purpose of the present investigation was to examine the accuracy of a handheld POC spectrophotometric device, and compare, the handheld POC spectrophotometric (Sp) device and classical ELISA, for lactate measurement in transition period cows. The authors hypothesized that Sp and ELISA measurements would closely correlate at field testing.

Materials and Methods

Animals and blood collection

A total of 72 Holstein dairy cows belonging to a commercial dairy herd located in Aydin province, Turkey, were involved in the study. All procedures were approved by the Adnan Menderes University Animal Experimentation Local Ethics Committee. The period of study was November 2016 to January 2017, when climatic conditions were cool. Blood samples were taken via jugular venipuncture to Li-heparine tubes, two weeks before parturition (-2 weeks), one week before parturition (-1 week), at parturition (0 week), one week after parturition (+1 week), and two weeks after parturition (+2 weeks).

Blood samples were centrifuged at 6000 g for 5 minutes, as appropriate for cow-side laboratory device procedures. The obtained plasma samples were separated into two Eppendorf tubes for ELISA and cow-side analysis. Plasma samples for ELISA measurement were stored at -20°C prior to analysis.

Plasma L-lactate measurement by POC lactate meter and ELISA

Plasma samples were kept at -20°C for 2 months for L-lactate measurement. Plasma L-lactate levels were determined by ELISA with 96-well plates and the use of lactate dehydrogenase specific to L-lactate. The resultant intensity of colour was quantitated at 490 nm with an l-Quant microplate spectrophotometer.

All POC lactate meters were calibrated and used in accordance with the manufacturer’s recommendations. Lactate analysis was conducted with the Vet Photometer 700 DP (Diaglobal, Germany) cow-side measurement device, based on the enzymatic-colorimetric method. The principle of this analysis is as follows: the enzymatic conversion of lactate by lactate oxidase to pyruvate, and the subsequent conversion of the intermediary-generated H₂O₂ to a dye, with the concentration of the quinonimine dye measuring the lactate concentration in the blood at 520 nm wavelengths – this final result being read automatically by the device. The analysis was performed in a series of steps: each of the separated 10 μL blood samples were added to lactate test cuvettes containing buffer and mixed thoroughly. Confirmed required test in the device, the cuvette, with the sample, was inserted into the photometer, and a blank stored in the memory. After the final sample blank was memorized in the device, all cuvettes with their samples were fitted with starter reagent caps and mixed thoroughly. After selecting ‘on/enter’, the cuvettes were inserted into the device and the results read.

Statistical analysis

Agreement between the cow-side POC meters and the ELISA laboratory method for lactate measurement was determined using the Bland-Altman method (Bland and Altman 1986, Seed 2000, Glantz 2005). The accuracy and precision of the POC lactate meters
Comparative interpretation of lactate measurement ...

were determined by concordance and Pearson’s correlation coefficients. The data obtained from both measurements were not normally distributed. Passing-Bablok regression analysis was used to compare the two lactate measurements for the best linear fit. Precision was described in terms of the 95% confidence interval (CI) in limits of agreement with a p value of 0.05. Statistical analysis was performed using a commercially available software program (GraphPad Prism 6.0, GraphPad Software Inc, San Diego, California).

Results

Descriptive statistics for the ELISA and cow-side lactate measurement method levels are shown in Fig. 1. No significant differences were found between the two measurement methods. This comparison is represented in the correlation analyses of the linear regression equation, and the 95% CIs (Fig. 2). The correlation between the two different lactate analysis methods for the same blood sample was \( r = 0.71 \).

The level of agreement (±2 SD) between the Vet Photometer method and the ELISA method is presented (Fig. 3); it was found to be higher for the ELISA analysis (bias +1.31 lower and 0.99 upper, limit).

Discussion

Plasma lactate levels might show variation in relation to disease severity. A brief explanation of this...
could be the disproportion between anaerobic and aerobic metabolism, and a likely elevated imbalance in the production and clearance of Lc (Tashkin et al. 1972, Levraut et al. 1998). On the other hand, Lc concentration can be solely down to elevations at the end stages of disease, due to certain ‘reserve’ steps involving oxygen transportation and sufficient oxygen supply to peripheral tissues (Metcalfe and Dhindsa 1970).

The literature on lactate measurements has highlighted several comparisons of POC meters, such as Accutrend Plus, i-STAT, Lactate Pro, and Lactate Scout, pointing out these devices are used extensively in ambulatory and veterinary clinics. All of these devices are based on the principles of enzymatic spectrophotometry and the enzymatic amperometric system for Lc measurement (Acierno et al. 2008, Karagiannis et al. 2013, Karapinar et al. 2013). Researches comparing the reliability of amperometric measuring devices has been conducted on cats (Acierno et al. 2008), dogs (Ferasin et al. 2007, Zacher et al. 2010, Karagiannis et al. 2013), horses (Tennet-Brown et al. 2010, Dechant et al. 2011, Radcliffe et al. 2012), foals (Castagnetti et al. 2010), cattle (Coghe et al. 2000), and calves (Burfein and Heuwiesser 2012). Apart from the data discussed at the beginning of this study, comparative interpretation of spectrophotometric analysis was the method of choice.

The results obtained from the present study evidenced the use of POC spectrophotometric devices for accurate and reliable lactate measurement, especially under field conditions. There was a positive and strong ($r=0.71$) correlation between the POC spectrophotometric device and ELISA, this result supporting findings by Makita (1997) and Pyne et al. (2000), who also detected a strong relationship.

The POC spectrophotometer allows measurement of several parameters, such as lactate, calcium, magnesium, and NEFA. In particular, measurements of NEFA and calcium are inappropriate and expensive under field conditions. The Vet Photometer is ready for use on turning on, and calibration is not needed. The Vet Photometer 700 POCT device is based on photometric analysis according to the Lambert-Bair law of enzymatic-kinetic principles, with a 520 and 546 nm measuring wave. Considerable research has been done on the reliability of measurements made according to these principles. Numerous conservational factors are able to affect the rate of enzyme-catalysed reactions through reversible or irreversible changes; of these, the effects of temperature and pH are well recognized. Optimum temperature is therefore a major variable to be considered (Robinson 2015). The Vet Photometer 700 is a cow-side device that needs to be studied in light of changes in ambient temperature. In the present study the differences between the measurements recorded by the devices can be explained by the influence of variations in ambient temperature on enzymatic kinetic reactions.

Under field conditions, lactate determination in bovines requires laboratory-based, expensive, and specific equipment. Patient-side lactate meters have been well recognized for use with horses and dogs, however (Acierno and Mitchell 2007, Thorneloe et al. 2007, Tennent-Brown 2010). In equine neonatal intensive care, Lactate Scout is regarded as reliable for determining lactate levels (Castagnetti et al. 2010), but limited research has been conducted to date on the use of POC lactate meters with ruminants. In cows with pneumonia, plasma L-lactate levels have been significantly overestimated in POCT devices, despite a high positive correlation with ELISA (Coghe et al. 2000). In another study, different anticoagulants were used for determining the accuracy of the Lactate Scout device. In cows, Lactate Scout has a positive correlation ($r = 0.75$) reference method with the use of Li-heparin tubes.
(Burfeind and Heuwieser 2012). In the present study, differences in the mean values of plasma L-lactate levels calculated by the ELISA method were positively correlated ($r = 0.71$) with those calculated by Vet Photometer DP 700.

Correlation analysis is insufficient for comparing the reliability of two measurements; thus, Passing-Bablok regression analyses were therefore used, as this is recommended for data which are not normally distributed (Passing and Bablok 1983); in the present study, the L-lactate data obtained from the two measurement techniques were not normally distributed (see Fig. 2). The evaluation of bias between the two measurement methods was plotted using the Bland-Altman method (Bland and Altman 1986); the mean bias was lower for the ELISA method than for the Vet Photometer DP 700 (see Fig. 3).

In conclusion, the Vet Photometer DP 700 is likely to be useful for cow-side measurement of lactate levels in cows. It should be borne in mind, however, that under field conditions the enzymatic kinetic reaction procedure has to be performed in optimal ambient climatic conditions.

References


