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RESEARCH ARTICLE

Clinical Utilization of Point-of-Care Blood L-Lactate Concentrations in Naturally Occurring Respiratory Disease in Feedlot Cattle

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ABSTRACT

Assessment of blood L-lactate concentration (LAC) as point-of-care (POC) biomarkers is turning into a typical procedure for different diagnostic purposes in the food animal industry. The purpose of our study was to figure out whether blood LAC measured by a hand held lactate analyzer with different variables could be utilized to anticipate the event of bovine respiratory disease (BRD) in feedlot cattle. Moreover, assess the blood LAC stability over different time point in clinically healthy feedlot cattle. Blood sample was collected at entry during processing (n=104) and at initial diagnosis of BRD (n=24). In addition, clinically healthy pen matched controls calves (n=24) were sampled at the same time of pulling diseased calves for determination of blood LAC using a handheld portable lactate analyzer. In a separate study, selected clinically healthy calves (n=9) were sampled and blood LAC stability at the different time point (0, 30, 60, 90 and 120 minute) was assessed. Logistic regression model revealed blood LAC during initial processing was significantly associated with odds of becoming a BRD case in calves revealing lung score 2. Moreover, blood LAC was significantly different (P=0.02) between clinically healthy and those calves that developed BRD and was significantly correlated (P<0.01**)** within the different time points. Our results demonstrated that analyzing blood LAC at initial diagnosis of BRD together with other clinical variable might help in the treatment decision. Therefore, further investigation should be designed to correlate blood LAC measurements for prediction of BRD clinical outcomes.

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INTRODUCTION

Bovine respiratory disease (BRD) is one of the prevalent diseases in feedlot calves. It keeps being one of the main leading causes of morbidity and mortality especially in intensely raised and newly transported calves **(**Buckham Sporer *et al.,* 2008; Pardon *et al.,* 2013**).** Current strategies for BRD identification in recently received calves comprise of measuring rectal temperature and visual appraisal of the clinical symptoms. These current methods are subjective and frequently lack affectability to predict BRD in its initial phases of development. To minimize the negative economic impact of BRD, distinctive biomarkers have been implemented at feedlot arrival to evaluate health status for adequate management of these cases according to their anticipated outcome **(**Aich *et al.,* 2009**).** Blood L-lactate is a recently introduced biomarker used to assess impact of physiological activity, which favors anaerobic metabolism and pathological phenomena due to systemic tissue or peripheral hypo perfusion **(**Coghe *et al.,* 2000**).** In cattle, the significance of rising blood LAC has been studied in cases of right displaced abomasum or abomasal volvulus (Boulay *et al.,* 2014) and in case of BRD **(**Delesalle *et al.,* 2007; Buczinski *et al.,* 2015). Lactate analysis is based either on standard photometric or biosensor-based procedure **(**Hauss *et al.,* 2014**).** Photometric procedures are accepted to give high exactness and reliability; nonetheless, the turnaround time coming about because of sample transport and preparation is too long to take into consideration fast decision making **(**Hauss *et al.,* 2014**).** Therefore, there is a need in the cattle industry for

advantageous and reliable techniques for measuring L-lactate in field situation. With the recent availability of different handheld blood L-lactate analyzers **(**Burfeind *et al.,* 2012), blood LAC can now be quickly assessed on the farm with results available in less than 1 min and at relatively low cost. The Lactate Plus (Nova Biomedical, Waltham, MA, USA) is a handheld lactate meter that gives a rapid result of blood LAC in the range from (0.3 to 25) mmol/Lin 13 seconds, uses 0.7 m*l* of whole blood and can be used easily at the farm-side**.** Our hypothesis was that blood LAC assessment of BRD-affected calves with other clinical variables could be used to influence treatment decisions at the time of initial diagnosis. The purpose of our study was to figure out whether blood LAC measured by a hand-held lactate analyzer could be utilized to anticipate the event of BRD in recently received feedlot cattle. Moreover, assess the blood LAC stability over the different time point.

MATERIALS AND METHODS

A total of one hundred thirty-five (n=135) Charolais feedlot calves were transported to the commercial feedlot located in South Farms- Beef Cattle and Sheep Field Laboratory at university of Illinois at Urbana-Champaign-USA. The calves were processed within 24 hrs after arrival. During processing, each calf was individually weighed and vaccinated with a modified live virus vaccine (5 m*l* IM; Bovi-Shield Gold FP5 L5 HB Cattle Vaccine, Zoetis Animal Health, Florham Park, NJ, USA). All calves were dewormed with Noromectin Pour-On Solution (Noromectin, Norbrook® Inc. Lenexa, KS, USA). The use of animals for this study was approved by University of Illinois Institutional Animal Care and Use Committee (IACUC Protocol: #15064).

Examination procedures were performed while the calves are being processed; rectal temperature was measured using digital thermometer. The automated lung score was recorded using microphone of an automated Whisper stethoscope (Whisper®, Geissler Corp, Plymouth, MN, USA) and software rendering a 5-point lung score (1=normal, 2=mild acute, 3=moderate acute, 4=severe acute, and 5=chronic) (Zeineldin *et al.,* 2016).

Blood samples were obtained from selected healthy calves (n=104) and blood LAC was recorded using Lactate Plus handheld lactate meters (Nova Biomedical, Waltham, MA, USA) (Hauss *et al.,* 2014).

Following processing, the calves were monitored daily for detection of clinical signs of respiratory disease according to the unit health management protocols. A BRD case was defined when the calf presenting a rectal temperature>104°F and lung score≥2. In the event that an animal develops BRD during the first month after entry, blood samples for blood LAC determination were collected when the calf is handled for treatment.

A separate study was performed for determining the blood LAC stability over different time in clinically healthy feedlot calves. After 2 month of arrival in the beef unit, total of twelve calves were examined. From all examined calves, nine clinically healthy calves with rectal temperature <104°F and lung score <2 were selected for blood sampling. Blood samples were immediately placed on ice after collection and analyzed with hand held Lactate plus meter at 0, 30, 60, 90 and 120 minute after sampling.

Statistical analysis: The statistical analyses were performed using the SPSS version 22 (IBM Corp, Version 22.0, Armonk, NY, USA, 2013) statistical package. Normal distribution of all variables was assessed by Shapiro-Wilk Test (Shapiro and Wilk, 1965). Student's t- test was used for the analysis of the significance of the differences (P). The level of significance was accepted at 5% (P<0.05).

The relationship between the Log transformed blood LAC (Log LAC), variables of rectal temperature and lung score data was assessed using the linear regression analysis including the linear correlation coefficient (R) and significance of correlation (P). Moreover, logistic regression model was performed to evaluate the effectiveness of rectal temperature, lung score data, Log LAC during the initial processing to predict the BRD cases. Goodness of Fit for the logistic regression model was evaluated using Hosmer–Lemeshow test. Lung score were added to the logistic regression model to evaluate the interaction between Log LAC during the initial processing and the potential effects of the other variables. The odd ratio (OR) and 95% Confidence interval (CI) were evaluated for the significant variables. Finally, to better evaluate the diagnostic value of blood LAC in diagnosis of BRD, receiver operating characteristic (ROC) analysis (plots a curve of sensitivity versus specificity) was performed.

The blood LAC stability over the time was investigated using Pearson correlation coefficients. Bootstrapping method was computed to calculate the suggested 95% confidence interval (CI) around the lower and upper values.

RESULTS

During the initial processing, all blood samples analyzed by the hand-held lactate analyzer were within the meter detection range. Mean blood LAC was **2**.94mmol/*l* and was ranged from (0.8 to7.10). While, mean rectal temperature was 102.9°F, and ranged from (101.4 to 104.6). Only 0.9% (1) calf were observed has chronic lung sound (lung score 5) and no calf showed sever lung sound (lung score 4); 21.1% (22) of the calves were with moderate acute lung sound (lung score 3); 45.1% (47) calves showed mild acute lung sound (lung score 2) and 32.6% (34) calves were healthy with no abnormal lung sound (lung score 1). Median blood LAC of 3.1, 3.2 and 2.4 mmol/*l* was observed for calves with lung score of 3, 2 and 1 respectively.

Linear regression analysis was modeled to evaluate the impact of rectal temperature and lung score data on blood LAC. These analyses showed significant correlation (P=0.01; R=0.25) between Log LAC and variables of lung score data during the initial processing. While, no significant correlations (P=0.59; R=0.05) were observed when assess the relationship between Log LAC and variables of rectal temperature in calves during the initial processing (Fig. 1).

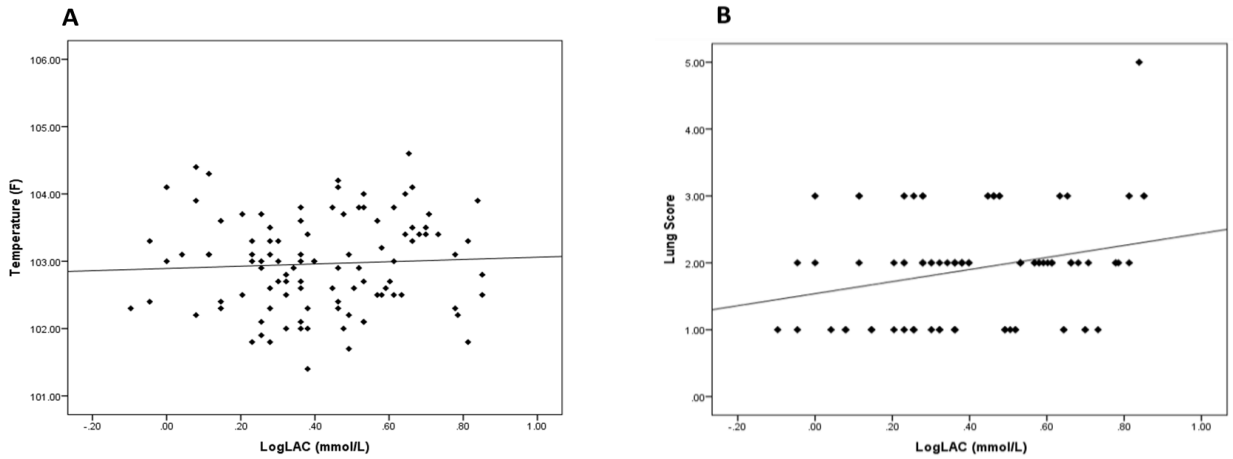

Fig. 1: Linear regression analysis of the relationship between log transformed blood LAC (Log-LAC) (mmol/*l*), rectal temperature (F°) (A) and lung score (B) during the initial management processing.

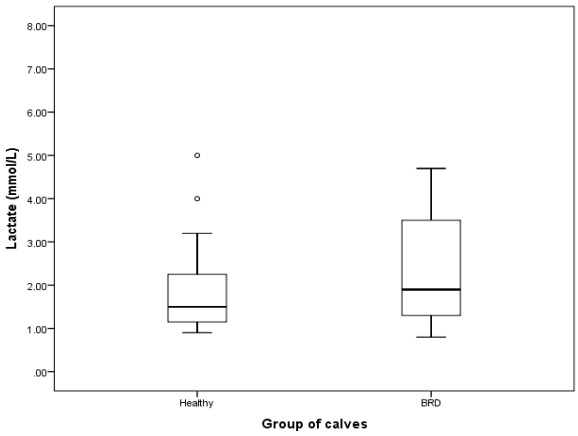

Fig. 2: Distribution of the blood LAC (mmol/*l*) in clinically healthy calves and BRD affected calves. The line within each box represents the median value, the bottom and top of the box represent the 25th and 75th percentiles, respectively. The whisker represents minimal and maximal values. The outside values, represented as circle.

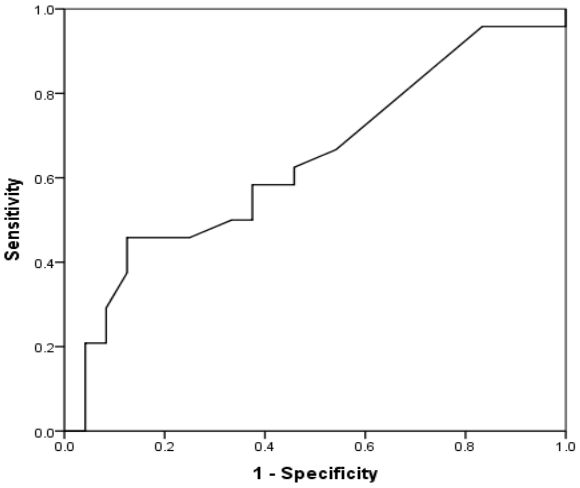

Fig. 3: ROC curve for blood LAC in all diseased calves. The ROC tests were able to accurately separate BRD cases from healthy controls. Area under the ROC curve for blood LAC= 0.64.

In a logistic regression model, which distinguished rectal temperature, lung score data and LAC as the predictor variables for BRD, rectal temperature during initial processing was significantly associated with probability of becoming a BRD case (P=0.001) with (OR=202.74; 95% CI=12.97: 3168.66). Lung Score data at processing was significantly associated with odds of becoming a BRD case (P=0.03) with (OR=2.74; 95% CI=1.4:5.34). When evaluating the relationship between LAC value at processing and odd of becoming a BRD case, statistically logistic regression analysis was modified by lung score data. In calves revealing lung score 2, Log LAC during initial processing was significantly associated with odds of becoming a BRD case (P=0.001) with (OR=1.46; 95% CI=0.99: 2.16). While, in calves showing lungs score 1 and 3, Log LAC during initial processing was not significantly associated with odds of becoming a BRD case (P=0.9; P=0.6) respectively, indicating that measurement of blood LAC during initial processing was not a better indicator for BRD cases. However, together with other variables such as lung score data, it may help in evaluating BRD risk. Hosmer–Leme show fit test did not report any problem with the entire logistic regression model (P>0.05).

During the study period, twenty-four calves were diagnosed as BRD cases (increased respiratory rate, induced cough, dyspnea, variable amount of mucopurulent discharge with rectal temperature≥104°F and lung score≥2). Our result revealed a significant difference (P=0.02) between the blood LAC in pen matched clinically healthy calves and that had BRD. The mean blood LAC was (2.3 and 1.86 mmol/*l*) for the BRD affected calves and the pen matched healthy control calves respectively (Fig. 2).

ROC analysis was performed in our study to determine an ideal cutoff value for blood LAC. Sensitivities, specificities and percent correct classifications are listed as a function of cutoff value in (Table 1). Another important measure of the accuracy of the clinical test is the area under the ROC curve. The area under ROC curve for blood LAC in our study was 0.64 (Fig. 3).

In a separate study, nine clinically healthy feedlot calves based on the clinical examination were selected to detect the values and stability of blood LAC at different time points. The mean and the 95% CI around upper and lower value for each measured variable were depicted in (Table 2). The stability of blood LAC over different time point was assessed using Pearson correlation coefficient. These analyses indicated that the blood LAC from the same blood sample was significantly stable with the different time point (P<0.01) as illustrated in (Table 3).

Table 1: The table represents the sensitivity and specificity of blood LAC at different cut offs point.

Blood LAC Cut off point	Sensitivity (%)	1 – Specificity (%)
.8500	95.80	100.00
.9500	95.80	83.30
1.0500	91.70	79.20
1.1500	87.5	75.00
1.2500	83.30	70.80
1.3500	75.00	62.50
1.4500	66.70	54.20
1.5500	62.50	45.80
1.6500	58.30	45.80
1.7500	58.3	37.50
1.9000	54.20	37.50
2.0500	50.00	37.50
2.1500	50.00	33.30
2.2500	45.80	25.00
2.3500	45.80	20.80
2.5500	45.80	16.70
2.8000	45.80	12.50
3.0500	37.50	12.5
3.3000	29.20	8.30
3.4500	25.00	8.30
2.5500	45.80	16.70
3.7500	20.80	8.30
4.2000	20.80	4.20
4.4500	16.70	4.20
4.5500	12.50	4.20
4.6500	4.20	4.20

Table 2: Rectal temperature (F°), lung score and blood LAC (mmol/*l*) at different time point (0, 30, 60, 90 and 120 minute (m)) in clinically healthy feedlot calves.

Variable	Mean±SD	Minimum	Maximum	95% Confidence Interval	
				Upper value	Lower value
Temp (F°)	102.75±0.68	101.40	103.90	102.32	103.15
Lung score	1.67±0.50	1	2	1.33	1.88
LAC 0 m	1.81±0.68	0.9	2.9	1.40	2.23
LAC 30 m	1.92±0.73	1.2	3.1	1.51	2.36
LAC 60 m	2.02±0.85	1.1	3.3	1.52	2.53
LAC 90 m	1.97±0.84	0.8	3.3	1.51	2.51
LAC 120 m	1.66±0.84	1	3	1.42	2.44

Bootstrap results are based on 1000 bootstrap samples.

Table 3: Pearson correlations and level of significance (P) of blood LAC with the different time point (0, 30, 60, 90 and 120 minute (m)).

Blood LAC	LAC 0 m	LAC 30 m	LAC 60 m	LAC 90 m	LAC 120 m
LAC 0 m	1	.988**	.986**	.986**	.991**
LAC 30 m	.988**	1	.991**	.990**	.994**
LAC 60 m	.986**	.991**	1	.994**	.989**
LAC 90 m	.986**	.990**	.994**	1	.987**
LAC 120 m	.991**	.994**	.989**	.987**	1

**Correlation is significant at the 0.01 level.

DISCUSSION

Early prediction of BRD particularly in recently received calves is considered to be a prerequisite for successful treatment **(**Helena *et al.,* 2015). It widely recognized that, the clinical examinations of the calves with BRD are not the suitable way to estimate the damage severity and functional changes of lung tissue in a sufficient manner **(**Helena *et al.,* 2015). Therefore, there is a need for other specific individual biomarker for forecasting individual disease risk with greater accuracy for improving the management strategies (Buczinski *et al.,* 2015). Of the potentially interesting predictor, blood LAC has been proven to be a prognostic tool for evaluating disease severity (Green *et al.,* 2011). Because of a limited information is available regarding the association between BRD and change in blood LAC, we analyzed these parameters during initial management processing after entry and at initial identification of BRD in recently received feedlot calves. Several methods are available for blood LAC determination including laboratory analyzer methods, blood gas analyzers and hand-held lactate meters (Chua *et al.,* 2013)**.** Because of the delayed turnaround time, laboratory analyzer methods are not ideal when rapid decision making is required (Chua *et al.,* 2013)**.** Conversely, hand-held lactate analyzer offers greater ease of use, shorter time to result, and lower cost compared to blood gas analyzers (Tanner *et al*., 2010). In our study, we evaluated blood LAC using hand held Lactate Plus analyzer (Nova Biomedical, Waltham, MA, USA) that gives a rapid result of blood LAC in 13 seconds using single drop of whole blood. The accuracy of this portable clinical analyzer has been validated in both humans (Labrecque *et al.,* 2014) and veterinary medicine **(**Hauss *et al.,* 2014; Nieto *et al.,* 2015)**.** During initial processing, mean blood LAC was 2.94 mmol/*l*. The blood LAC in these feedlot calves were nearly similar to the whole blood lactate measured by handheld lactate analyzer at processing from the jugular vein in feedlot steer **(**Buczinski *et al.,* 2015)**.** This higher value of blood LAC during the initial processing might be due to the stressful condition, suggesting that higher LAC at processing would not indicate higher risk of future BRD (Montgomery *et al.,* 2009; Buczinski *et al.,* 2015). This finding was supported by study of **(**Buczinski *et al.,* 2015) which proved that acute stress is associated with a rapid increase of blood LAC. In the other hand, an interesting interaction between lung score and blood LAC was observed during initial processing (P=0.01; R=0.25).

When evaluating the relationship between blood LAC value at processing and odd of becoming a BRD case, the logistic regression analysis was modified by lung score data. In calves revealing lung score 2, Log LAC during initial processing was significantly associated with odds of becoming a BRD case (P=0.001). Additionally, our study demonstrated significant differences in blood LAC between the BRD affected calves and those pen matched clinically healthy calves (P=0.02). ROC curves were calculated in our study to determine an ideal cutoff value for blood LAC (Fig. 3). These data suggest that single blood LAC assessment was not accurate predictor in identifying individual feedlot calves at risk for BRD infection. However, together with other variable it might be useful in diagnosis of BRD at initial identification and may help in treatment decision. Up to now, our result corresponded to those reported by (Aich *et al.,* 2009; Montgomery *et al.,* 2009; Hanzlicek *et al.,* 2010), who study the relationship between the respiratory affection and increase of blood lactate concentration. In the study reported by (Coghe *et al.,* 2000), who observed increased blood lactate levels in calves with respiratory disease within 24 h before the death. These findings were supported in hospital study where lactate concentration was measured in calves with chronic respiratory disease (Nagy *et al.,* 2006). For our knowledge, the lactate plus analyzer has not been investigated in feedlot cattle. Therefore, we measured blood LAC in healthy feedlot calves specifically from the same population at risk for developing BRD. As well as evaluating the blood LAC stability over different time point. Descriptive statistics in our study shows that the blood LAC in clinically healthy

feedlot calves were ≥2.0 mmol/*l*. Moreover, our study demonstrated significant correlation between blood LAC over different time point (P<0.01). In contrast, previous research using blood samples for evaluating the blood lactate has showed increase in lactate over time for blood samples stored in vitro at room temperature (Seymour *et al.,* 2011)**.** Therefore, assessment of blood LAC should be performed immediately after blood sample collection, as L-lactate will continue to rise because of ongoing erythrocyte metabolism (Wotman *et al.,* 2009). From the previous studies, it is obvious that the evaluation of the effects of respiratory diseases in calves on blood LAC is not uniform. This may result from the complexity of the disease severity, etiology and pathogenesis. In addition, result from different methods of lactate analysis (venous or arterial whole blood, blood plasma, blood chemistry analyzers, handheld portable analyzers, enzymatic or photometric methods). The principal limitation to our study is the small sample size of diseased calves. Another point that also needs to be taken into account is the extra-time when pulling sick animals to perform the test. Therefore, future investigation with larger sample sizes is warranted to determine the ability of blood LAC with clinical variables in prediction of future BRD and define the better specificity and sensitivity of this evaluating method.

Conclusions: In conclusion, blood LAC are higher in BRD affected claves than in pen matched clinically healthy calves. In addition, combination of blood LAC and lung score had significantly higher probability of becoming BRD cases. Finally, blood LAC>2 mmol/*l* seemed to be a best value to express the blood LAC in clinically healthy calves. Following our observations, assessment of the BRD by analysis of single blood LAC assessment were not accurate predictor in identifying individual feedlot calves at risk for BRD infection. However, together with other variable it might be useful in diagnosis of BRD at initial identification and may help in treatment decision. In our opinion, these studies are important because blood LAC measurement using hand held portable lactate analyzer as POC biomarkers in BRD in recently received calves is seldom. Its use might be useful in the evaluation and management strategies of respiratory disease during early feeding periods in feedlot industry

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