

Field Method for Monitoring Blood Glucose in Beef Cattle^{1,2}

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ABSTRACT: The purpose of this study was to determine the applicability of the Accu-Chek Easy (ACE) human self-monitoring system for monitoring glycemic status in cattle. The ACE method was compared with the Yellow Springs Instrument (YSI) analytical laboratory method in two studies. A preliminary study (62 samples) and a primary study (434 samples) involved a nine-fold range and a 10-fold range, respectively, of glucose concentrations obtained during the acute phase response of growing beef cattle to injections of varying dosages of endotoxin. The ACE monitoring method compared with the YSI analytical method resulted in similar patterns of glucose concentration change, similar ranking of glucose means across endotoxin dosages during hyper- and hypoglycemia, and a close relationship between

paired YSI and ACE concentrations from common samples. The ACE method identified all nine animals that displayed hypoglycemic distress during the acute phase response to endotoxin injection. The relationship between the YSI analytical method and the ACE monitoring method was found to be nonlinear ($YSI = -38.2 + 13.6 \cdot ACE^{.50}$; $R^2 = .99$; $S_{y \cdot x} = 7.3$ mg/dL), and the use of this equation to predict YSI values from ACE values in an independent data set resulted in linearity when YSI was regressed on the predicted YSI values ($YSI = -.78 + 1.00 \cdot \text{Predicted YSI}$; $R^2 = .87$; $S_{y \cdot x} = 6.9$ mg/dL). Even though variation seemed greater for ACE than for YSI, we concluded that a system developed for human self-monitoring of blood glucose, such as the ACE, can be used to monitor the glycemic status of cattle.

Key Words: Cattle, Glucose, Blood Sugar, Monitoring, Methodology

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Introduction

Several methods exist for measuring glucose concentration in various biological media (AOAC, 1995), and the underlying chemistry has been adapted for the analytical determination of biological fluids. The YSI method (Model 2700 SELECT Yellow Springs Instrument, Yellow Springs, OH) for the analytical determination of glucose in fresh blood samples for beef cattle has been used in our laboratory for several years. However, like most analytical methods, the

inconvenience of transporting samples to a laboratory setting, the complexity of sample handling, and instrumentation costs reduce its desirability as a method for monitoring on a near real-time basis in the field.

For several years, technology and portable instrumentation have been available for self-monitoring blood glucose by humans. Typically, current methods rely on a finger or toe prick to provide a droplet of fresh capillary blood that is delivered directly to a reaction strip containing the appropriate reagents, and either a color change or electron flow on the reaction strip is read by a portable, digital instrument. The analysis is conducted on site, and the entire process takes approximately 1 min per sample. However, these monitoring methods may not produce the accuracy required for monitoring cattle because they have been developed and standardized specifically for monitoring human capillary blood.

The purpose of this research was to determine the applicability of the blood glucose monitoring method Accu-Chek Easy (ACE, Boehringer Mannheim, Indianapolis, IN, one of several developed for human self-monitoring of blood glucose) for on-site monitoring of

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the glycemic status of cattle. The ACE monitoring method was compared with the YSI analytical method for measuring jugular blood glucose concentrations in beef cattle before and during the acute phase reaction (APR) of an endotoxin challenge.

Materials and Methods

Animal Care. The animal protocols for the research in this report were approved by the Beltsville Agricultural Research Center Institutional Animal Care and Use Committee.

YSI Method. Fresh jugular blood samples collected in heparinized 10-mL monovette tubes (Sarstedt, Newton, NC) were transported on ice to the laboratory and analyzed within 1 h. Glucose was measured in a 25- μ L sample aliquot using a calibrated YSI model 2700 SELECT instrument (YSI, 1996).

The YSI method utilizes an automated analytical instrument in which glucose is measured using an electronic probe containing an immobilized enzyme membrane. Measurement of β -D-glucose is based on a glucose oxidase/hydrogen peroxide reaction. Glucose in blood is oxidized by glucose oxidase to form gluconolactone and hydrogen peroxide, and the hydrogen peroxide, in the presence of a platinum electrode, generates electrons. Electron flow is linearly proportional to glucose concentration. The system automatically calibrates itself every five samples to assure the maintenance of linearity (up to 250 mg/dL) or to signal the need to change the enzyme membrane. In our laboratory, the analytical CV is typically within the range of 2 to 4%.

ACE Method. For the ACE method (Boehringer Mannheim, 1993, 1995), a kit was purchased (available in many pharmacies) that contains the battery-powered instrument for reading disposable reaction test-strips, a bottle of glucose standard (80 mg/dL), a container of reaction test-strips, and a standardization chip that is inserted into the monitoring instrument to electronically align the monitoring instrument with the specific lot of disposable test-strips. Containers of test strips and their standardization chips can be purchased separate from the monitor. Before analysis, the standardization chip is inserted into the instrument, the instrument is turned on, and, after a signal is received that the instrument is ready (usually about 5 s), one drop of standard glucose solution is dispensed to a test-strip, and the test-strip is read. If the instrument determines the correct glucose concentration within the provided statistical limits, then the instrument is calibrated properly. Usually it is not necessary to repeat this procedure for up to 50 samples or unless the instrument is shut down for several hours. In our experience, the standard concentration readings have been within 1 mg/dL of the documented standard glucose concentration.

Measurement of glucose with the ACE method is based on a glucose oxidase/ferricyanide reaction for measuring β -D-glucose. The test strips contain potassium ferricyanide, ferric sulfate-4-hydrate, glucose oxidase (*Aspergillus niger*), and buffer. Glucose in blood is oxidized to gluconolactone, and potassium ferricyanide is reduced to potassium ferric ferrocyanide (Prussian Blue). Color intensity measured at 660 nm is proportional to glucose concentration. The analytical CV is typically less than 4%.

For analysis of bovine blood, jugular blood was collected in heparinized 10-mL monovette tubes (Sarstedt, Newton, NC). In the preliminary study, an unmeasured droplet of blood was dispensed onto the test strip as recommended for human self-monitoring. In the primary study, a 5- μ L sample aliquot was obtained immediately from the fresh blood sample, using a mechanical micropipetter with a disposable tip, and dispensed to a disposable test-strip. The test-strip was inserted into the monitoring instrument and read. The test-strip was removed from the instrument and discarded, and within 5 s the instrument reset automatically and was ready to receive the next test-strip.

The ACE instrument does not read below a concentration of 20 mg/dL. Values below 20 mg/dL are indicated by a readout of LOW by the instrument. Blood glucose concentrations below 20 mg/dL may be observed in cattle as part of the hypoglycemic stage of the APR following endotoxin challenge. For these instances we had prepared a laboratory standard containing 50 mg/mL of glucose. The high concentration for the standard used for this purpose was to minimize the dilution of blood with standard solution. If a sample read LOW, then 10 μ L of standard solution followed by 1 mL of blood was added to a 2-mL disposable conical vial with a snap top. The sample was shaken vigorously for approximately 10 s and then analyzed as described. Concentration in the original sample was then determined by subtraction.

Preliminary Study. The purpose of this study was to determine whether the ACE method was a feasible method for monitoring blood glucose in beef cattle. This was part of a preliminary study that tested doses of *E. coli* endotoxin (lipopolysaccharides; LPS, Sigma, 055:B5) estimated as appropriate for developing a response curve but without causing animal death. This limited study involved the analysis of 62 jugular blood samples. Each of nine beef steers (Angus \times Hereford; 229 ± 11 kg BW) that had been accustomed to individual pens and rope halters and were gaining approximately 1.2 kg of BW/d were fitted with a jugular cannula. On the day following cannulation, the steers (three steers/LPS dose level) were injected with either .2, 1.0, or 2.5 μ g LPS/kg BW, and blood samples for glucose analysis were collected via the jugular cannula at 0 h (before injection) and at 1, 2,

3, 4, 6, and 8 h after injection. For all blood samples, glucose was immediately determined by ACE, and then the samples were placed on ice, transported to the laboratory, and analyzed by YSI.

Primary Study. The purpose of this study was to more fully determine the applicability of the ACE method for field monitoring of glycemic status in pathophysiologically challenged beef animals. The study involved the analysis of 434 jugular blood samples. This was part of a larger study that compared the effects of two diet regimens (60% forage vs all-concentrate), daily injection of somatotropin (.1 mg bovine somatotropin/kg BW; Elsasser et al., 1996) vs placebo, and four levels of LPS. Levels of LPS were 0, .2, 1.0, and 3.0 $\mu\text{g}/\text{kg}$ BW given as a one-time intravenous bolus injection. Twenty-eight steers and four heifers (Angus \times Hereford cattle, 241 ± 19 kg BW), which were assigned to a factorial arrangement of treatments, were sampled via a jugular cannula before and after LPS injection as described in the preliminary study and sampled again when injected with LPS 8 wk later. Two of the steers died at the end of the first sampling period; thus, only 30 steers were sampled during the second sampling period.

For the first sampling period, blood samples from two animals selected at random within each diet \times LPS level were assigned to a standardization data set, and samples from the other steers were assigned to a prediction data set (112 samples per data set). Sample assignment was switched for the second sampling thus adding 112 samples to the standardization group and 98 samples (minus two steers that died) to the prediction group for a total of 224 samples used for standardization and 210 samples for prediction. The standardization data set contained actual YSI and ACE values, and the prediction data set contained actual YSI and ACE values plus predicted YSI values calculated from its ACE values and the regression equations determined from the standardization data set.

Statistical Analysis. For the preliminary study, the ACE and YSI glucose values were evaluated by analysis of variance using the GLM procedure of SAS (1989) and by regressing YSI glucose on ACE glucose. For the primary study, both ACE and YSI were evaluated by analysis of variance as in the preliminary study. Regression analysis was also used to evaluate the relationship between YSI and ACE values over the entire range of data and over a more limited but linear range (25 to 80 mg/dL).

Results

Analytical Recovery. Recovery of added glucose was determined for both the YSI analytical laboratory method and the ACE monitoring method by determining the difference between fortified and unfortified

samples. Individual jugular samples from 10 beef steers were fortified with glucose standard (100 μL of appropriate standard in 10 mL of blood to result in either 0, 25, or 50 mg/dL additional glucose). Recoveries of added glucose for YSI were $98.7 \pm 7.0\%$ and $93.1 \pm 4.7\%$ for the 25 and 50 mg/dL additional glucose, respectively, and for ACE the respective recoveries were $107.0 \pm 18.9\%$ and $104.3 \pm 8.4\%$. Recoveries were calculated as the difference between concentrations measured in the fortified (25 and 50 mg/dL) and unfortified samples and expressed as a percentage of the fortification concentration. Even though recovery seemed to be complete for ACE, a greater analytical variation was associated with this method than was apparent for the sample analyses results in the preliminary and primary studies.

Preliminary Study. Figure 1 shows a plot of the YSI glucose values (top graph) over the sampling times for each LPS treatment and a similar plot for the ACE values (bottom graph). Glucose values by YSI ranged from 13 to 132 mg/dL of whole blood. Overall average glucose concentrations were similar for YSI and ACE (61.0 and 58.9 mg/dL, respectively; $P > .10$; SEM = 3.1 mg/dL). Even though not statistically significant, ACE seemed numerically to overestimate YSI glucose at high concentrations and underestimate YSI glucose at low concentrations (method \times time and method \times LPS not significant, $P > .10$) for this preliminary data set. At time 0, mean glucose concentrations were 70.1 and 68.3 mg/dL ($P > .10$; SEM = 4.3 mg/dL) for the YSI and ACE determinations, respectively. Respective means at peak concentration (1 h after injection) were 95.2 and 112.9 mg/dL ($P > .10$; SEM = 10.3 mg/dL) and during the lowest concentrations (3 to 4 h after injection) were 43.0 and 35.3 mg/dL ($P < .09$; SEM = 3.0 mg/dL). Based on the absolute ACE and YSI values, the relative concentration patterns for YSI and ACE across time and treatments seemed to be similar.

Figure 2 is a plot of the paired YSI versus ACE values from each sample. Over a nine-fold concentration range (20 to 208 mg/dL for ACE), the relationship between methods was curvilinear ($\text{YSI} = -50.2 + 24.6 \cdot \text{ACE}^{.38}$, $R^2 = .99$). Visually, the middle portion of this concentration range (25 to 80 mg/dL) appeared to show a linear relationship between methods.

Based on these limited data, it appeared that the ACE could be used to monitor glycemic status in cattle and that a mathematical relationship could be developed between ACE and an analytical laboratory method such as YSI to enhance the analytical capability of ACE when used for monitoring glucose in cattle.

Primary Study. Figure 3 shows a plot of the YSI glucose values (top graph) over the sampling times for each LPS treatment and a similar plot for the ACE values (bottom graph) from the primary study.

Glucose values by YSI ranged from 11.5 to 163 mg/dL whole blood. Overall average glucose concentrations were 61.9 and 56.7 mg/dL ($P < .01$; SEM = 1.7 mg/dL) for YSI and ACE, respectively. As in the preliminary study, similar patterns of glucose change were obtained using both methods of analysis, and ACE seemed to overestimate glucose at high concentrations and underestimate glucose at low concentrations (method \times time, $P < .05$; method \times LPS was not significant ($P > .10$). At time 0, mean glucose concentrations were 70.1 and 68.3 mg/dL ($P > .10$; SEM = 4.3 mg/dL) for the YSI and ACE determinations, respectively. At peak concentration (1 h after injection), the YSI glucose concentrations were 69.8,

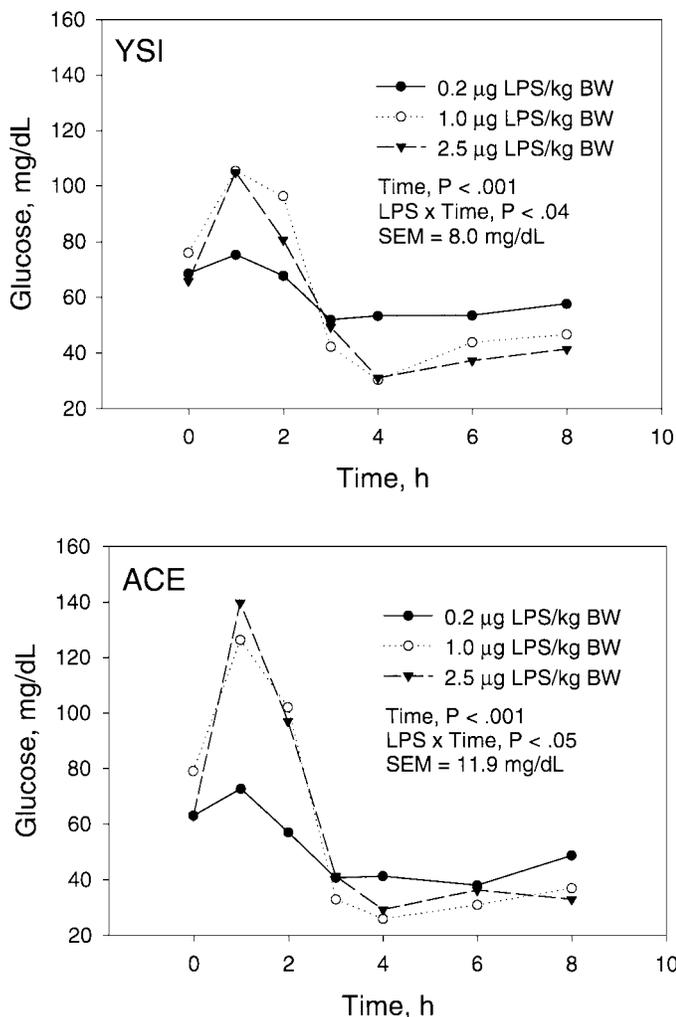


Figure 1. Blood glucose concentrations in growing beef cattle (preliminary study) before (0 h) and after an intravenous bolus injection of *E. coli* endotoxin (LPS, Sigma 55:B5; .2, 1.0, and 2.5 $\mu\text{g}/\text{kg}$ BW) and analyzed with the Yellow Spring Instrument (YSI) analytical laboratory method (top panel) or the Accu-Check Easy (ACE) glucose monitor for human self-monitoring (bottom panel). Similar patterns of glucose change were noted using both methods of analysis.

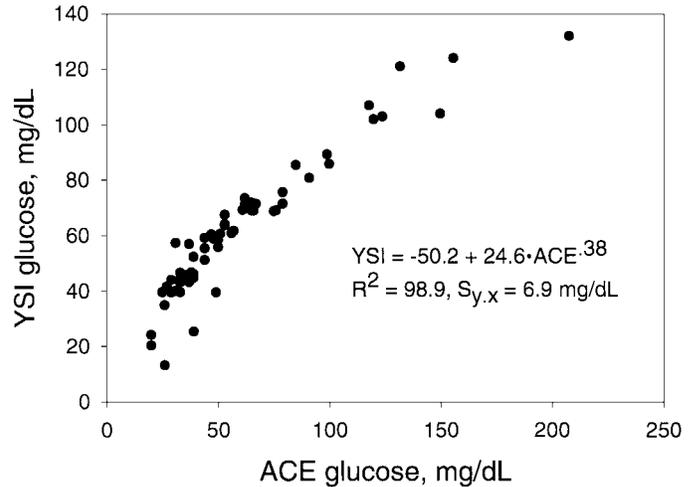


Figure 2. Blood glucose concentrations in growing beef cattle for the preliminary study determined by Yellow Spring Instrument (YSI) analytical laboratory method plotted against concentrations determined by the Accu-Check Easy (ACE) monitor for human self-monitoring. Over a nine-fold concentration range (20 to 208 mg/dL for ACE), the relationship between methods was curvilinear.

81.9, 90.4, and 83.9 mg/dL for the saline, .2, 1.0, and 3.0 LPS doses, respectively, and the respective ACE glucose concentrations were 57.5, 78.4, 94.8, and 83.9 mg/dL (method, $P > .10$; method \times LPS, $P > .10$; SEM = 3.7 mg/dL). At trough concentrations (3 to 4 h after injection), the respective YSI glucose concentrations for the saline, .2, 1.0, and 3.0 LPS doses were 66.2, 51.7, 42.5, and 39.7 mg/dL, and the respective ACE glucose concentrations were 59.0, 45.6, 42.0, and 37.3 mg/dL (method, $P < .01$; method \times LPS, $P > .10$; SEM = 2.1 mg/dL). During both the highest and lowest periods of glucose concentration, mean glucose rank among LPS treatments was similar for ACE and YSI determinations.

Figure 4 is a plot of the paired YSI vs ACE values from each sample from the standardization data set of the primary study. Over a 12-fold concentration range (17 to 228 mg/dL for ACE), the relationship between methods was curvilinear ($\text{YSI} = -38.2 + 13.6 \cdot \text{ACE}^{.50}$, $R^2 = .99$, $S_{y,x} = 7.3$ mg/dL). Over a smaller range of 25 to 80 mg/dL, the relationship between YSI and ACE was linear ($\text{YSI} = 7.8 + .97 \cdot \text{ACE}$, $R^2 = .71$, $S_{y,x} = 7.3$ mg/dL). As in the preliminary study, the regression of these values from YSI and ACE was curvilinear over the entire range of data, and, in this study, a linear relationship was obtained over a limited range of concentrations.

Figure 5 shows a plot of predicted YSI concentrations vs YSI analytical values for the entire range of data (top graph) and for the range from 25 to 80 mg/dL (bottom graph) using the prediction data set. The YSI glucose concentrations (Y axis) were the actual

YSI concentrations in the prediction data set. The predicted YSI concentrations were calculated using the ACE values from the prediction data set and the regression equation determined from the standardization data set. For the full range of data, the relationship between the actual and predicted glucose values ($YSI = -.78 + 1.00 \cdot \text{predicted YSI}$, $R^2 = .87$) was linear with the regression coefficient indistinguishable from unity and the intercept value not different from zero ($P > .10$). For the limited range of data, the relationship between predicted and actual ($YSI = -1.16 + 1.03 \cdot \text{Predicted YSI}$, $R^2 = .77$) was linear. As with the relationship for the full range of data, the slope was indistinguishable from unity, and the intercept was not different from zero ($P > .10$). The intercept of -1.16 suggested only a slight underestimation of the analytical concentration of glucose by ACE between 25 and 80 mg/dL.

Discussion

Pathophysiological stress can have a major impact on animal well-being and growth. One objective of research with beef cattle in our laboratory is to increase our understanding of the biology underlying the interplay between the immune system and growth mechanisms. Glycemic status of the animal is an important factor for determining the response to immune challenge. For example, using an intravenous challenge of endotoxin as a model, blood glucose will typically rise to twice normal levels by 1 h after endotoxin injection, and then can fall to half normal levels by 3 h (Elsasser et al., 1996). In beef cattle, some animals can exhibit glucose concentrations at or below 25 mg/dL, at which time immediate antidotal treatment is required to prevent death.

A quick and reliable method was not found in the literature for monitoring blood glucose in cattle that would give a near real-time result. The YSI methodology (YSI, 1996) has been routinely used in our laboratory for the analytical determination of blood glucose in cattle, but this was not satisfactory for field monitoring purposes. Several systems are available for human self-monitoring of capillary blood obtained from finger or toe prick; however, these systems have not been tested for monitoring jugular blood in cattle.

The Accu-Chek Easy system (Boehringer Mannheim, 1993; one of several systems available for human self-monitoring of blood glucose) was found in this study to adequately monitor changes in glycemic status. On the basis of two studies, under conditions in which endotoxin injection resulted in a nine-fold range in analytical glucose concentrations (preliminary study) and a 10-fold range in analytical glucose concentrations (primary study), the ACE monitoring system gave results comparable to the YSI method. In

both studies, mean glucose concentrations ranked the same for the YSI and ACE determinations across different dosages of endotoxin. Based on regression analysis, a close relationship was obtained between paired YSI and ACE determination across the entire range of glucose concentrations studied. Subsequently, applying the relationship between YSI and ACE glucose concentrations to an independent data set demonstrated an improvement in the precision of the ACE method as evidenced by the zero intercept and unity of slope when YSI concentrations were regressed on predicted YSI concentrations. Furthermore, based

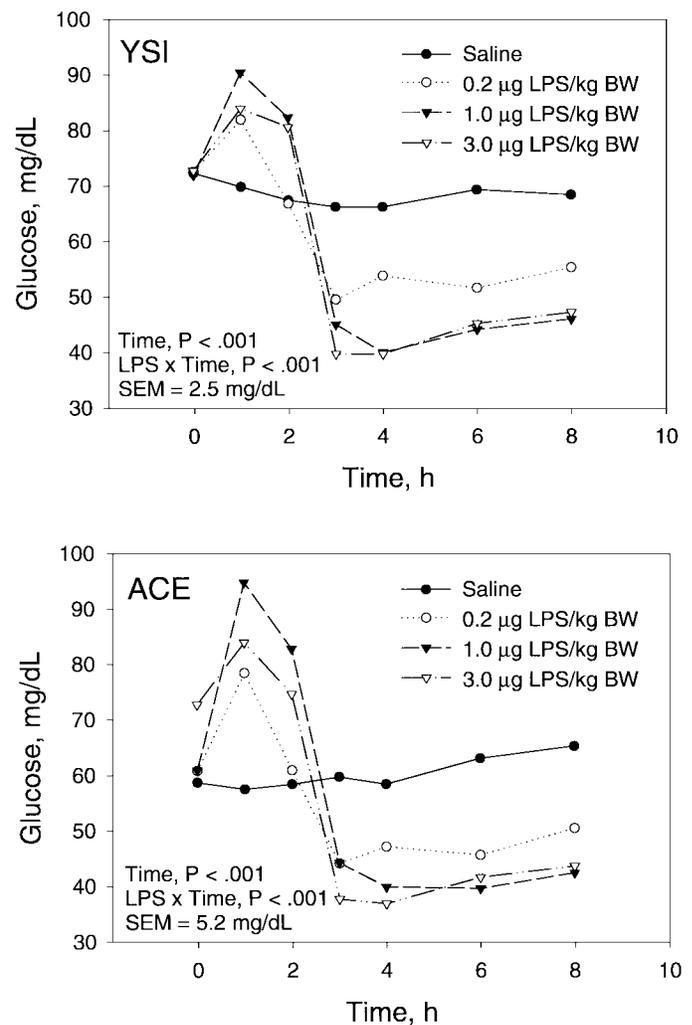


Figure 3. Blood glucose concentration in growing beef cattle for the primary study before (0 h) and after an intravenous bolus injection of saline or *E. coli* endotoxin (LPS, Sigma 55:B5; .2, 1.0, and 3.0 $\mu\text{g}/\text{kg BW}$) and analyzed by the Yellow Spring Instrument (YSI) analytical laboratory method (top panel) or the Accu-Chek Easy (ACE) glucose monitor for human self-monitoring (bottom panel). For this primary data set, similar patterns of glucose change were noted using both methods of analysis.

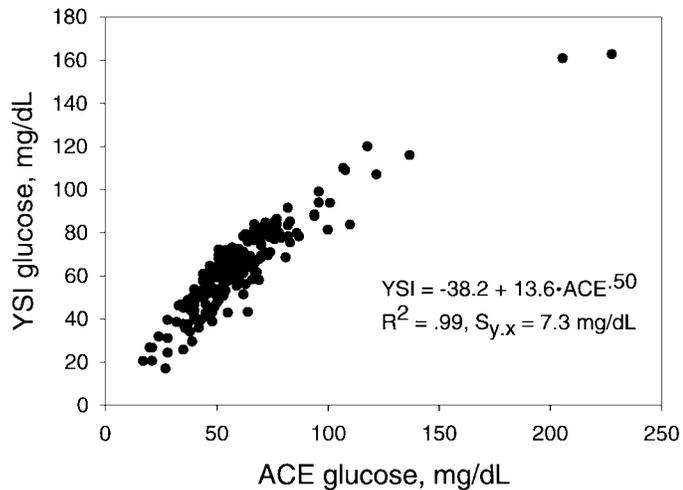


Figure 4. Blood glucose concentrations in growing beef cattle for the primary study determined by Yellow Spring Instrument (YSI) analytical laboratory method plotted against concentrations determined by the Accu-Check Easy (ACE) monitor for human self-monitoring. Over a 12-fold concentration range (17 to 228 mg/dL for ACE), the relationship between methods was curvilinear. Over a smaller range of 25 to 80 mg/dL the relationship between YSI and ACE was linear ($YSI = 7.9 + .97 \cdot ACE$, $R^2 = .71$, $S_{y,x} = 7.3$ mg/dL).

on blood glucose concentrations, both methods identified the nine cases for which animals displayed hypoglycemic distress.

Even though the ACE method appears analytically to be more variable and less precise than the YSI method, analytical precision is improved when the ACE method is standardized against a calibrated analytical laboratory procedure. It is not understood why the ACE method appears to be nonlinear in relation to the YSI method outside the midrange of 25 to 80 mg/dL, particularly at high concentrations where ACE appeared to overestimate glucose concentration. However, this overestimation may be due to the physical nature of loading the sample droplet onto the test strip. The droplet of blood is placed on the test strip within the strip's measurement window but disperses outside of that window so the end result can be a uniform sample density on the test strip regardless of droplet volume. The immediacy of the enzyme reaction, droplet dispersion time, and sample viscosity may work together to cause a slightly greater amount of reaction product within the measurement window, which becomes apparent at high glucose concentrations. This is consistent with the apparent greater overestimation of glucose by ACE for the preliminary study than for the primary study.

Based on the variability of the scatter plots, variability did not appear to be less in the primary study than in the preliminary study, even though more precise droplet volume was pipetted onto the test

strips for the primary study and ambient temperature at the animal pens was warmer during the primary study (16 to 30°C) than during the preliminary study (2 to 10°C). The ACE monitor must be buffered from low ambient temperatures because it will automatically shut down at temperatures below 5°C. Thus, based on the results of this study, we concluded that a human self-monitoring system for blood glucose such as the ACE is satisfactory for monitoring glycemic status in cattle.

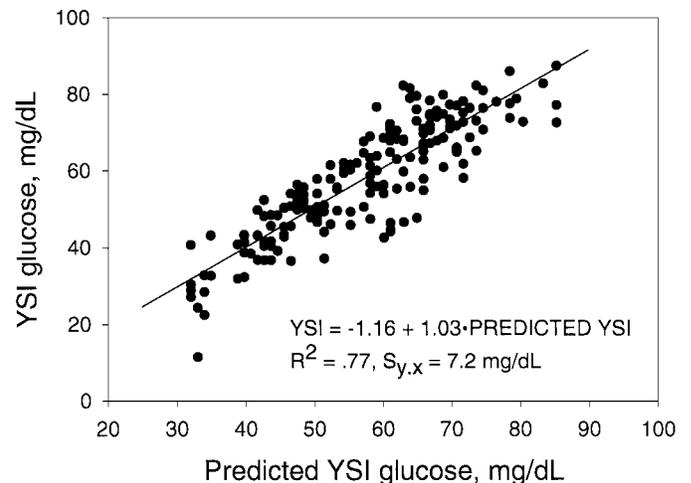
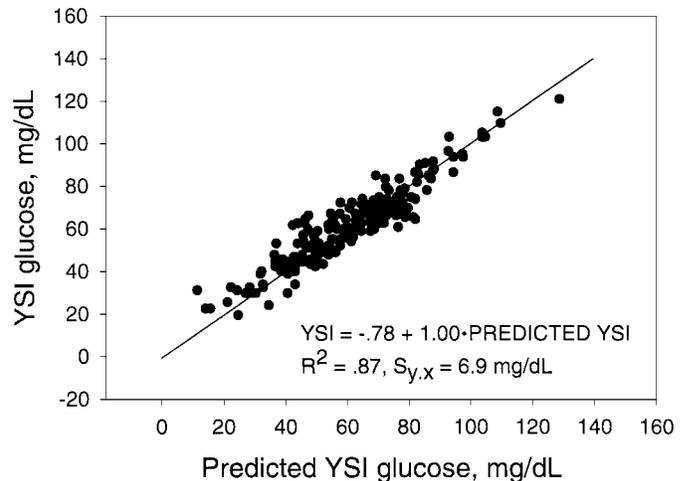


Figure 5. Predicted YSI glucose from the ACE values of the prediction data set of the primary study (top panel) and the nonlinear prediction equation from the primary study (Figure 4) plotted against YSI glucose values of the prediction data set of the primary study. The regression is represented graphically by the solid line, with a slope of unity and an intercept not different from zero ($P > .10$). The relationship between the predicted YSI and measured YSI for a smaller linear range of 25 to 80 mg/dL (bottom panel) was $YSI = -1.16 + 1.03 \cdot \text{PREDICTED YSI}$ ($R^2 = .77$, $S_{y,x} = 7.2$ mg/dL). The regression consisted of a slope equal to unity and an intercept not different from zero ($P > .10$).

Implications

We determined whether a monitor for human self-monitoring of glucose in capillary blood could be used as a convenient means for near real-time monitoring of jugular blood in cattle. The Accu-Chek Easy monitor from Boehringer Mannheim Corp. (Indianapolis, IN) was compared in this study to the laboratory analysis using the YSI Model 2700 SELECT Analyzer from Yellow Spring Instrument Co. (Yellow Springs, OH). Good agreement between methods for determining hypoglycemic, euglycemic, and hyperglycemic blood glucose was obtained. Even though results obtained with the Accu-Chek Easy monitor were somewhat more variable than with the YSI Analyzer, we concluded that the Accu-Chek Easy monitor could be used as a field monitor for blood glucose in cattle, providing a more convenient means for monitoring blood glucose remotely from a laboratory.

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