Journal of Avian Medicine and Surgery 40(1):46–55, 2026

Original Study

High Level of Agreement Between Blood Glucose Concentrations Measured With the AlphaTrak 3 Point-of-Care Glucometer and by Colorimetry in a Psittacine (Rosy-faced Lovebird, *Agapornis roseicollis*), but Not in a Passerine (House Finch, *Haemorhous mexicanus*)

Pierre Deviche, Nadia Upah, Leanna Watts, and Karen Sweazea

Abstract: Commercial point-of-care (POC) devices are commonly used to rapidly and cost-effectively measure the concentrations of vertebrate blood components such as metabolites and electrolytes. However, these devices can provide inaccurate results, and their utility must be rigorously tested by comparing results with those obtained from a reference assay. This study evaluated the accuracy of the veterinary AlphaTrak 3 glucometer (Zoetis, Kalamazoo, MI, USA) for use in 2 phylogenetically distant avian taxa, the house finch (*Haemorhous mexicanus*, order Passeriformes) and the rosy-faced lovebird (*Agapornis roseicollis*, order Psittaciformes), compared with an enzyme end-point colorimetric assay (Cayman Chemical, Ann Arbor, MI, USA) as the reference. This POC device was used to measure blood glucose in 30 house finch and 32 rosy-faced lovebird samples. The level of agreement between assays and the POC device reliability were determined with the Bland-Altman method and the Pearson product moment correlation. All the samples contained detectable glucose concentrations, but 9 finch samples had glucose concentrations exceeding the glucometer's upper detection limit and were eliminated. The POC device produced consistent results for both species. Relative to the reference assay, the glucometer overestimated glucose concentrations by 3.8% in house finch samples and by 8.7% in rosy-faced lovebird samples. There was good agreement between glucose concentrations measured with the 2 assay methods in the rosy-faced lovebird, but not in the house finch (allowable error $<15\%$). These findings confirm the need to test the validity and accuracy of measurements with commercial glucometers, and they highlight that the reliability of these devices should be tested for each species under consideration.

Key words: blood, plasma, parrot, avian, bird, passerine, psittacine

INTRODUCTION

The use of point-of-care (POC) devices for the measurement of soluble blood components, including electrolytes, metabolites, and ions, has gained considerable popularity with the veterinary and clinical diagnostic communities, wildlife rehabilitators, and scientists investigating the physiology of free-ranging and captive animals.[1–4] Several factors contribute to this popularity. In particular, POC devices are generally portable and thus advantageous for field studies during which the use of large or heavy laboratory equipment or biohazardous chemicals (eg, organic solvents, radioactive tracers) that are needed for traditional assays is not practical. In addition, POC devices are easy to use, generally only require small (microliter range) volumes of blood, and can be run with samples from small animals or repeated samples from the same individual. Finally, these assays can generally be performed without sample preprocessing (eg, centrifugation, purification) and the results are rapid, often within minutes.

Point-of-care devices have great potential to benefit veterinary clinical medicine and, more generally, animal physiology, including research that primarily uses wild animals in their natural settings. However, they also

From the School of Life Sciences, Arizona State University, 1151 S. Forest Avenue, Tempe, AZ 85287, USA (Deviche, Upah, Watts); and the College of Health Solutions, Arizona State University, 550 N Third Street Phoenix, AZ 85004, USA (Sweazea). Current address: Department of Molecular and Cell Biology, University of Connecticut, 75 North Eagleville Road, Storrs, CT 06269, USA (Upah).

Corresponding Author: Pierre Deviche, deviche@asu.edu

have potential limitations, in particular their accuracy and reliability.[1] With regard to accuracy, POC device readings can be affected by ambient temperature and humidity. For example, the AlphaTrak 3 glucometer (Zoetis, Kalamazoo, MI, USA), which was evaluated in the present study, is designed to operate at temperatures between 4–40°C (39–104°F) and relative humidities between 5 and 90% (manufacturer's specifications), and so would not be suitable for use in environmental situations (eg, winter conditions, polar regions, and hot deserts) that are outside these boundaries. However, in practice, a POC device's reliability may be more important to consider than its sensitivity to extreme ambient conditions. The question of reliability has received considerable attention on the part of investigators interested in using POC devices to measure blood glucose (GLU) in avian taxa. Some avian studies found results to be inconsistent with those of laboratory analyses[5,6] and to differ from 1 model to another.[7,8] Other studies measuring blood electrolyte concentrations with different models found results to have good agreement for some electrolytes but not for others.[9,10] In yet another study, the plasma concentrations of multiple metabolites in samples from 18 species belonging to diverse taxonomic groups were compared with 6 models.[11] In that study, relative metabolite concentrations were generally consistent across models, but this was not the case for absolute concentrations.[11] Finally, the performance of POC devices can vary across species, even those belonging to a same order (eg, Strigiformes).[10] This variation indicates that the accuracy and reliability of POC device results should be rigorously tested for each taxon being investigated by comparing these results with those obtained with a reference assay.[5–11] Proper validation is particularly important when the results are to be used in comparative studies.[1,12,13]

To address the above issues, the suitability of the veterinary AlphaTrak 3 glucometer, which is designed to measure GLU concentrations in mammalian blood samples, was investigated for use in avian research. For this, the present study assessed the level of agreement between measurements with this glucometer and those obtained with a widely used enzyme end-point colorimetric assay in 2 avian species, the house finch (HOFI, *Haemorhous mexicanus*) and the rosy-faced lovebird (RFLO, *Agapornis roseicollis*). These 2 species belong to distantly related orders: Passeriformes and Psittaciformes, respectively.[14] House finches are native to the southwest United States[15] and RFLOs are native to Africa.[16] However, this small parrot is commonly kept and bred in captivity, and it has established a large feral breeding population in the Phoenix, AZ, USA metropolitan area, where the study was conducted.[17]

A literature search identified only 4 studies using AlphaTrak glucometers in birds: canvasback ducks (*Aythya valisineria*),[18] mourning doves (*Zenaida macroura*),[19] raptors of several species,[6] and the Hispaniolan Amazon parrot (*Amazona ventralis*).[7] Notably, the latter 2 studies recommended against the use of this POC device to determine blood GLU in the taxa investigated.

METHODS

Bird capture and maintenance in captivity

Twenty HOFIs (10 adult males, 2 adult females, and 8 fully grown hatch-year birds of undetermined sex) and 16 adult RFLOs (8 birds of each sex) were caught in Phoenix, AZ, USA (33°25′14″N, 111°56′01″W; 350 m above sea level) with Japanese mist nets and seed-baited traps. The studies were done in accordance with the guidelines for the use of wild birds in research.[20] They were conducted under Arizona State University approved institutional animal care and use protocols (22-1902R and 22-1917R) and with permits issued by the Arizona Game and Fish Department, the US Fish and Wildlife Service, and the Bird Banding Laboratory (US Geological Survey). Consistent with the stipulations of the approved institutional animal care and use protocols and scientific collecting permits, the RFLOs were adopted by members of the local community at the end of the study. House finches were marked with a numbered US Geological Survey tarsal aluminum band and released at their respective capture sites.

The sex (adults) and age of HOFIs were determined based on plumage coloration and degree of skull pneumatization, respectively.[21] The plumage of adult lovebirds is sexually monomorphic, and their sex was identified by polymerase chain reaction analysis of individual blood samples (2–3 drops of blood per sample; IQ Bird Testing, Miami, FL, USA), following an experimental protocol designed by this company specifically for small bird species. At the time of capture, the health of each bird was assessed using a combination of body mass (reference intervals: HOFI, 19–22 g[15]; RFLO, 43–63 g[16]), plumage condition, and lack of visible signs of pathologies (eg, avian pox–related skin lesions). Only birds that appeared to be healthy based on these criteria were brought into captivity and used for the study.

Birds were brought to the Association for Assessment and Accreditation of Laboratory Animal Care International-accredited animal care facility at Arizona State University. They were held in light- and temperature-controlled environmental chambers under artificial light, with the photoperiod approximating natural conditions.

Finches were held in individual cages[22] for the duration of the study. Lovebirds were initially held up to 2 birds per cage for approximately 2 weeks until sex was determined, transferred to large flight aviaries (up to 3 birds of each sex per aviary) for 3 weeks, and then separated into identified pairs that were placed in smaller visually isolated cages for the remainder of the study (21 weeks). Birds were never fasted and always received food and water ad libitum. Finches were fed commercial pelleted food (Mazuri 56A6; Mazuri Exotic Animal Nutrition, St Louis, MO, USA) and RFLOs received a diet consisting of a commercial seed mix supplemented with fresh vegetable and bean mix obtained from local suppliers. The birds were under the supervision of an institutional veterinarian. They were regularly weighed to ensure that they maintained a normal weight and were observed daily for any signs of abnormal behavior such as lethargy, fluffed feathers, or anorexia.

House finches were being used in a noninvasive experiment aimed at determining the physiological effects of ambient temperature and interactions between temperature and acute stress.[22] Lovebirds were being used in a noninvasive study investigating the behavioral and physiological effects of environmental enrichment (unpublished data). Some RFLOs from the local population were infected with *Chlamydia psittaci*, the bacterium responsible for psittacosis.[23] There was no evidence found on examination that the RFLOs used in the present study carried this disease; however, as a preventive measure, all birds were treated with doxycycline (50 mg/100 mL drinking water × 7 days; Sun Pharmaceutical, Mumbai, India), starting on the second day after capture. Previous research on psittacine birds established the effectiveness of oral treatment with doxycycline in preventing *C. psittaci* replication[24] and eliminating clinical signs of the disease.[25]

Blood sample collection

Two blood samples were collected from each HOFI after 3 weeks and again after 5.5 weeks of captivity.[22] One sample was collected from each RFLO starting after 7 weeks of captivity and then approximately monthly until the end of the study.

In both species, a blood sample (baseline) was collected from each individual within 3 minutes of removing it from its home cage. Blood was collected either from the brachial vein with a heparinized microhematocrit tube (VWR International, Radnor, PA, USA; RFLO, max. 250 μL) or from the jugular vein with a 0.3-mL single-use heparinized plastic syringe (VWR International; HOFI, 80 μL).[22,26–28] Lovebirds were weighed and released in their home cage after sample collection, whereas HOFIs were held for 30 minutes in an individual opaque paper bag to stimulate a stress response;[29,30] a second blood sample (70 μL) was collected at that time and the birds were weighed and then returned to their home cage. For both species, the volume of blood collected on any given day was <1% of the species' average body weight. No adverse effect of the restraint or the venipuncture was noticed, except for occasionally producing a small hematoma at the blood collection site. After determination of whole blood GLU concentrations with the POC device, the remainder of each sample was immediately placed on ice and then centrifuged at 11 200 g for at least 10 min to separate plasma from formed elements. Plasma was stored at −80°C (−176°F) for 4–5 months before the colorimetric assay.

Glucose measurements

Glucose concentrations in freshly collected whole blood samples were measured in duplicate and within minutes of collection with the AlphaTrak 3 glucometer (2 μL per sample). It was operated according to the manufacturer's recommended protocol using the cat settings.[18] The same glucometer was used for all the measurements, and it was calibrated with the control solution per the manufacturer's guidelines at the beginning of most blood collection sessions. The glucometer was used to measure plasma GLU in 30 randomly selected HOFI samples. Nine samples contained GLU at a concentration exceeding the upper detection limit of the device (>400 mg/dL; manufacturer's specification) and were excluded from the study. The remaining 21 samples were obtained from 13 of the 20 HOFIs. Eleven samples were collected at baseline and 10 samples were collected after restraint for 30 minutes. The assay was also used to measure plasma GLU in 2 samples from each of the 16 RFLOs for a total of 32 samples, collected 7 weeks apart. All the RFLO samples contained GLU at concentration <400 mg/dL.

Plasma GLU concentrations were measured in the above samples with a commercial enzyme end-point colorimetric assay (Cayman Chemical, Ann Arbor, MI, USA) and following the manufacturer's recommended protocol. The assay is based on the oxidation of GLU with concomitant reduction of GLU oxidase, which is then regenerated to produce hydrogen peroxide. Hydrogen peroxide then oxidizes a clear substrate to result in the formation of a stable pink-colored product. The assay has been used to measure plasma GLU in multiple passerine species,[31–34] including HOFI,[26] as well as nonpasserine species.[11,35,36] Samples were diluted 15 times and assayed in duplicate; all the samples from each

species were processed in a single assay. Plasma GLU in 10 of the above HOFI samples was also measured in a separate assay to determine interassay variation. Each assay included a complete standard curve consisting of 8 GLU solutions at concentrations ranging from 0 to 100 mg/dL. Absorbance of the wells was read at 514 nM.

Statistical analyses

The study aimed at evaluating the consistency of GLU measurements with a POC device and the level of agreement between these measurements and those obtained for the same blood samples with a reference colorimetric assay. It was not intended to investigate whether GLU concentrations were intraindividually consistent over time or the potential influence of factors such as acute stress (HOFI) or environmental enrichment (RFLO) on glycemia. These variables were accordingly not included in the statistical analyses.

Each data set was tested for the presence of statistical outliers by the Grubb test and for normal distribution by the Kolmogorov-Smirnov test. Standard curves of the colorimetric assay were analyzed by linear regression to determine the relationship between GLU concentrations and corresponding absorbance. The paired Student t-test was used to compare GLU concentrations measured in the same HOFI samples during 2 independent assays, and the Pearson product moment correlation was used to measure the relationship between these concentrations.

Whether the 2 assay methods yielded equally consistent results was assessed with the Bland-Altman method (percentage difference between measurements versus measurement averages[37]) and by examination of the coefficients of repeatability (CR; values below which the absolute differences between 2 measurements would lie with 95% probability[38–40]). Bias was defined as the mean percentage difference between duplicates, and limits of agreement (LOAs) as the lower and upper 95% confidence intervals of the bias.[41]

The Pearson product-moment correlation was used to determine the strength and direction of the relationship between values obtained with the glucometer and the colorimetric assay. However, this correlation does not inform on the level of agreement between variables.[39,41] In particular, it does not estimate bias (ie, 1 method giving systematically lower or higher values than the other), and pairs of variables that are in visually poor agreement can result in relatively high correlation coefficients.[42] To address these limitations, data for each species were analyzed by Passing-Bablok regression,[43,44] and, in order to specifically measure the level of agreement between assays, also by the Bland-Altman method. Coefficients of reliability were calculated as described above (ie, by percentage differences in GLU concentrations measured with the 2 methods).[37] The lower and upper acceptance limits (allowable error) were set at a predefined 15% difference from the bias.[41] As recommended,[39] standard deviations (SD) were calculated by the following formula, in which SD_1 and SD_2 are the SDs of the differences between duplicate values obtained with the glucometer (SD_1) and by colorimetry (SD_2), and SD_3 is the SD of the differences between average values obtained with the 2 assays:

$$\sqrt{0.25 \times SD_1^2 + 0.25 \times SD_2^2 + SD_3^2}$$

Following Bland and Altman's[38] recommendation, whether absolute differences between pairs of values varied systemically as a function of the corresponding individual means of the 2 values (ie, heteroscedasticity test[45]) was assessed with the Pearson product moment correlation.

Data were analyzed using GraphPad Prism 10 (GraphPad Software, Boston, MA, USA) and SigmaPlot 13.0 (Grafiti LLC, Palo Alto, CA, USA), and graphed with GraphPad Prism 10. The threshold for statistical significance of all tests was set at $P = 0.05$, and unless otherwise indicated, data are presented as means ± standard errors.

RESULTS

Quality controls and mean glucose concentrations

In the colorimetric assay standard curves, absorbance was in both species linearly related to GLU concentrations across the range of these concentrations used in the assay (in both species $R^2 > 0.999$, $P < 0.0001$; Supplemental Fig S1), with average coefficients of variation of replicates equal to 1.3% (HOFI) and 3.9% (RFLO). Glucose concentrations measured by colorimetry in the same HOFI samples (n = 10) in 2 separate assays were correlated (Pearson product moment correlation: $R^2 = 0.60$, $P = 0.009$) but differed by 15% (397 ± 9 mg/dL versus 345 ± 10 mg/dL; paired Student t test: $t_9 = 8.09$, $P = 0.004$; interassay coefficient of variation = 10%). The lower limit of detection of the assay was 0.23 mg/dL.

No data set contained statistically significant outliers ($P > 0.05$), and all data sets were normally distributed ($P > 0.10$). Mean plasma GLU concentrations measured by colorimetry equaled 359.9 ± 9 mg/dL for HOFIs and 303 ± 4 mg/dL for RFLOs (Table 1). The intra-assay coefficients of variation for HOFI

Table 1. Agreement between duplicate blood glucose measurements with the AlphaTrak 3 glucometer (glucometer) and by enzyme end-point colorimetry assay (colorimetry) in the house finch (*Haemorhous mexicanus*) and the rosy-faced lovebird (*Agapornis roseicollis*) as determined by the Bland-Altman method (percentage differences between measurements versus averages). For each comparison, the table includes the blood glucose concentration (mean ± SE mg/dL, percentage bias, associated SD, lower and upper 95% confidence intervals of the bias [lower and higher limits of agreement (LOAs)], and the coefficients of reliability [CRs]).

	Sample size	Mean	% Bias	SD	Lower LOA	Upper LOA	CR
House finch							
Glucometer	21	359 ± 9	−0.29	5.53	−11.13	10.56	15.33
Colorimetry	21	345 ± 7	0.59	6.62	−12.38	13.55	18.33
Rosy-faced lovebird							
Glucometer	32	303 ± 4	1.13	2.43	−3.64	5.89	6.73
Colorimetry	32	278 ± 5	3.73	3.73	−3.58	11.04	10.33
Comparison of assays							
House finch	21	352 ± 7	3.77	10.98	−17.74	25.28	30.40
Rosy-faced lovebird	32	290 ± 4	8.73	6.86	−4.72	22.17	19.00

samples (n = 21) equaled 2.6% (glucometer) and 3.6% (colorimetric assay), respectively. Corresponding coefficients of variation for RFLO samples (n = 32) equaled 1.5% and 3.1%, respectively.

Measurement consistency and agreement between assays

Twenty-one HOFI blood samples were obtained from 13 birds and 32 RFLO blood samples were obtained from 16 birds. When comparing duplicate GLU concentrations measured with each type of assay, all LOAs were within the predefined 15% acceptance limits (ie, the 2 assays provided similarly consistent measurements; Table 1). In addition, within each species CRs were similar for the 2 types of assays (Table 1), indicating that the measurement consistency did not depend on the assay type.

Measurements with the glucometer and by colorimetry were positively related (HOFI: correlation coefficient [r] = 0.55, $P = 0.01$; RFLO: $r = 0.73$, $P < 0.001$; Fig 1). Passing-Bablok regression analysis revealed in both species that the 95% confidence interval for the regression line slope included 1 and the 95% confidence interval for the Y-intercept included 0, suggesting that the 2 measurement techniques provided similar results (Table 2). On average, the glucometer mildly overestimated GLU concentrations in both species (Fig 1). This overestimation (systematic bias) equaled 25.0 ± 3.3 mg/dL (8.7%) in the RFLO and 34.3 ± 7.5 mg/dL (3.8%) in the HOFI (Table 1) and was significantly larger in the RFLO than in the HOFI (Student t test: $t = 2.08$, $P = 0.04$).

Absolute differences in GLU concentrations between the 2 assays did not correlate with corresponding mean concentrations in either species (HOFI: $r = 0.03$, $P >$

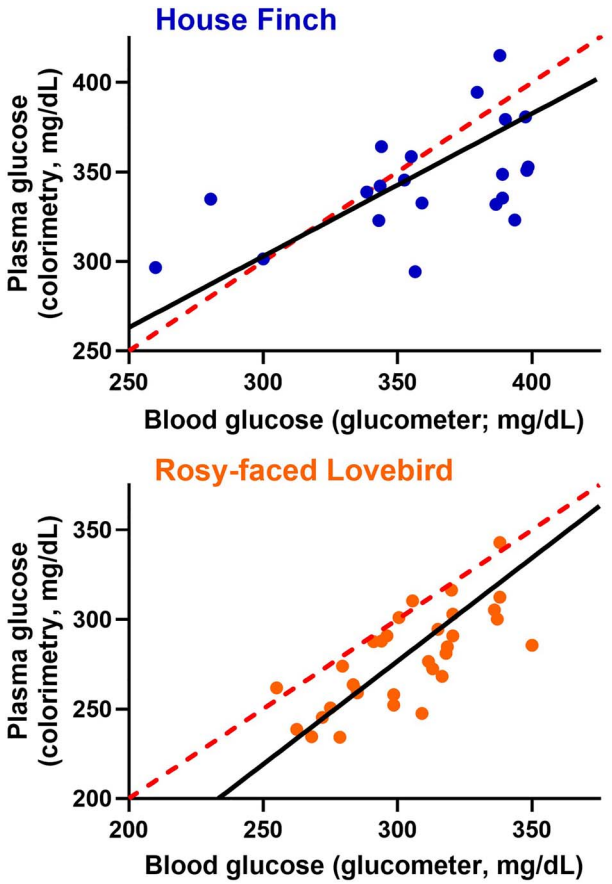

Figure 1. Scattergrams of the relationship between blood glucose concentrations measured by the AlphaTrak 3 glucometer and plasma concentrations measured by enzyme end-point colorimetry in the house finch (*Haemorhous mexicanus*; n = 21 samples) and rosy-faced lovebird (*Agapornis roseicollis*; n = 32 samples). For each species, the figure includes the regression line calculated based on Passing-Bablok analysis parameters (black) and the line of equality (red dotted line).

Table 2. Passing-Bablok regression analysis of the relationship between blood glucose measurements with the AlphaTrak 3 glucometer and by enzyme end-point colorimetry assay (colorimetry) in the house finch (*Haemorhous mexicanus*) and the rosy-faced lovebird (*Agapornis roseicollis*). For each species, the table shows the sample size, slope of the regression, and Y-intercept, as well as associated 95% confidence intervals (CIs).

	House finch	Rosy-faced lovebird
Sample size	18	32
Slope (95% CI)	0.8 (0.43 to 1.54)	1.15 (0.91 to 1.45)
Y-intercept (95% CI)	63.79 (−206 to 183.86)	−68.81 (−159.36 to 0.47)

0.90; RFLO: $r = 0.12$, $P > 0.50$; Fig 2). Values obtained with 1 assay did not vary systemically as a function of those obtained with the other assay, thereby providing no indication of heteroscedasticity.

When comparing the 2 assays, it was found that limits of agreement were within those of the allowable error (15%) in the RFLO, but outside the allowable error boundaries in the HOFI (Table 1; Fig 3).

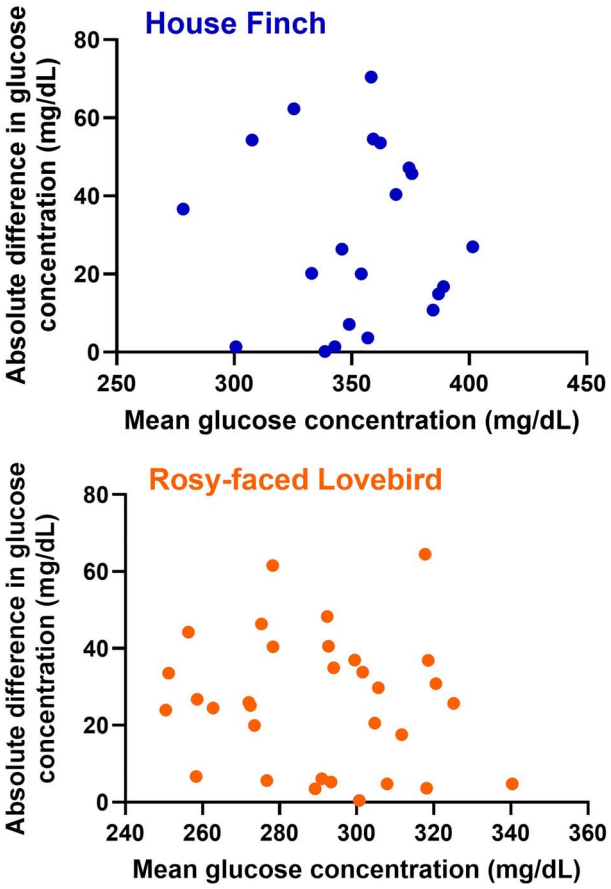

Figure 2. Scattergrams of the relationship between absolute differences in blood glucose concentrations measured using the AlphaTrak 3 glucometer and by enzymatic end-point colorimetry, and the corresponding mean glucose concentrations in the house finch (*Haemorhous mexicanus*; n = 21 samples) and the rosy-faced lovebird (*Agapornis roseicollis*; n = 32 samples).

We interpret these and associated CR values to indicate a high level of agreement between results obtained with the 2 assay types in the RFLO, but not in the HOFI.

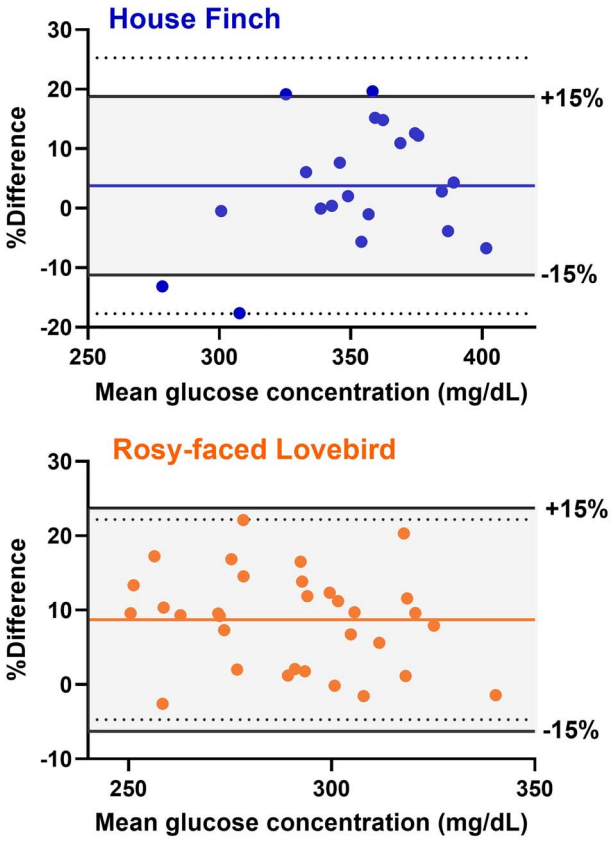

Figure 3. Bland-Altman plots showing the agreement between measurements of blood glucose concentrations with the AlphaTrak 3 glucometer and by enzyme end-point colorimetry in the house finch (*Haemorhous mexicanus*) and the rosy-faced lovebird (*Agapornis roseicollis*). Graphs show the individual differences (%) between the 2 assays as a function of the average blood glucose concentrations measured with the 2 assays. On each graph, the solid-colored horizontal line represents the mean percentage difference, the dotted lines represent the lower and upper 95% confidence intervals (concentrations of agreement), and the solid black lines represent the 15% acceptance limits.

DISCUSSION

Glycemia is an essential indicator of metabolic condition. Abnormally low or high GLU can signal a pathological state, and so glycemia is often measured to diagnose disease and during assessment of the health profile of captive as well as free-ranging avian species. The determination of blood GLU in veterinary settings relies increasingly on the use of commercial POC devices, which are portable, are inexpensive, require a small volume of blood, and often provide results within minutes. Reliance on these devices assumes that they provide consistent and accurate measurements. The present study found that the AlphaTrak 3 veterinary glucometer measures blood GLU consistently in a psittacine bird, the RFLO, and in a passerine bird, the HOFI. However, only in the RFLO are blood GLU measurements with the glucometer in agreement with those obtained with the colorimetric assay. A main implication of these results is that the use of this glucometer in avian veterinary settings must be validated for every species considered.

Blood samples of both species were assayed for GLU with the glucometer in duplicate and the LOAs of differences between duplicates were $<15\%$, indicating that the glucometer provided consistent results. Consistency was particularly high for the RFLO samples, for which LOAs were less than 6% (Table 1). Furthermore, in both species, CR and LOA values were generally similar whether samples were assayed with the POC or by colorimetry (Table 1). Thus, result consistency was similar for the 2 types of assays.

Glucose concentrations measured with the glucometer and the colorimetric assay were positively correlated in both species. However, as discussed elsewhere,[41] a positive relation between results obtained with 2 different methods does not necessarily establish agreement between these methods, which must therefore be evaluated by a separate approach such as the Bland-Altman method. Using this approach, a high level of agreement between GLU concentrations was determined with the 2 assay types for the RFLO. In contrast, LOAs in the HOFI exceeded the 15% allowable error (Table 1; Fig 3), thereby indicating in this species that the POC device and colorimetric assay measurements were not in good agreement. Accordingly, the AlphaTrak 3 glucometer is not recommended for the measurement of blood GLU in HOFI samples. Other studies likewise concluded that this device does not reliably measure blood GLU in some other avian taxa,[6] including the Hispaniolan Amazon parrot.[7] Thus, the accuracy of the glucometer measurements cannot be taken for granted and must be rigorously tested for each species.

Several factors need be considered in the interpretation of these findings and their significance. One of these factors is that data obtained from the colorimetric assay were used as the reference to compare with those obtained with the glucometer. The same colorimetric assay has been used to measure plasma GLU in multiple avian species, including in the HOFI, and these studies found that glycemia can, for instance, be affected by acute stress[31,32,46] and ambient light conditions.[34] Furthermore, average GLU concentrations measured by colorimetry in the HOFI (359.9 ± 9 mg/dL) and the RFLO (303 ± 4 mg/dL) are within the range of those reported for other avian taxa belonging to the same taxonomic orders (Passeriformes: 162-522 mg/dL; Psittaciformes: 180-432 mg/dL).[12,47,48] These observations are consistent with the conclusion that the colorimetric assay accurately measures plasma GLU in normoglycemic birds. However, to our knowledge, the level of agreement between results obtained with this assay and a "gold standard" method (laboratory chemistry analyzer[6,49]) has not been evaluated. Future work is needed to address this question to unambiguously establish the reliability and validity of data obtained with the colorimetric assay. It would also be important to determine whether blood GLU concentrations measured with the POC device correlate to those measured with the colorimetric assay in HOFI and RFLO that are experimentally manipulated to induce hypoglycemia or hyperglycemia.

The glucometer mildly overestimated GLU concentrations relative to the colorimetric assay in both study species. Two factors may have contributed to this overestimation. First, blood GLU was measured with the glucometer within minutes of sample collection, whereas plasma GLU was measured by colorimetry after blood samples were kept on ice for up to 2 hours before centrifugation and plasma samples were then stored at -80°C for 4–5 months. Some loss of GLU due to enzymatic breakdown may have occurred during sample storage on ice and/or at -80°C. This possibility is, however, unlikely, based on the results of previous research. Indeed, GLU concentrations in human serum decreased by $\leq 3\%$ during storage either at 17°C for 25 days or at 2–8°C for 30 days.[50] In addition, GLU concentrations in human plasma did not decrease significantly during storage at -20°C for up to 3 months.[51] Second, LOAs are statistical estimates that are sample specific.[39] Thus, as these authors state, statistically analyzing a different set of samples from the same species will yield different LOAs, and it cannot be discarded that this would in turn lead to different conclusions about the level of agreement between POC and the colorimetric assay results.

The overestimation of GLU measurements with the glucometer was substantially larger in RFLO (bias = 8.7%) than in HOFI (bias = 3.8%). The cat setting was used on the glucometer because, similar to the situation in birds, 93% of felid blood GLU is contained in plasma and only 7% contained within erythrocytes (glucometer manufacturer's specifications). Blood GLU in birds is for the most part located in plasma,[52] and variation in blood GLU distribution results from differences in permeability of erythrocytes to GLU. There is evidence that this permeability in birds is quite low. The erythrocytes of mature chickens (*Gallus gallus domesticus*) take up little glucose.[53] In addition, the GLUT1 transport proteins that facilitate unidirectional glucose transport into red blood cells are not normally active in these cells during normal aerobic conditions, and transport rather occurs bidirectionally through antiporters.[54] Furthermore, passerine red blood cells contain a low level of glycated hemoglobin (mean of 1.3% in the collared flycatcher, *Ficedula albicollis*[55]), although it is unclear whether this is due to lower GLU uptake by these cells or to relative resistance of avian hemoglobin to glycation. Psittacine birds have some different hematological characteristics from other birds,[56] and it is conceivable that the rate of GLU exchange between plasma and erythrocytes differs between psittacine birds and other birds, which could account for the larger overestimation of blood GLU concentrations measured with the glucometer in the RFLO relative to HOFI. Future research aimed at evaluating this hypothesis is warranted, as GLU distribution in the blood and its uptake by red blood cells have been investigated in few avian species, and to our knowledge not in psittacine birds.

Acknowledgments: NU and LW received financial support from the National Science Foundation–funded CAP Long-term Ecological Research Project (FP00014165). LW's research was also supported by an Arizona State University (ASU) Graduate Research Support Program award. Additional support was provided by a grant from the ASU Global Drylands Center.

REFERENCES

1. Stoot LJ, Cairns NA, Cull F, et al. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Conserv Physiol.* 2014; 2:cou011.
2. Perrault JR, Arendt MD, Schwenter JA, et al. Comparison of 2 glucose analytical methodologies in immature Kemp's ridley sea turtles: dry chemistry of plasma versus point-of-care glucometer analysis of whole blood. *J Vet Diagn Invest*. 2021;33:595–599.
3. Smyth BJ, Polaski RS, Safer A, et al. Point-of-care glucose and lipid profile measures using a human point-of-care device in mouse models of type 2 diabetes mellitus, aging, and Alzheimer disease. *J Am Assoc Lab Anim Sci*. 2021; 60:609–615.
4. St Clair SL, Ulyatt CM, Corkin MT, et al. Glucose testing methods: a systematic review and meta-analysis of diagnostic accuracy of point-of-care devices for neonatal hypoglycemia. *J Pediatr*. 2025;278:114438.
5. Acierno MJ, Mitchell MA, Schuster PJ, et al. Evaluation of the agreement among three handheld blood glucose meters and a laboratory blood analyzer for measurement of blood glucose concentration in Hispaniolan Amazon parrots (*Amazona ventralis*). *Am J Vet Res*. 2009;70: 172–175.
6. Hiebert K, Reich S, Allender MC. Evaluation of the agreement between 2 point-of-care glucometers and a laboratory automated analyzer in North American raptors. *J Avian Med Surg*. 2022;36:278–286.
7. Acierno MJ, Schnellbacher R, Tully TN Jr. Measuring the level of agreement between a veterinary and a human point-of-care glucometer and a laboratory blood analyzer in Hispaniolan Amazon parrots (*Amazona ventralis*). *J Avian Med Surg*. 2012;26:221–224.
8. Lieske L, Ziccardi MH, Mazet JAK, et al. Evaluation of 4 handheld blood glucose monitors for use in seabird rehabilitation. *J Avian Med Surg*. 2002;16:277–285.
9. Rettenmund CL, Heatley JJ, Russell KE. Comparison of two analyzers to determine selected venous blood analytes of Quaker parrots (*Myiopsitta monachus*). *J Zoo Wildl Med*. 2014;45:256–262.
10. Ammersbach M, Beaufrere H, Gionet RA, et al. Laboratory blood analysis in Strigiformes, part II: plasma biochemistry reference intervals and agreement between the Abaxis Vetscan V2 and the Roche Cobas c501. *Vet Clin Pathol*. 2015;44:128–140.
11. Morales A, Frei B, Leung C, et al. Point-of-care blood analyzers measure the nutritional state of eighteen free-living bird species. *Comp Biochem Physiol A Mol Integr Physiol*. 2020;240:110594.
12. Braun EJ, Sweazea KL. Glucose regulation in birds. *Comp Biochem Physiol B Biochem Mol Biol.* 2008;151:1–9.
13. Tonyushkina K, Nichols JH. Glucose meters: a review of technical challenges to obtaining accurate results. *J Diabetes Sci Technol*. 2009;3:971–980.
14. Prum RO, Berv JS, Dornburg A, et al. A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. *Nature*. 2015;526:569–572.
15. Badyaev AV, Belloni V, Hill GE. House finch (*Haemorhous mexicanus*), version 1.0. In: Poole AF, ed. *Birds of the World*. Cornell Lab of Ornithology, Ithaca, NY; 2020.
16. Collar N, Boesman PFD. Rosy-faced lovebird (*Agapornis roseicollis*), version 1.0. In: Hoyo J, Elliott A, Sargatal J, et al, eds. *Birds of the World*. Cornell Lab of Ornithology; 2020.
17. Radamaker KA, Corman TE. Status of the rosy-faced lovebird in Phoenix, Arizona. *Ariz Birds*. 2011;6:8–14.
18. Anderson NL, De La Cruz SEW, Brenn-White M, et al. Reference values and comparison of blood chemistry and plasma protein values between gold standard analyzers and four point-of-care devices in free ranging

canvasbacks (*Aythya valisineria*). *J Zoo Wildl Med.* 2022;53:302–318.
19. Basile AJ, Kreisler A, Hassen R, et al. Acute metformin induces hyperglycemia in healthy adult mourning doves, *Zenaida macroura. Comp Biochem Physiol A Mol Integr Physiol*. 2024;291:111594.
20. Fair J, Paul E, Jones J, et al, Eds. *Guidelines to the Use of Wild Birds in Research*. Washington, D.C. Ornithological Council; 2023.
21. Pyle P. *Identification Guide to North American Birds. Part I. Columbidae to Ploceidae*. Slate Creek Press, Bolinas, CA; 1997.
22. Deviche P, Sweazea K, Upah N. Adjustments to energy provisioning and oxidative balance in response to temperature in a wild passerine. *Comp Biochem Physiol A Mol Integr Physiol.* 2025;305:111864.
23. Dusek RJ, Justice-Allen A, Bodenstein B, et al. *Chlamydia psittaci* in feral rosy-faced lovebirds (*Agapornis roseicollis*) and other backyard birds in Maricopa County, Arizona, USA. *J Wildl Dis*. 2018; 54:248–260.
24. Flammer K, Whitt-Smith D, Papich M. Plasma concentrations of doxycycline in selected psittacine birds when administered in water for potential treatment of *Chlamydophila psittaci* infection. *J Avian Med Surg*. 2001;15:276–282.
25. Guzman DS, Diaz-Figueroa O, Tully T Jr, et al. Evaluating 21-day doxycycline and azithromycin treatments for experimental *Chlamydophila psittaci* infection in cockatiels (*Nymphicus hollandicus*). *J Avian Med Surg*. 2010;24:35–45.
26. Valle S, Eagleman D, Kieffer N, et al. Disruption of energy homeostasis by food restriction or high ambient temperature exposure affects gonadal function in male house finches (*Haemorhous mexicanus*). *J Comp Physiol B*. 2020;190:611–628.
27. Gao S, Deviche P. The causative effects of corticosterone on innate immunity during the stress response in the house sparrow, *Passer domesticus. Gen Comp Endocrinol.* 2019;275:30–37.
28. Deviche P, Desaivre S, Giraudeau M. Experimental manipulation of corticosterone does not influence the clearance rate of plasma testosterone in birds. *Physiol Biochem Zool*. 2017;90:575–582.
29. Wingfield JC, Monk D. Behavioral and hormonal responses of male song sparrows to estradiol-treated females during the non-breeding season. *Horm Behav*. 1994;28:146–154.
30. Deviche P, Valle S, Gao S, et al. The seasonal glucocorticoid response of male rufous-winged sparrows to acute stress correlates with changes in plasma uric acid, but neither glucose nor testosterone. *Gen Comp Endocrinol*. 2016;235:78–88.
31. Davies S, Rodriguez NS, Sweazea KL, et al. The effect of acute stress and long-term corticosteroid administration on plasma metabolites in an urban and desert songbird. *Physiol Biochem Zool*. 2013;86:47–60.
32. Deviche P, Beouche-Helias B, Davies S, et al. Regulation of plasma testosterone, corticosterone, and metabolites in response to stress, reproductive stage, and social challenges in a desert male songbird. *Gen Comp Endocrinol*. 2014;203:120–131.
33. Fokidis HB, des Roziers MB, Sparr R, et al. Unpredictable food availability induces metabolic and hormonal changes independent of food intake in a sedentary songbird. *J Exp Biol*. 2012;215:2920–2930.
34. Pham K, Lazenby M, Yamada K, et al. Zebra finches (*Taeniopygia castanotis*) display varying degrees of stress resilience in response to constant light. *Gen Comp Endocrinol.* 2025;114644.
35. Basile AJ, Jasbi P, Clark W, et al. A four-week white bread diet does not alter plasma glucose concentrations, metabolic or vascular physiology in mourning doves, *Zenaida macroura. Comp Biochem Physiol A Mol Integr Physiol.* 2020;247:110718.
36. Funk A, Hutton P, Earl S, et al. Short communication: levels of land use and land cover in Phoenix, Arizona are associated with elevated plasma triglycerides in the Gambel's quail, *Callipepla gambelii*. *Comp Biochem Physiol A Mol Integr Physiol.* 2020; 247:110730.
37. Stockl D, Cabaleiro, DR, Van Uytfanghe K, et al. Interpreting method comparison studies by use of the Bland–Altman plot: Reflecting the importance of sample size by incorporating confidence limits and predefined error limits in the graphic. *Clin Chem*. 2004; 50:2216–2218.
38. Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet*. 1995;346:1085–1087.
39. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Int J Nurs Stud*. 2010;47:931–936.
40. Vaz S, Falkmer T, Passmore AE, et al. The case for using the repeatability coefficient when calculating test–retest reliability. *PLoS One*. 2013;8: e73990.
41. Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. *Statistician*. 1983;32:307–317.
42. Ludbrook J. Linear regression analysis for comparing two measurers or methods of measurement: but which regression? *Clin Exp Pharmacol Physiol*. 2010;37: 692–699.
43. Hintze JL. Passing-Bablok regression for method comparison. NCSS. Accessed 30 June 2025. https://www.ncss.com/wp-content/themes/ncss/pdf/Procedures/NCSS/Passing-Bablok_Regression_for_Method_Comparison.pdf. 2020.
44. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in Clinical Chemistry, Part I. *J Clin Chem Clin Biochem*. 1983;21: 709–720.
45. Lindholm C, Altimiras J. Point-of-care devices for physiological measurements in field conditions: a smorgasbord of instruments and validation procedures.

Comp Biochem Physiol A Mol Integr Physiol. 2016;202:99–111.
46. Cooper CE, Hurley LL, Deviche P, et al. Physiological responses of wild zebra finches (*Taeniopygia guttata*) to heatwaves. *J Exp Biol.* 2020;223:jeb225524.
47. Sweazea KL. Revisiting glucose regulation in birds—a negative model of diabetes complications. *Comp Biochem Physiol B Biochem Mol Biol*. 2022;262:110778.
48. Harr KE. Clinical chemistry of companion avian species: a review. *Vet Clin Pathol*. 2002;312:140–151.
49. Mohsenzadeh MS, Zaeemi M, Razmyar J, et al. Comparison of a point-of-care glucometer and a laboratory autoanalyzer for measurement of blood glucose concentrations in domestic pigeons (*Columba livia domestica*). *J Avian Med Surg*. 2015;29:181–186.
50. Hirigo AT. Effect of storage time and temperature on the stability of serum analytes. *Health Sci J*. 2020;15:6255.
51. Pleus S, Freckmann G, Baumstark A, et al. Stability of glucose concentrations in frozen plasma. *J Diabetes Sci Technol*. 2022;16:1096–1100.
52. Bell DJ. The distribution of glucose between the plasma water and the erythrocyte water in hens' blood. *J Exp Physiol Cogn Med Sci*. 1957;42:410–416.
53. Johnstone RM, Mathew A, Setchenska MS, et al. Loss of glucose transport in developing avian red cells. *Eur J Cell Biol*. 1998;75:66–77.
54. Cloherty EK, Diamond DL, Heard KS, et al. Regulation of GLUT1-mediated sugar transport by an antiport/uniport switch mechanism. *Biochemistry* 1996;35:13231–13239.
55. Recapet C, Sibeaux A, Cauchard L, et al. Selective disappearance of individuals with high levels of glycated haemoglobin in a free-living bird. *Biol Lett*. 2016;12:20160243.
56. Polo FJ, Peinado VI, Viscor G, et al. Hematologic and plasma chemistry values in captive psittacine birds. *Avian Dis.* 1998;42:523–535.