Research Note: Comparison of chicken blood chemistry and electrolyte parameters between the portable i-STAT1 clinical analyzer and VetScan VS2 serum biochemistry panel using Hy-Line commercial white-egg laying hens

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ABSTRACT The i-STAT1 clinical analyzer has become an increasingly popular tool in clinical production animal medicine as it can provide pen-side results in a cost effective and timely manner when compared to standard benchtop serum biochemistry blood gas and chemistry analyses. This study compares the results of the portable Abbott i-STAT1 analyzer and the Abaxis VetScan VS2 for glucose (Glu, mg/dL), ionized Ca (mmol/L), Na (mmol/L), and K (mmol/L) values. Three genetically distinct commercial varieties (CV) of Hy-Line white-egg laying hens are used in this study (Hy-Line W-36, Hy-Line W-80, and Hy-Line W-80+). Thirty blood samples (n = 10 per CV) were obtained in the production house from the brachial vein and concurrently analyzed by the i-STAT1 portable device. Serum from 22 of these same samples was analyzed via VetScan VS2, a benchtop serum clinical biochemistry analyzer, using VetScan Avian/Reptilian Profile Plus reagent rotors. A paired T-test was used to test for statistical differences in means between the 2 instruments for each of the parameters. Parameters with significant mean differences were then subject to correlation and regression analysis to further evaluate relationships between the results from the 2 methods. Significant differences between means were found for Glu, Na, and K levels. Ca levels were found to be not directly comparable by the 2 analysis instruments. This comparison elucidates the importance of clinical analyzer validations when applying different strategies of diagnostic medicine in poultry.

Key words: blood gas, blood chemistry, laying hen, white egg layer, i-STAT1

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INTRODUCTION

The i-STAT1 clinical analyzer allows for pen-side results for blood chemistry and electrolytes in a cost effective and timely manner when compared to standard benchtop serum biochemistry blood gas and chemistry analyses. As a result, the i-STAT1 has become an increasingly popular tool in poultry medicine due to its ability to report quick and consistent results (Schaal et al., 2016). In recent years, the device has been implemented in various investigations including, but not limited to, reference interval establishment, Ca tetany in broiler-type birds, and various heat-stress studies (Steinmetz et al., 2007; Martin et al., 2010, 2011; Schaal et al., 2016; Van Goor et al., 2016, 2017; Wang et al., 2018; Rowland et al., 2019). Considerable reference interval variability is observed between commercial types and varieties of chickens making accurate inferences on blood gas and chemistry data difficult. Comparisons between different analysis instruments and understanding of potential confounding factors are important for practical application of this information for clinicians and field veterinarians (Sauer et al., 2019). The i-STAT1 has been compared to other analysis instruments, with one of the first such comparisons utilizing a chicken model being published in 2007 by Steinmetz et al. which used Lohmann leghorn layer-type birds to validate results of the i-STAT1 against an unspecified conventional serum chemistry analyzer. This study by Steinmetz et al. also utilized the EG7+ cartridge, rather than the CG8+ cartridge more commonly used today. Nonetheless, it was

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concluded that the i-STAT1 can reliably measure pH, pO_2, pCO_2, Na, iCa, and PCV, and accurately calculate HCO_3, tCO_2, Hb, and sO_2 compared to the unspecified benchtop analysis instrument (Steinmetz et al., 2007). Our study provides a comparison of the results of the Abbott i-STAT1 portable analyzer (Abbott, Abbott Park, IL) and the Abaxis VetScan VS2 benchtop clinical analyzer (Abaxis, Union City, CA) with a *Gallus gallus* species model. The specific parameters used for comparison are glucose (Glu), Ca, Na, and K as information from these 4 analytes is provided by both analysis instruments.

MATERIALS AND METHODS

Bird Husbandry, Blood Collection, and Analysis

Birds were handled according to the company animal welfare policy approved by the veterinarian on staff. All animal procedures were approved by the Institutional Animal Care and Use Committee of Iowa State University before the initiation of sampling. Three commercial varieties (CVs) of white-egg laying hens were utilized in this study (Hy-Line W-36, Hy-Line 80, and Hy-Line 80+). The laying hens representing each CV were chosen from a clinically normal, actively laying population at the time of blood collection. Hens were 66 wk (461 D) of age at the time of sampling. The American Society for Veterinary Clinical Pathology recommends a minimum sample size of at least 20 healthy reference individuals for direct validation (Friedrichs et al., 2012). As a result, 30 blood samples (n = 10 per CV) were obtained in this study via venipuncture of the brachial wing vein using 1 mL syringes with no anticoagulant and needles. All samples were subject to analysis by both instruments; thus, randomization was not performed.

Each sample of fresh, uncoagulated blood was directly loaded into a CG8+ cartridge, and immediately analyzed by an i-STAT1 clinical analyzer following manufacturer recommendations. Excess blood from each collection was reserved, allowed to clot, and serum was collected from these samples. The serum samples were then submitted to Iowa State University's Department of Veterinary Pathology for analysis via VetScan VS2, a benchtop serum clinical biochemistry analyzer, using VetScan Avian/Reptilian Profile Plus reagent rotors. Out of multiple blood parameters reported by the 2 instruments, 4 were shared: Glu in mg/dL, iCa in mmol/L from i-STAT1 and total Ca in mg/dL from VetScan VS2, Na in mmol/L from i-STAT1 and in mEq/L from VetScan VS2, and K in mmol/L from i-STAT1 and in mEq/L from VetScan VS2.

Statistical Analysis

Results from all samples were aggregated as the number sampled per CV was too low to detect potential confounding factors or bias. Statistical analysis was performed via paired T-test to test for statistical difference in the means between the 2 analysis instruments for each pair of common parameters. Pairs of parameters with significantly different means (P-value <0.01) were then subjected to correlation and regression analysis. Statistical analysis was performed using the computing environment R (R Development Core Team, 2019) using function t.test in R (R: a language and environment for statistical computing; R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/) to generate tables and figures described in this manuscript.

RESULTS AND DISCUSSION

Of the 30 serum samples submitted to the Iowa State University's Department of Veterinary Pathology for VetScan VS2 analysis, only a subset of successful results was obtained for Ca (n = 22), Glu (n = 22), Na (n = 21), and K (n = 17). The submission failures were reportedly due to either insufficient sample volume or hemolysis, neither of which were readily visually observed at the time of collection and submission to the pathology laboratory. The comparison of the analysis instruments indicated significant differences in means between i-STAT1 and VetScan VS2 results for both Glu and Na (Table 1). Glu and Na were found to be significantly correlated (P-value <0.05) between the 2 analysis instruments (rGluIstat, GluVetScan = 0.81; rNaIstat, NaVetScan = 0.78), but with a correlation coefficient significantly different from 1 (95% CI not including 1). Other correlations between instruments or parameters were not significantly different from zero (Figure 1). Regression analysis showed that i-STAT1 had a positive proportional analytic bias for Glu and Na relative to

Table 1. Results of the paired T-test analysis between the i-STAT1 clinical analyzer and VetScan VS2 including means and SD for Ca, glucose, Na, and K parameters.

	Ca		Glucose[1]		Na[1]		K	
	i-STAT[2]	VetScan	i-STAT	VetScan	i-STAT[3]	VetScan	i-STAT[3]	VetScan
Units	mmol/L	mg/dL	mg/dL	mg/dL	mmol/L	mEq/L	mmol/L	mEq/L
Mean	1.69 (19.35)	>20.0	241.91	220.91	146.61	143.05	4.62	5.31
SD	0.11	N/A	9.03	9.98	2.09	2.22	0.26	0.46
n	22	22	22	22	21	21	17	17

[1]P-value significance <0.001.
[2]Estimated calculated value in mg/dL comparable to the VetScan value is indicated within parentheses.
[3]Value in mEq/L is 1:1 for Na and K (Tully et al., 2019).

Figure 1. Correlation coefficients of glucose, Na, and K between the i-STAT1 clinical analyzer and VetScan VS2. The X indicates correlations that are not significantly different from zero.

VetScan VS2. A change of 1 mg/dL of Glu in i-STAT1 is equivalent to a change of 0.73 (±0.12) mg/dL in VetScan VS2, and a change of 1 mmol/L of Na in i-STAT1 is equivalent to a change of 0.73 (±0.13) mEq/L in VetScan VS2. Statistically significant differences between the 2 analysis instruments for Glu and Na illustrate the importance of caution when comparing results utilizing different instruments. The mean K values were significantly different between the 2 instruments (P-value <0.001), but there was no significant linear relationship between the values of K (R = 0.35) for the 2 instruments, suggesting lack of concordance between the results. Although the K parameter lacked the minimally sufficient number of samples (n = 17), adding 3 more data points likely will not result in a linear relationship

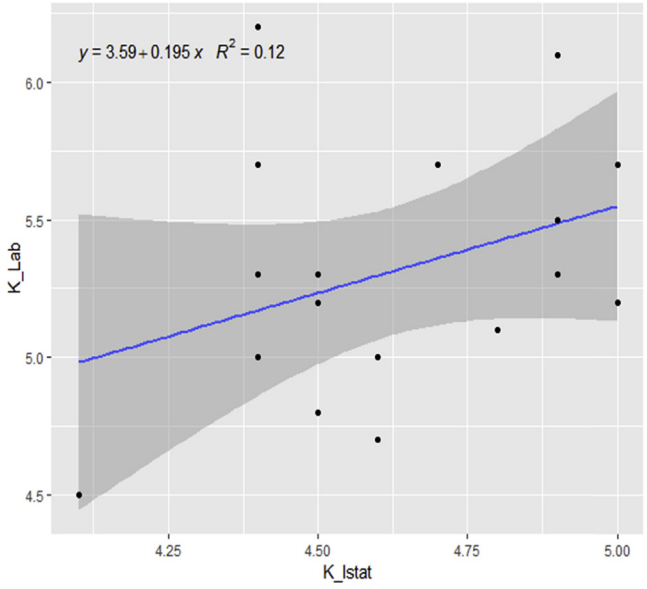

Figure 2. Scatter plot chart with data points (n = 17) of VetScan VS2 to calculate the regression coefficient (R = 0.35). Data indicate that that there is no significant linear relationship between the 2 assays.

(Figure 2). In addition, there seems to be an issue with low sample viability with VetScan VS2 (17/30 = 57%).

Concerning the iCa parameter, the VetScan VS2 analytical range was reported to be 4 to 16 mg/dL. This set of blood samples collected from actively laying hens consistently yielded a reported total iCa value of >20.0 by VetScan VS2, a result outside of the reported range for this instrument. In contrast, the i-STAT1 clinical analyzer did yield a mean iCa value of 1.69 mmol/L (SD = 0.11), a value within the analytical range of 0.25 to 2.50 for the i-STAT1 clinical analyzer. The mean iCa value of 1.69 mmol/L is equivalent to 6.77 mg/dL using the following conversion principle: (mg/dL × 0.2495 = mmol/L) (Stockham and Scott, 2008; Tully et al., 2009). Blood iCa comprises approximately 50% of the total Ca in a blood sample in a mammalian host; however, this value was reported to be 35% in a study utilizing a turkey model (McHurtry et al., 1984; Goff, 2015). By using the 35% value from an avian model rather than a mammalian estimation, the mean estimated total Ca from the i-STAT1 clinical analyzer was estimated to be 19.35 mg/dL (estimated total Ca = iCa × [1/0.2495] × [1/0.35]). The estimated values of 19.35 mg/dL falls outside of the previously indicated VetScan VS2 dynamic range, which suggests that only qualitative results can be obtained for Ca in laying hens from VetScan VS2 whereas i-STAT1 provides quantitative measurements of iCa. i-STAT1 was not only able to report a continuous numerical value for Ca, but also able to provide the iCa blood concentrations. The metabolically active form of Ca in systemic circulation is iCa, thus serving as an applicable and precise method of reporting blood Ca when conducting research with laying hens, which will inevitably have blood Ca levels consistently out of the reference interval in benchtop analyzers. A major limitation of this study is its applicability to other analysis instruments beyond the i-STAT1 clinical analyzer and the benchtop analyzer. As neither i-STAT1 nor VS2 is technically considered an official standardized instrument, this comparison could be considered problematic if either of the 2 analysis instruments is indeed imprecise.

CONCLUSIONS

This study has provided statistical evidence that mean Glu and Na were different between analysis instruments; i-STAT1 had a relative positive proportional analytic bias but the instruments remained correlated. Although viable sample numbers fall short of the recommendation by the American Society for Veterinary Clinical Pathology, no obvious relationship was shown for K between the 2 analysis instruments. The Ca parameter was found to fall outside the dynamic range of VetScan VS2 and thus could not be compared between the 2 devices. Although a large reference chemistry instrument was not used as the gold standard reference method, these 2 analysis instruments are considered mainstream in today's veterinary clinical chemistry laboratories in terms of both practicality and availability. The use of

CG8+ cartridge in this study ensures conviction in the use of i-STAT1 for Glu values vs. the 2007 study which used EC7+, which does not include Glu. In addition, specific avian panels are better suited in this sort of diagnostic inquiries in comparison to a more comprehensive panel. In summary, the Abbott i-STAT1 clinical analyzer has proven to be an easy-to-use device, providing immediate interpretation of blood gas and chemistry results in field settings.

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Conflict of Interest Statement: The authors do not declare any conflict of interest.

REFERENCES

Friedrichs, K. R., K. E. Harr, K. P. Freeman, B. Szladovits, R. M. Walton, K. F. Barnhart, and J. Blanco-Chavez. 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet. Clin. Pathol. 41:441–453.

Goff, J. P. 2015. Minerals. Pages 567–592 in Dukes' Physiology of Domestic Animals. 13th ed. W. O. Reece, ed. Wiley Blackwell, Ames, IA.

Martin, M. P., M. Wineland, and H. J. Barnes. 2010. Selected blood chemistry and gas reference ranges for broiler breeders using the i-STAT®1 handheld clinical analyzer. Avian Dis. 54:1016–1020.

Martin, M. P., M. Wineland, O. J. Fletcher, and H. J. Barnes. 2011. Selected blood chemistry values in mobility-impaired broiler breeder hens with suspected calcium tetany using the i-STAT handheld clinical analyzer. Avian Dis. 55:340–345.

McMurtry, J. P., R. W. Rosebrough, and N. C. Steele. 1984. Studies on blood calcium relationships in the Turkey. Poult. Sci. 10:2094–2099.

Rowland, K., M. E. Persia, M. F. Rothschild, C. Schmidt, and S. J. Lamont. 2019. Venous blood gas and chemistry components are moderately hertiable in coomercial white egg-laying hens under acute or chronic heat exposure. Poult. Sci. 0:1–5.

Sauer, Z. S., K. Taylor, A. Wolc, A. Viall, N. O'Sullivan, J. E. Fulton, I. Rubinoff, T. Schaal, and Y. Sato. 2019. Establishment of Hy-Line commercial laying hen whole blood gas and biochemistry reference intervals utilizing portable i-STAT1 clinical analyzer. Poult. Sci. 98:2354–2359.

Schaal, T. P., J. Arango, A. Wolc, J. V. Brady, J. E. Fulton, I. Rubinoff, I. J. Ehr, M. E. Persia, and N. P. O'Sullivan. 2016. Commercial Hy-Line W-36 pullet and laying hen venous blood gas and chemistry profiles utilizing the portable i-STAT®1 analyzer. Poult. Sci. 95:466–471.

Steinmetz, H. W., R. Vogt, S. Kastner, B. Riond, and J. M. Hatt. 2007. Evaluation of the i-STAT portable clinical analyzer in chickens (Gallus gallus). J. Vet. Diagn. Invest. 19:382–388.

Stockham, S. L., and M. A. Scott. 2008. Calcium, phosphorus, magnesium and regulatory hormones. Fundamentals Vet. Clin. Pathol. 2:597.

Tully, Thomas N., G. M. Dorrestein, and Alan K. Jones. 2009. Conversions to système international (SI) units. Handbook Avian Med. 2:447–448.

Van Goor, A., C. M. Ashwell, M. E. Persia, M. F. Rothschild, C. J. Schmidt, and S. J. Lamont. 2016. Quantitative trait loci identified for blood chemistry components of an advanced intercross line of chickens under heat stress. BMC Genomics 17:287.

Van Goor, A., C. M. Ashwell, M. E. Persia, M. F. Rothschild, C. J. Schmidt, and S. J. Lamont. 2017. Unique genetic responses revealed in rna-seq of the spleen of chickens stimulated with lipopolysaccharide and short-term heat. PLoS ONE 12:e0171414.

Wang, Y., P. Saelao, K. Chanthavixay, R. Gallardo, D. Bunn, S. J. Lamont, J. M. Dekkers, T. Kelly, and H. Zhou. 2018. Physiological responses to heat stress in two genetically distinct chicken inbred lines. Poult. Sci. 97:770–780.