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Establishment of Hy-Line commercial laying hen whole blood gas and biochemistry reference intervals utilizing portable i-STAT1 clinical analyzer

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Abstract

Blood gas and biochemistry reference intervals were established for 3 genetically distinct commercial varieties (CVs) of Hy-Line laying hens: 2 brown-egg layers (Hy-Line Brown, Hy-Line Silver Brown) and a tint-egg layer (Hy-Line Sonia) utilizing the i-STAT1 analyzer. Each respective variety of laying hen was sampled on a replicate cycle of 2 wk for a total of 6 replicates (35 to 46 wk of age). Blood samples were obtained in the production house from the brachial vein, and subsequently analyzed by the i-STAT1 portable device. i-STAT1 clinical analyzer reports blood gas and biochemistry values for the following parameters: pH, partial pressure of carbon dioxide (pvCO2, mm Hg), partial pressure of oxygen (pvO2, mm Hg), bicarbonate (HCO3, mmol/L), base excess (BE, mmol/L), saturation of oxygen on hemoglobin (sO2%), glucose (Glu, mg/dL), sodium (Na, mmol/L), potassium (K, mmol/L), total concentration of carbon dioxide (TCO2, mmol/L), ionized calcium (iCa, mmol/L), hematocrit (Hct % packed cell volume [PCV]), hemoglobin (Hb, g/dL). A total of 1,800 individual hen i-STAT1 records were utilized in the establishment of reference interval values for the 13 parameters between the 3 CVs. Statistical analysis via ANOVA and Tukey's test revealed significant line differences for all 13 blood gas and chemistry parameters measured, with particularly interesting results in iCa. The blood gas and chemistry parameters collected in this study will serve as reference intervals to set the framework for potential future correlations to genetic markers, physiological abnormalities, and production performance.

Keywords

blood gas, blood chemistry, laying hen, colored egg layer, i-STAT1

Disciplines

Agriculture | Animal Sciences | Large or Food Animal and Equine Medicine | Poultry or Avian Science | Veterinary Pathology and Pathobiology

Comments

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ABSTRACT Blood gas and biochemistry reference intervals were established for 3 genetically distinct commercial varieties (CVs) of Hy-Line laying hens: 2 brown-egg layers (Hy-Line Brown, Hy-Line Silver Brown) and a tint-egg layer (Hy-Line Sonia) utilizing the i-STAT1 analyzer. Each respective variety of laying hen was sampled on a replicate cycle of 2 wk for a total of 6 replicates (35 to 46 wk of age). Blood samples were obtained in the production house from the brachial vein, and subsequently analyzed by the i-STAT1 portable device. i-STAT1 clinical analyzer reports blood gas and biochemistry values for the following parameters: pH, partial pressure of carbon dioxide ($pvCO_2$, mm Hg), partial pressure of oxygen (pvO_2, mm Hg), bicarbonate (HCO_3, mmol/L), base excess (BE, mmol/L), saturation of oxygen on hemoglobin (sO_2%), glucose (Glu, mg/dL), sodium (Na, mmol/L), potassium (K, mmol/L), total concentration of carbon dioxide (TCO_2, mmol/L), ionized calcium (iCa, mmol/L), hematocrit (Hct % packed cell volume [PCV]), hemoglobin (Hb, g/dL). A total of 1,800 individual hen i-STAT1 records were utilized in the establishment of reference interval values for the 13 parameters between the 3 CVs. Statistical analysis via ANOVA and Tukey's test revealed significant line differences for all 13 blood gas and chemistry parameters measured, with particularly interesting results in iCa. The blood gas and chemistry parameters collected in this study will serve as reference intervals to set the framework for potential future correlations to genetic markers, physiological abnormalities, and production performance.

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

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INTRODUCTION

The portable i-STAT1 (2006) blood gas and biochemistry analyzer (Abbott Laboratories, East Windsor, NJ) allows for very prompt and accurate on-site analyses of various physiological parameters for the interpretation of human and animal health. This technology has been proven to be a useful research tool in various animal industries including but not limited to cattle, equine, pigs, fish, cats, dogs, exotic parrots, falcons, and poultry (Peiró et al., 2010; Rettenmund et al., 2014; Raghav et al., 2015). Although utilization of i-STAT1 technology is an efficient mode of data collection, it is still a relatively novel method in poultry medicine, when compared to more conventional in-house laboratory analyzers.

One of the earliest validations of i-STAT1 technology utilized Rhode Island Red hens, a classic brown-egg laying breed of chicken, in 2007 (Steinmetz et al., 2007). The utilization of i-STAT1 technology for the collection of blood gas and biochemistry data has recently been increasing in popularity in the layer and broiler facets of the poultry industry. Broiler breeder reference intervals have been established (Martin et al., 2010). The established broiler reference intervals were then utilized in an investigation of the relationship between blood chemistry values and calcium tetany, a clinical disease in breeder hens (Martin et al., 2011). A recent heat stress study identified quantitative trait loci pertinent to various response mechanisms to heat stress with an advanced intercross line of broiler birds (Van Goor et al., 2016). The blood gas and chemistry data obtained via i-STAT1 technology from that study were subsequently utilized for correlation to genotypic data on the broiler birds (Van Goor et al., 2016). Another heat stress study investigated the differences between blood gas and chemistry values in Leghorn and Fayoumi genetic lines aiming to identify their significance when trying to improve heat tolerance through genetic improvement (Wang et al., 2018). In the commercial layer industry, reference intervals were established for commercial Hy-Line International W-36 pullets and laying

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hens, a common white-egg laying Leghorn strain in the US Midwest (Schaal et al., 2016).

Genetically distinct laying bird lines may vary in blood gas and chemistry profiles, and one purpose of this study was to determine if this assumption was correct. Samples were collected and blood chemistry analyzed from 2 brown-egg and 1 tint-egg laying commercial varieties (**CVs**), allowing for the establishment of specific population reference intervals for commercial use. The global market of laying hens remains stable, with approximately 55% brown, 40% white, and 5% tint-egg layers according to N.P. O'Sullivan (Hy-line International, Dallas Center, Iowa, personal communication); thus, study on these different varieties has international relevance. The study herein expands upon the initial work published by Schaal et al. (2016) to broaden the scope of reference intervals available to include 2 brown-egg and a tint-egg laying **CVs** beyond the previously investigated white-egg laying W-36 variety. The 3 specific varieties included in the investigation were Hy-Line Brown (**HYB**), Hy-Line Silver Brown (**HYSB**), and Hy-Line Sonia (**HYS**), a tint variety. Accurate reference intervals for these laying bird lines have the potential to guide advances in genetic selections if correlated with production parameters. Additionally, blood gas and chemistry data can be valuable when experimentally modulating poultry nutrition specific to genetic lines. Environmental factors such as temperature and humidity may be evaluated and correlated to blood gas and biochemistry data (Van Goor et al., 2017; Wang et al., 2018). Furthermore, establishment of reference intervals for particular blood gas and chemistry parameters can provide useful clinical application when applied to identified disease states and general physiological abnormalities. The practical application of i-STAT1 clinical analyzers is still in the beginning stages in the poultry industry, and the implementation possibilities are vast.

MATERIALS AND METHODS

Bird Husbandry

Three varieties of commercial production birds were used in this study: 2 brown-egg (HYB and HYSB) and 1 tint-egg variety (HYS). Birds were 35 wk and 5 d of age at the beginning of the study with an average minimum sampling size of 100 birds for each of the 6 CVs. The birds were 46 wk of age at the end of the sample collection process. All hens were individually housed in modern, conventional cages. All birds utilized in the study were under Hy-Line ownership housed in facilities in Dallas County, Iowa. Diet formulation and bird management were determined via Hy-Line product guides and company policy. The birds used for sample collections were apparently healthy and clinically normal at the time of each blood collection.

Blood Collection and Analysis

Birds were handled according to 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 the sampling. The study included 6 replicates occurring on a rotating, approximately 2-wk interval schedule between blood collections beginning 2016 May 23 and ending 2016 August 3.

Blood samples were obtained via venipuncture of the brachial wing vein using 1 mL non-heparinized syringes and needles. Fresh, uncoagulated blood was directly loaded into a CG8+ cartridge following the manufacturer's recommendations. After loading and closure, the cartridge was inserted immediately into the i-STAT1 device. CG8+ cartridges inserted and analyzed by i-STAT analyzers allow for the collection of 13 blood gas and chemistry parameters: **pH**, partial pressure of carbon dioxide (**pvCO_2** mm Hg), partial pressure of oxygen (**pvO_2**, mm Hg), bicarbonate (**HCO_3**, mmol/L), base excess (**BE**, mmol/L), saturation of oxygen on hemoglobin (**sO_2**%), glucose (**Glu**, mg/dL), sodium (**Na,** mmol/L), potassium (**K**, mmol/L), total concentration of carbon dioxide (**TCO_2**, mmol/L), ionized calcium (**iCa**, mmol/L), hematocrit (**Hct**, %), hemoglobin (**Hb**, g/dL). Average total time for each sampling per bird was between 2 and 3 min. Test results were subsequently uploaded to a computer to allow statistical analysis of the data.

Statistical Analysis

Blood chemistry values were obtained from 1800 iSTAT1 records, and were utilized in the statistical analysis of the blood gas and chemistry data between the 3 CVs in the investigation, with information compiled collectively across replicate. The SAS 9.4 statistical software package was utilized to determine reference intervals, means, and standard deviations. Additionally, SAS was utilized to perform statistical ANOVA and Tukey's studentized range test procedures for each of the 13 applicable blood gas and chemistry parameters to test for significant differences between varieties. Parameter correlations of the data obtained from the 3 brown-egg laying hen varieties were obtained via SAS Proc Corr function with statistical significance set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Reference intervals for the 13 previously mentioned blood gas and chemistry were established for the 3 egg production varieties (Table 1). Simple statistics of the 13 investigated parameters across all varieties (combined) are included in Table 2. There was a significant effect of bird variety on all 13 blood chemistry parameters. Tukey's studentized range tests were used to identify which of the varieties were different from each other

Table 1. Blood gas and chemistry parameter means, standard deviations, minimums, maximums, and counts for the 2 brown-egg (Hy-Line Brown, Hy-Line Silver Brown) and 1 tint-egg (Hy-Line Sonia) commercial varieties of laying hens.

		pH	PCO_2 ($pvCO_2$ mm Hg)	PO_2 (pvO_2 mm Hg)	HCO_3 (mmol/L)	BE (mmol/L)	sO_2 (%)	Glu (mg/dL)	Na (mmol/L)	K (mmol/L)	TCO2 (mmol/L)	iCa (mmol/L)	Hct (% PCV)	Hb (g/dL)
Hy-Line Brown	Mean	7.33^{A}	50.5^{B}	39.0^{B}	26.2^{B}	0.2^{C}	67.7^{A}	243.4^{A}	150.7^{A}	5.2^{A}	27.7^{B}	$1.7^{A,B}$	22.9^{C}	7.8^{C}
	SD	0.07	7.6	5.6	2.0	2.6	7.8	10.0	2.6	0.4	2.1	0.1	2.2	0.8
	Min	7.12	34.9	26.0	20.1	−8.0	44.0	208.0	144.0	4.2	21.0	1.4	16.0	5.4
	Max	7.52	74.6	56.0	32.4	7.0	88.0	279.0	158.0	6.3	35.0	2.1	29.0	9.9
	Count	541	540	540	541	541	539	540	540	542	541	542	540	540
Hy-Line Sonia	Mean	7.32^{B}	51.1^{B}	41.0^{A}	26.1^{B}	-0.1^{B}	69.9^{A}	241.6^{B}	148.9^{B}	5.1^{B}	27.6^{B}	1.7^{A}	23.4^{B}	8.0^{B}
	SD	0.06	7.0	6.0	1.7	2.2	8.3	10.3	2.7	0.3	1.8	0.1	2.1	0.7
	Min	7.12	29.4	27.0	20.4	−6.0	42.0	216.0	141.0	4.2	21.0	1.4	17.0	5.8
	Max	7.50	74.3	58.0	31.6	6.0	86.0	278.0	159.0	6.2	34.0	2.2	34.0	11.6
	Count	545	540	549	546	546	544	549	548	548	546	548	546	546
Hy-Line Silver Brown	Mean	$7.33^{A,B}$	52.7^{A}	38.3^{B}	27.4^{A}	1.4^{A}	66.5^{A}	241.9^{B}	150.5^{A}	5.2^{A}	29.0^{A}	1.7^{B}	23.7^{A}	8.1^{A}
	SD	0.06	7.3	4.8	1.8	2.3	7.1	7.7	2.4	0.4	1.9	0.1	2.3	0.8
	Min	7.11	36.0	26.0	21.5	−7.0	43.0	217.0	144.0	3.9	23.0	1.4	14.0	4.8
	Max	7.47	76.3	66.0	33.5	7.0	94.0	267.0	159.0	6.5	36.0	2.1	32.0	10.9
	Count	527	520	522	527	527	522	524	527	525	527	527	524	524

Superscript lettering A–C indicates statistically significant differences between varieties when letter is not shared ($P \leq 0.05$) for each of the respective parameters: pH, partial pressure of carbon dioxide ($pvCO_2$, mm Hg), partial pressure of oxygen (pvO_2, mm Hg), bicarbonate (HCO_3, mmol/L), base excess (BE, mmol/L), saturation of oxygen on hemoglobin (sO_2, %), glucose (Glu, mg/dL), sodium (Na, mmol/L), potassium (K, mmol/L), total concentration of carbon dioxide (TCO_2, mmol/L), ionized calcium (iCa, mmol/L), hematocrit (Hct, % packed cell volume [PCV]), hemoglobin (Hb, g/dL).

Table 2. Blood gas and chemistry summary statistics for 13 parameters: pH, partial pressure of carbon dioxide ($pvCO_2$, mm Hg), partial pressure of oxygen (pvO_2, mm Hg), bicarbonate (HCO_3, mmol/L), base excess (BE, mmol/L), saturation of oxygen on hemoglobin (sO_2, %), glucose (Glu, mg/dL), sodium (Na, mmol/L), potassium (K, mmol/L), total concentration of carbon dioxide (TCO_2, mmol/L), ionized calcium (iCa, mmol/L), hematocrit (Hct, % packed cell volume [PCV]), hemoglobin (Hb, g/dL) utilizing combined data from 2 brown-egg layer (Hy-Line Brown, Hy-Line Silver Brown) and 1 tint-egg layer (Hy-Line Sonia) varieties.

Variable	N	Mean	Std Dev	Minimum	Maximum
pH	1,613	7.32	0.06	7.10	7.52
PCO_2 ($pvCO_2$, mm Hg)	1,600	51.3	7.3	29.4	76.3
PO_2 (pvO_2, mm Hg)	1,611	39.5	5.6	26	66
HCO_3 (mmol/L)	1,614	26.6	1.9	20.1	33.5
BE (mmol/L)	1,614	0.5	2.4	−8	7
sO_2 (%)	1,605	68.0	7.8	42	94
Glu (mg/dL)	1,613	242.3	9.5	208	279
Na (mmol/L)	1,615	150.0	2.7	141	159
K (mmol/L)	1,615	5.1	0.4	3.9	6.5
TCO_2 (mmol/L)	1,614	28.1	2.0	21	36
iCa (mmol/L)	1,617	1.7	0.1	1.38	2.16
Hct (% PCV)	1,610	23.33	2.23	14	34
Hb (g/dL)	1,610	7.93	0.76	4.8	11.6

(Table 1). Several correlations between the 13 parameters reported by i-STAT1 clinical analyzers were found to be statistically significant amongst the data collected from these 3 egg layer varieties.

pH, $pvCO_2$, pvO_2, TCO_2, HCO_3, and BE

The 3 investigated CVs investigated had statistically significant differences in pH, $pvCO_2$, pvO_2, TCO_2, HCO_3, and BE. This particular group of parameters serves as important indicators of physiological acid–base balance, respiratory ventilation, and the avian cardiovascular system (Steinmetz et al., 2007; Reece et al., 2015).

The most fundamental of the acid–base parameters in this group, pH, was previously reported to be 7.58 (SD 0.116) for the species *Gallus gallus* (Steinmetz et al., 2007). Reference intervals presented in Tables 1 and 2 possibly derange from this previously established reference range serving as an illustration of the importance of establishing reference intervals for specific CVs using i-STAT1 clinical analyzers Physiologically, pH is negatively correlated to $pvCO_2$ and positively correlated to pvO_2. The parameter correlation values for these colored-egg laying lines follow this generality, especially $pvCO_2$ with a notable correlation of –0.864 (Table 3). Previous investigation of the Hy-Line W-36 CV revealed that $pvCO_2$ increases and pvO_2 decreases as the variety ages (Schaal et al., 2016). This finding may be something to consider in future work since this particular study covers a narrow age range of 3 CVs of colored-egg laying hens.

Because of its variability based on metabolic differences or environmental factors altering individual bird results, the usefulness of BE was previously indicated as unreliable in the realm of chicken i-STAT1 implementation (Steinmetz et al., 2007; Schaal et al., 2016). Resulting values from this investigation of brown-egg layers are in agreement with this conclusion.

Both TCO_2 and HCO_3 had similar Tukey test grouping results when comparing the 3 varieties, with model significance noted between the varieties. The strong correlation value of 0.984 between the TCO_2 and HCO_3 parameters illustrates the physiological relationship of the 2 parameters. Clinically, TCO_2 and HCO_3 are functionally analogous parameters with a large proportion of the calculated TCO_2 value being derived from the HCO_3 calculated value. Bicarbonate (HCO_3) is a major buffering molecule circulating in the bloodstream. HCO_3 had positive correlation values exceeding >0.9 not only with TCO_2, but also BE. The high correlation values between these parameters can be readily explained physiologically under the general principles of the circulatory buffering system for the regulation of acid–base homeostasis employed by a vast majority of living organisms (Reece et al., 2015). Collectively, these may still serve as useful parameters for acid–base balance in avian medicine.

sO_2, Hct, and Hb

All 3 varieties shared statistically similar values of saturation of oxygen on hemoglobin (sO_2). The parameter pvO_2 was previously noted to have statistical differences between CVs; thus, the statistical similarity of sO_2 between CVs should be interpreted carefully as they share a relatively strong correlation value (0.856). When compared to analysis by an automated whole blood analyzer, i-STAT was found to report lower Hct values. It has been suggested that a possible reason for this trend is the large size of the avian nucleated erythrocyte size and subsequent potential interference during mechanical motion through the device as the device was originally developed as a human medical device (Steinmetz et al., 2007). As expected, Hct and Hb were found to be highly correlated with a correlation value of >0.999 indicating their physiological interdependence. The 2 parameters are highly related

Table 3. Estimates of correlations between 13 blood gas and chemistry parameters: pH, partial pressure of carbon dioxide ($pvCO_2$, mm Hg), partial pressure of oxygen (pvO_2, mm Hg), bicarbonate (HCO_3, mmol/L), base excess (BE, mmol/L), saturation of oxygen on hemoglobin (sO_2, %), glucose (Glu, mg/dL), sodium (Na, mmol/L), potassium (K, mmol/L), total concentration of carbon dioxide (TCO_2, mmol/L), ionized calcium (iCa, mmol/L), hematocrit (Hct, % packed cell volume [PCV]), hemoglobin (Hb, g/dL) utilizing combined data from 2 brown-egg layer (Hy-Line Brown, Hy-Line Silver Brown) and 1 tint-egg layer (Hy-Line Sonia) varieties (above diagonal) and their corresponding *P*-values (below diagonal).

	pH	PCO_2 ($pvCO_2$ mm Hg)	PO_2 (pvO_2 mm Hg)	HCO_3 (mmol/L)	BE (mmol/L)	sO_2 (%)	Glu (mg/dL)	Na (mmol/L)	K (mmol/L)	TCO_2 (mmol/L)	iCa (mmol/L)	Hct (% PCV)	Hb (g/dL)
pH		*–0.864*	–0.366	0.265	0.635	0.128	–0.158	–0.571	–0.081	0.158	–0.285	–0.392	–0.391
PCO_2 ($pvCO_2$ mm Hg)	–0.864		0.164	0.216	–0.187	–0.274	0.180	0.587	0.248	0.312	0.230	0.427	0.425
PO_2 (pvO_2 mm Hg)	–0.366	0.164		–0.397	–0.467	*0.856*	0.014	0.069	–0.320	–0.363	0.107	0.028	0.028
HCO_3 (mmol/L)	0.265	0.216	–0.397		0.904	–0.308	0.057	0.040	0.342	0.984	–0.105	0.090	0.091
BE (mmol/L)	0.635	–0.187	–0.467	0.904		–0.185	–0.024	–0.212	0.231	*0.845*	–0.204	–0.098	–0.097
sO2 (%)	0.128	–0.274	0.856	–0.308	–0.185		–0.063	–0.244	–0.382	–0.326	–0.049	–0.194	–0.193
Glu (mg/dL)	–0.158	0.180	0.014	0.057	–0.024	–0.063		0.159	0.193	0.081	0.061	0.087	0.087
Na (mmol/L)	–0.571	0.587	0.069	0.040	–0.212	–0.244	0.159		0.233	0.109	0.087	0.230	0.229
K (mmol/L)	–0.081	0.248	–0.320	0.342	0.231	–0.382	0.193	0.233		0.362	0.017	0.132	0.130
TCO_2 (mmol/L)	0.158	0.312	–0.363	0.984	0.845	–0.326	0.081	0.109	0.362		–0.081	0.133	0.134
iCa (mmol/L)	–0.285	0.230	0.107	–0.105	–0.204	–0.049	0.061	0.087	0.017	–0.081		0.049	0.048
Hct (% PCV)	–0.392	0.427	0.028	0.090	–0.098	–0.194	0.087	0.230	0.132	0.133	0.049		0.999
Hb (g/dL)	–0.391	0.425	0.028	0.091	–0.097	–0.193	0.087	0.229	0.130	0.134	0.048	0.999	

physiologically with Hct being a direct measurement and Hb being a parameter resulting from a calculation by the i-STAT device (Steinmetz et al., 2007). Additionally, the reported ANOVA Tukey test results of Hb mirrored the results of Hct by CVs (Table 1).

Glu, Na, and K

Blood glucose levels varied significantly between HYB vs. HYS and HYSB. Similar differences were also noted for the blood electrolyte, Na, with the HYS having the lowest value overall. Sodium levels assuredly play a crucial role in bird hydration status and affect an array of body systems such as the cardiovascular system (Steinmetz et al., 2007; Reece et al., 2015).

Circulating potassium levels mimicked the results of the blood sodium levels, with differences noted between CVs. The results of this parameter were previously found to be denoted as unreliable when compared to traditional serum biochemistry analyzers (Steinmetz et al., 2007). This previous conclusion could have been confounded due to the timing differences of sample analysis resulting in artifactual increases in whole blood K levels via i-STAT as compared to the traditional methods (Steinmetz et al., 2007). The different results of K in Table 1 between CVs should be carefully interpreted under the context of i-STAT sampling method. In general, the clinical implications of blood chemistry Na and K value derangements have not been strongly characterized, unlike ionized calcium (Steinmetz et al., 2007).

iCa

HYS was found to have higher blood iCa than the other 2 CVs. Although the CVs of colored-egg layers varied statistically, the overall difference in circulating ionized calcium between the 3 colored-egg laying lines was not found to be particularly striking. This could be explained from the genetic similarity that the 3 CVs share. Assuredly, the colored-egg laying hens did have a mean value of 1.7, which is numerically higher than the W-36 commercial hens in the initial i-STAT investigation by Hy-Line International (Schaal et al., 2016). Additionally, this parameter may be of particular interest when correlating findings to production data.

CONCLUSIONS

Previously, the i-STAT measurements pH, pvO_2, $pvCO_2$, Na, iCa, and PCV and device calculations of HCO_3, TCO_2, Hb, and sO_2 were found to be reliable in this study when compared to benchtop values in a generalized investigation of the species *G. gallus* (Steinmetz et al., 2007). Of the 13 blood gas and chemistry parameters established in this study, one most interesting and significant findings was the limited degree of dissimilarity of iCa between these genetically related brown-egg layers (Table 1). The importance of

iCa homeostasis has been a topic of particular interest in the avian species, such as its implication of the calcium tetany disease process in broiler breeder hens (Martin et al., 2011). Intuitively, the high amount of calcium output from egg production renders this parameter of special pertinence in the layer industry. The iCa value obtained from the i-STAT device can serve as a more precise measurement of the electrolyte calcium than a total serum measurement in clinical cases of acid–base disturbances (Steinmetz et al., 2007). The inconsistency of BE was noted in a previous study and will most likely be disregarded in the process of correlating reference intervals to production data (Schaal et al., 2016). The statistically similar grouping by variety of the parameters, HCO_3 and TCO_2, Na and K, Hct and Hb, were anticipated as they are highly related physiologically (Reece et al., 2015). The numerically inverse relationships between the parameter PCO_2 and the parameters pH and PO_2 are similarly expected based on physiological relationships too. The discord between the brown-egg laying varieties is confounded and can potentially be influenced by many factors including age, stage of production, genetic selection, and nutrition among other factors.

The initial work of reference range establishment of the Hy-Line W-36 in 2016 hypothesized that other CVs of white-egg layers would share similar reference ranges as they share a very similar breed foundation comprising their respective CVs (i.e., Hy-Line W-36, Hy-Line 80, and Hy-Line 80+). Although colored-egg CVs of laying hens are also foundationally composed of similar breeds when compared to other colored-egg CVs, white and colored-egg laying hens have been divergently bred. For example, the Hy-Line W-36 is primarily derived from the White Leghorn breed of chicken, whereas the brown CVs in this particular investigation are founded primarily from the Rhode Island Red and White Plymouth Rock breeds. This strong genetic independence could reveal strong differences in blood gas and chemistry data. To continue, the reference range establishment of colored-egg layers and subsequent differences elucidated between CVs found illustrate the need for reference range establishment for individual CVs rather than relying on speculation on a basis of general egg color alone. These values will serve as critical baseline information when trying to make accurate inferences for diagnostic investigations regarding bird health, especially for actively laying birds of these CVs in the 35 to 46 wk of age interval.

The mean values and overall reference intervals will be used to broaden the scope of implementation of i-STAT1 technology in the laying industry. Due to the health status of the birds being housed on a highly biosecured genetics research farm, these data could be used to create reference ranges for comparisons in future work focused on clinically diseased individuals. Specifically concerning the colored-egg laying varieties in this study, the authors will next investigate the differences in the reference range values found in Table 1 over time (by replicate), and correlate production data already collected on these particular birds (i.e., shell quality, shell color, persistency of lay, and growth curves).

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CONFLICT OF INTEREST

The authors do not declare any conflict of interest.

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