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Implementation of iSTAT®1 clinical technology in egg laying chickens blood gas and chemistry analyzation

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This thesis is being dedicated to so very many people in my life that have impacted my professional goals in life including but not limited to my family, friends, mentors and peers. More specifically, I would like to thank my parents, Thomas and Charlene Sauer, as well as my brother, Isaac Sauer. Together, they fueled my passion for animal science in my early years, and have continued to provide support to complete my professional education in veterinary medicine (D.V.M.) and veterinary preventative medicine (M.S.).

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NOMENCLATURE

CV	Commercial Variety
HYB	Hy-Line Brown
HYSB	Hy-Line Silver Brown
HYS	Hy-Line Sonia
pH	potential hydrogen
$pvCO_2$	partial pressure of carbon dioxide
pvO_2	partial pressure of oxygen
HCO_3	bicarbonate
BE	base excess
sO_2	saturation of oxygen on hemoglobin
Glu	glucose
Na	sodium
K	potassium
TCO_2	total concentration of carbon dioxide
iCa	ionized calcium
Hct	hematocrit
Hb	hemoglobin

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ABSTRACT

The commercial poultry industry utilizes a diverse set of production birds and lacks venous blood gas and biochemistry reference intervals for specific commercial varieties (CVs) of laying hens of significance to commercial production. This master's thesis aims to review the implementation of practical i-STAT®1 utilization in chickens, expand upon and provide reference intervals for some of the world's most popular CVs of brown and tint egg laying hens (Hy-Line Brown, Hy-Line Silver Brown and Hy-Line Sonia), and document notable findings therein. Chapter 1 provides an introduction to the i-STAT®1 clinical technology utilized in subsequent chapters, a review of 13 blood gas and biochemistry parameters obtained from i-STAT®1 clinical technology. The 13 applicable parameters within the context of this study are 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).

The portability of the i-STAT®1 is one of the key advantages in its successful implementation in the commercial poultry industry. The primary objective of Chapter 2 is to compare and validate the accuracy of the i-STAT®1 portable clinical analyzer and the VetScan VS2® benchtop clinical analyzer. Chapter 3, an already published work in the *Journal of Poultry Science*, includes work establishing blood gas and biochemistry reference intervals for the three colored egg laying commercial varieties in peak production, the Hy-Line Brown, Hy-Line Silver Brown and Hy-Line Sonia. Chapter 3 of

this thesis also illustrates the importance of such reference interval establishments for individual CVs by providing strong evidence of apparent differences between CVs. Chapter 4 provides a summary of the aforementioned chapters therein. Overall, results obtained from i-STAT®1 clinical analyzers should be very cautiously interpreted when compared against VetScan VS2® benchtop results, especially for the parameters iCa and K. In addition to effect of analyzation method, bird genetics (by CV) had a significant effect on blood gas and biochemistry values, with significantly different reference intervals reported for each of the CVs sampled. Practical application of the established reference intervals for the 3 CVs of colored egg laying hens may only be extended to birds 35-46 weeks of age of each respective variety. Future works with i-STAT®1 implementation with poultry can include correlation of blood gas and biochemistry data to production data, nutrition, clinical disease and genetic selection.

CHAPTER 1. GENERAL INTRODUCTION

i-STAT®1 Clinical Technology Background

Diversified laboratory testing has proven to be crucial in avian medicine under a variety of circumstances. In addition to a thorough physical examination and/or necropsy, an evaluation of blood gas and biochemistry values may allow for a more comprehensive clinical profile of avian(s). Analyzation of avian blood samples has proven very useful when trying to better understand avian physiology and the pathophysiology behind various disease states. In today's ever advancing field of clinical medicine, it is vital investigators know the diagnostic options available to them. Similarly, it is of equal importance to accurately interpret such information yielded from diagnostic pursuits. Advancements in diagnostics have propelled the veterinary medicine forward by increasing ease of use, timeliness, accuracy and utility. One of such relatively recent implementations is the i-STAT®1 clinical analyzer.

The i-STAT clinical technology by Abaxis was originally developed for human medical use, but eventually the i-STAT®1 handheld analyzer was modified for veterinary use. This technology has the ability to provide information from a patient or specimen with approximately 95 µL of fresh whole blood using electrochemical sensing technology. Chemically sensitive films coat electrodes which act as sensors when in contact, to minute chemical reactions in a blood sample. Each sensor is either potentriometric, amperometric or conductometric. Potentriomectric sensors act by measuring changes in electric potentials, whereas amperometric sensors measure changes in electric current. Conductometric sensors fundamentally measure changes in conductivity. A particular concentration of analyte is then determined by the apparent

difference of the sample from the calibrant. Finally, an internal processor of the device transforms this numerical difference into units of concentration most applicable to the analyte being measured (i-STAT®1, 2006).

Results from the device are available on the digital device within two minutes of inserting a pre-loaded cartridge into the device, and easily uploaded to computer devices for medical records and use in statistical comparisons. Various single-use cartridges are available for use with i-STAT® clinical analyzers which have the ability to yield values on acid-base status, blood gases, chemistries, electrolytes, hematology profiles, and even cardiac troponin I (i-STAT®1, 2006).

As mentioned previously, i-STAT®1 clinical analyzers are paired with a single-use cartridge for individual sample analyzation. Shelf-life of such cartridges varies between types from 5-11 months (i-STAT®1, 2006). Cartridges currently available include i-STAT EC8+, CG4+, G, CG8+, Chem8+, 6+, Crea, ACT Celite®, E3+ and Cardiac Troponin (i-STAT®1, 2006). Selection of cartridge type is highly dependent on the goal of analyzation. Not all of the aforementioned cartridges are readily applicable to poultry diagnostics, the Cardiac Troponin cartridge is an example of such cartridges; however, it has been useful in investigations of other food producing species (Labonte et al., 2015). The CG8+ cartridge is one of the most commonly implemented cartridge types in recent publications involving poultry (Martin et al., 2010; Schaal et al., 2016; Van Goor et al., 2016; Van Goor et al., 2017; Wang et al., 2018; Rowland et al., 2019). All total, the CG8+ cartridges measure 13 valuable blood gas and biochemistry parameters as follows: 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]), and hemoglobin (Hb g/dl). A majority of the analytes of the CG8+ cartridge are measured by the device, however some of the analytes (Hb, HCO_3, TCO_2, sO_2 and BE) are based on calculations (i-STAT®1, 2006). The i-STAT®1 clinical analyzer and associated CG8+ cartridges allow researchers to capture and profile a large variety of parameters like the previously mentioned above in a very efficient manner, and aid in the prevention of artifactual changes witnessed in these parameters when subject to prolonged analyzation times.

13 Blood Gas and Biochemistry Parameters

Electrolyte Parameters

As with other components, electrolytes circulating in avian plasma can help identify changes in avian physiology and general health. Approximately 95% of the osmotic pressure from avian plasma comes from electrolytes such as sodium, potassium and chloride. The remaining 5% of osmotic pressure attributable to plasma comes from larger freely circulating molecules such as glucose, urea, amino acids and proteins. Concentrations of the aforementioned plasma components, particularly electrolytes, are very transient. This particular section is dedicated to the four blood electrolytes researchers are able to obtain values of from the CG8+ cartridge which include ionized calcium (iCa), glucose (Glu), sodium (Na) and potassium (K).

Calcium

Among all plasma constituents in birds, calcium is perhaps one of the most highly regulated although subject to change upon metabolic demands just as any other blood

biochemical parameter (Dacke, 1979). Calcium comprises approximately 1.5% of a birds body weight, making it the most abundant mineral in the body (Bolukbasi et al., 2005). An early paper published by Heller et al., classified the various components of total blood calcium into the categories protein-bound calcium, absorbable calcium, absorbable non-filterable calcium and ionized calcium (1934). Of the four aforementioned calcium components in whole blood, it was concluded that the absorbable non-filterable form of calcium is most closely associated with egg-shell formation (Heller et al., 1934). A more recent publication has cited that a vitamin D_3 dependent Ca-binding protein is likely responsible for the active transport across the intestinal and subsequently uterine membrane for eggshell deposition (Bolukbasi et al., 2005).

Once in systemic circulation, a large portion of total blood calcium is bound to the protein molecule, albumin. As blood levels of albumin decrease, an artifactual decrease in serum total calcium will also be witnessed (Harris, 2009). The i-STAT®1 clinical analyzer reports only the ionized calcium which is believed to represent approximately 50% of the total blood calcium (Reece et al., 2015). This fraction of the total calcium in circulating blood is not directly subject to derangements of serum albumin (Harris, 2009).

Avian skeletal metabolism is very effective and efficient at calcium regulation compared to mammals. Derangements in calcium are often implicated in various pathological conditions, thus careful interpretation must be made with abnormal circulating calcium levels (Dacke et al., 2015). The parathyroid glands play a crucial role in the regulation of blood calcium levels in avian species. Subclinical dietary deficiencies of calcium are often not appreciable in blood samples due to the overriding function of the parathyroid glands (Harris, 2009). Nonetheless, prolonged periods of anorexia have

been cited as a cause of decreased iCa values (Martin et al., 2010). Dietary supplementation of with calcium sources such as $CaCl_2$ have been shown to disturb blood calcium:phosphorus ratios as well as result in a significant reduction of blood pH (Ahamd et al., 2005). Dietary supplementation of vitamin D_3 has been shown to increase calcium deposition in eggshells (Bolukbasi et al., 2005). Various deficiencies in iCa due to etiologies external to diet do exist particularly in laying chickens where the calcium demands for egg production purposes have the potential to result in various bone maladies (Heller et al., 1934).

Eggshell calcification of avian megalecithal dramatically increases the demand for calcium mobilization and utilization in laying hens (Dacke, 1979; Schweitzer et al., 2005). The eggshell calcification produced for a single egg represents approximately 10% the total calcium reserves of an individual bird (Kenny, 1986). An estimated 2.4 grams of calcium is required for a laying chicken to produce a 60-gram egg within an 18-hour time interval (Whitehead, 2004).

Upon onset of sexual maturity in avian species, skeletal bone biology dramatically changes in anticipation of such eggshell demands in response to estrogen production (Dacke, 2000; Whitehead, 2004). Although mammalian ionized calcium remains relatively constant, avian iCa has been shown to increase over 100 percent upon the onset of lay (Heller et al., 1934). Evidence has been provided that Hy-Line W-36 first cycle hens (20 to 68 weeks) have significantly higher iCa values than that of pullets (4 to 15 weeks) and second cycle layers (70 to 110 weeks) (Schaal et al., 2016). This study has provided modern evidence of the significant effects of age and sexual maturity of egg production on iCa values.

Hens performing at high production standards are more susceptible to developing osteoporosis (Fleming et al., 2006). Chronic deficiencies of calcium in laying hens results in osteoporosis of skeletal bone with increased fragility and subsequent susceptibility to fracture (Stamford, 2006; Whitehead and Fleming, 2000). Caged layer paralysis subsequent to osteoporosis has been a major disorder impacting laying hen welfare and production, notably since the introduction of battery-cage production (Couch, 1955). At one time, approximately 30% of laying hens housed in conventional battery cages experienced fractures during production through depopulation (Gregory and Wilkins, 1989). Chickens suffering from osteoporosis were found to have an iCa of 3.5 mg/100 ml serum compared to an iCa of 3.7 mg/100 ml in clinically normal chickens (Heller et al., 1934).

In attempt to reduce the incidence of caged layer paralysis, efforts were made to produce birds with increased bone strength. As a result, laying birds selected for bone strength have historically produced eggs with lower overall shell quality (Fleming et al., 1998). Research suggests estrogen activity is related to the onset of osteoporosis as the hormone stimulates medullary bone formation while simultaneously decreasing the density and thickness of cancellous and cortical bone (Turner et al., 1993; Wilson and Thorp, 1998). Medullary bone tissue develops in the bone marrow cavities in reproductively active females and is mobilized for calcium deposition into eggshells rather than for providing stability (Dacke, 1979). Exogenous administration estrogens to male birds has been shown to similarly increase medullary bone density, and can be blocked by simultaneous dosing of tamoxifen, an anti-estrogenic compound (Ohashi et al., 1987; Williams et al., 1991). Circulating blood estrogen levels are thought to decline

at 70 weeks of age in laying hens, decreasing calcium absorption from bone as well as intestinal calcium absorption (Beck and Hansen, 2004). As a result of decreased calcium absorption, blood calcium values have been shown to precipitously drop back to pre-egg production levels at the onset of molt in laying hens (Heller et al., 1934).

Decreased iCa in broiler breeders has been correlated to clinical mobility impairment in suspected calcium tetany cases in broiler breeder hens (Martin et al., 2010). Eight out of twelve birds (75%) in the study identified as birds with impaired mobility were found to have significantly low blood iCa values below previously established reference ranges (Martin et al., 2010). Venne et al. suggests sampling for iCa values in cases of suspected or anticipated calcium tetany be taken at 12 hours post-oviposition as this time period presents the lowest iCa utilizing i-STAT®1 clinical analyzers (2019). If using a more conventional benchtop analyzer such as the VetScan VS2®, the lowest total calcium values can be expected approximately 20 hours after oviposition (Venne et al., 2019). Birds suffering from hypernatremia or hypokalemia could also present with clinical signs consistent with calcium tetany resulting from hypocalcemia leading to a potentially inaccurate field diagnosis without a blood biochemical profile (Martin et al., 2010). A simple sampling from a population of affected birds could potentially differentiate calcium tetany from hypernatremia and/or sudden death syndrome resulting from hypokalemia (Martin et al., 2010).

Other conditions leading to a disregulations in blood calcium include insufficient exposure to full spectrum lighting, glucocorticoid administration and hypomagnesaemia (Harris, 2009). Laying hen stocking density and associated stress has not been shown to

affect iCa values (Kang et al., 2016). A trial involving a constant rate infusion of adrenocorticotropic hormone (ACTH), a hormone associated with physiologic stress responses, did not alter plasma concentrations of iCa as well as pH in broiler-type birds although it did affect other blood gas and biochemistry parameters (Olanrewaju et al., 2006). A genetically correlated hypocalcaemic syndrome of African grey parrots has been documented in clinical exotic avian literature, but such genetically attributed syndromes are not readily documented in modern poultry production (Harris, 2009). Additionally, birds in states of acidosis (blood pH <7.00) experience strong inhibition of bone matrix mineralization due to the activation of osteoclasts which aid in the mobilization of Ca^{2+} and OH^- ions to increase blood alkalinity thereby increasing blood calcium values. Conversely, when blood pH is above approximately 7.20, the two aforementioned ions are utilized in bone matrix formation (Venne et al., 2019). As a result, mild decreases in iCa can be attributed to physiologic alkalosis as well as periods of anorexia due to feed withholding (Martin et al., 2010). Overall, serum calcium values remain as a highly targeted biochemistry marker in the literature, especially in egg laying varieties of production birds.

Glucose

Regardless of poultry species or intent of production, glucose is an important molecule to meet the energy demands of cellular metabolism (Reece et al., 2015). Interestingly, normal avian blood glucose concentrations are over twice as high as their mammalian counterparts, yet their glomerular filtration rate (GFR) remains comparable (Scanes, 2015; Morgan and Braun, 2001). The avian kidney is very efficient in their ability to resorb filtered glucose, and is partly responsible for the high glucose levels

witnessed in avian species (Morgan, 1975). Excess circulating glucose is stored as glycogen, or is subject to lipogenesis in the liver rather than in adipose tissue as conventionally described in mammalian species. The expected circulating glucose concentration in avian species is 15.4 mmol/L when averaged across 139 species (Scanes, 2015). Broiler-type chickens have been shown to have mean serum glucose concentrations of 15.0 mmol/L (Soleimani and Zulkifli, 2010).

Theories have been suggested that domestication and artificial selection of avian species have altered glucose metabolism and circulating concentrations of the molecule (Scanes, 2015). In support of this theory, wild turkeys have been shown to have approximately 50% greater circulating concentrations of glucose than domesticated counterparts (Lisano and Kennamer, 1977; Anthony et al., 1990). The overriding theory of the impact of natural selection remains contentious as a study involving domestic chickens and their wild counterpart, red jungle fowl, revealed no statistical difference in mean blood glucose concentrations (Soleimani and Zulkifli, 2010). A study comparing Cobb and Ross broiler birds showed Cobb to have statistically lower blood glucose levels (Martin et al., 2010). These same birds also illustrated broiler breeder hens in post-peak production have significantly lower blood Glu than pre-peak and peak production birds (Martin et al., 2010). A relationship between circulating glucose levels and body weight/age have not yet been clearly established (Beuchat and Chong, 1998; Braun and Sweazea, 2008).

Avian individuals affected by pathological conditions will classically experience elevation in blood glucose in part due to the physiologic stress response of the host (Harris, 2009). Hyperglycemia is most commonly witnessed in birds suffering for stress

or birds that recently ingested a meal (Harris, 2009). Hyperglycemia secondary to stress is incited biochemically by increased catecholamine secretion especially the mineralocorticoid, cortisol (Olanrewaju et al., 2006). A study investigating the effects of a constant rate infusion of adrenocorticotropic hormone (ACTH) to commercial broilers resulted in an increased Hct, Hb, $pvCO_2$ and HCO_3, while lowering pvO_2, Na and K (Olanrewaju et al., 2006). In companion avian medicine, hyperglycemia in association with diabetes mellitus has been documented (Harris, 2009). Chickens have also been witnessed to have statistically elevated blood glucose levels approximately 12 hours after egg laying, which can potentially be explained by increased feed consumption by birds proceeding the event of lay (Venne et al., 2019). Oral administration of monosaccharides has been documented to increase blood glucose levels in chickens significantly (Sinsigalli et al., 1987). Subsequent return to a basilar level of blood glucose can occur in as little as one hour, although rates vary between bird ages with older birds having a slower return to basilar levels of glucose (Sinsigalli et al., 1987). Stocking density of birds actively in lay has not been shown to significantly alter blood Glu levels (Kang et al., 2016).

Witnessing hypoglycemia on blood chemistry panels is often quite rare in avian medicine. Nonetheless, hypoglycemia in avian species is most likely due to septicemia (Harris, 2009). The intuitive explanation of starvation as a primary factor inciting hypoglycemia is actually extremely rare, but can occur during prolonged periods of anorexia (Harris, 2009; Scanes, 2015). Simon et al. reported fasted chickens to have a mean blood glucose approximately 0.4 mmol/L lower than normally fed chickens (2011). Other publications have readily cited fasting to affect circulating glucose levels in chicken models (Dupont et al., 2008; Christensen et al., 2013).

Chickens often experience statistically lower levels of blood glucose during the night most logically explained by their tendency to be diurnal thereby roosting instead of accessing food at night (Christensen et al., 2013). Exogenous administration of insulin has also been shown to induce a state refractory hypoglycemia (Gleeson and Brackenbury, 1984). Interestingly, dietary supplementation with $CaCl_2$ has been shown to significantly lower Glu concentrations in broilers (Ahmad et al., 2005). Clinical hypoglycemia (avian blood glucose levels <150 mg/dL) has been documented in broilers suspected to suffer from spiking mortality syndrome and rickets (Burns et al., 2002; Davis et al., 1995).

Glucose is conventionally thought to be falsely lowered due largely in part to energy demands by avian erythrocytes, but glucose has been shown to be ineffective at maintaining erythrocyte ATP levels *in vitro* (Matthew et al., 1993). Glutamine has been shown to maintain erythrocyte ATP levels *in vitro* in chickens, and it is also believed avian erythrocytes employ the citric acid cycle (Scanes, 2015). The small amount of glucose that is transported across the cellular membrane of chicken erythroblasts for intracellular utilization is mediated via GLUT 1 and GLUT 3 transporters. As erythrocyte differentiation occurs, activity of the two aforementioned GLUT transporters declines significantly (Matthew et al., 1993). Passive transport of monosaccharides into erythrocytes via simple diffusion is greater in chicken embryos than in adult chickens (Ingermann et al., 1985). Once inside erythrocytes, approximately 50% of glucose is metabolized to lactate (Nicol et al., 1988). Continuing the theme of false blood glucose elevations, a study investigating the effects of CG8+ cartridge manufacturer expiration

date found glucose to be significantly increased in expired cartridges, with no other significant differences noted (Rettenmund et al., 2014).

Sodium

The parameter sodium is another major blood electrolyte in systemic circulation. The mean plasma concentration of sodium across 47 compiled avian species has been reported as 152.5 mmol/L (Scanes, 2015). After renal filtration, resorption of sodium as well as chloride and water occurs in the coprodeum, rectum and caeca as urine is not concentrated by avian kidneys (Tully et al., 2009). Sodium fluxes into the erythrocytes of turkeys have been reported to 2-3 times greater than that of humans in comparative research trials investigating beta-adrenergic receptors and adenylate cyclase (Palfrey and Greengard, 1981). Production status in modern poultry have been correlated with differences in Na in circulation. More specifically, post-peak production broiler breeder hens were found to have significantly higher blood Na than pre-peak and peak production birds (Martin et al., 2010).

Elevations in circulating sodium (hypernatremia) are often implicated with water depravation, hyperthermia, dehydration and dietary salt toxicity in chickens (Harris, 2009; Arad et al., 1983). Hyperthermia and subsequent dehydration have been implicated with elevated chloride levels as witnessed with Na (Scanes, 2015). Many of the aforementioned inciting causes of hypernatremia, are involved in the process of acute heat stress. Following an event of acute heat stress in chickens, one can anticipate a rebounding decrease in Na as well as K (Borges et al., 2004).

Hyponatremia is often experienced via renal excretion secondary to renal disease or gastrointestinal loss via diarrhea (Harris, 2009). Hyponatremia has also been cited to

occur following time periods of anorexia in chickens (Christensen et al., 2012). When dietary sources of sodium are low, microvilli on the coprodeal epithelial cells increase in height and density in attempt to enhance resorption of Na after it is filtered out of systemic circulation by the kidneys (Tully et al., 2009).

Potassium

The mean blood potassium levels across 46 avian species is reportedly 3.21 mEquiv/L (Scanes, 2015). Significant imbalances of circulating potassium are often indicative of severe, life threatening pathological conditions in birds. For instance, hyperkalemia has the propensity to develop during advanced adrenal or kidney disease as well as during transient episodes of systemic acidosis (Harris, 2009).

High blood K levels, as well as increased blood calcium values can classical present clinically as increased excitability via the elevation of cellular resting membrane potentials (Venne et al., 2019). High K and low Ca values in tandem could have particularly devastating effects on host homeostasis due to a poor balance of normal physiologic threshold potentials (Venne et al., 2019). The aforementioned combination of K and Ca values could clinically present as hyperexcitability and even cardiac fibrillation (Venne et al., 2019).There appears to be a positive correlation between serum increasing K and iCa suggesting host adaptations to avoid such drastic disparities in the circulating values of these two biochemical components of avian blood (Venne et al., 2019).

Low extracellular K has the propensity to lower membrane resting potentials, thereby increasing the energy demand and subsequent generalized lethargy ensues (Venne et al., 2019). As a bird ages, potassium transport into erythrocytes declines (Drew et al., 2002). Physiologic states of hypokalemia can present clinically similar to

hypocalcemia (Martin et al., 2010). On the contrary, hypokalemia is commonly witnessed in animals with diarrhea and generalized states of alkalosis (Harris, 2009). In cases of calcium tetany, the K electrolyte was not shown to vary statistically during a regular cycle of egg formation (Venne et al., 2019). Birds suffering from heat stress subsequent increase in pH stimulates the excretion of potassium, a positively charged ion, from the kidney in attempt to maintain homeostasis. Severe heat stress can then alter blood K levels at an appreciable level. Decreased feed and water intake as well as peripheral shunting of blood during heat stress can further exasperate electrolyte imbalances (Scanes, 2015).

Erythrocytic Parameters

This section is dedicated to three parameters measured by the CG8+ cartridge most heavily associated with circulating erythrocytes and their functionality. Avian erythrocytes are nucleated, and have also retained mitochondria resulting in larger erythrocytes compared to mammals. Adult chicken erythrocytes have a mean hematocrit/packed cell volume of 44%, and have a mean hemoglobin of 10.1% (Scanes, 2015). Compared to mammalian species, erythrocytes of the domestic chicken have a limited lifespan of approximately 35 days (Williams, 1972).

The Hct parameter is directly measured by the i-STAT®1 clinical analyzer (i-STAT®1, 2006). Hct values generated by the i-STAT®1 were found to be lower than traditional benchtop microhematocrit methods in samples obtained from gyr falcons (Raghav et al., 2015). Hematocrit has been shown to be inversely proportional to $\log_{10}$ body weight across all avian species, with lighter birds having an increased mean hematocrit percentage as compared to heavier structure birds (Scanes, 2015). Although

high growth rates have been associated with the development of ascites in broilers, increased blood hematocrit values are not necessarily correlated to susceptibility of ascites (Buys et al., 1999).

Chicken models have demonstrated a sex predilection for differences in hematocrit, with males having a statistically higher erythrocyte concentration and hematocrit (Scanes, 2015). Additionally, male Quaker parrots were shown to have increased Hb and Hct compared to females (Rettenmund et al., 2014). Generalized states of hypoxia have been shown to increase hematocrit percentages in both chickens and quail models (Rosse and Walkmann, 1966). Similarly, poultry raised in areas of high altitude may experience an significant increase in erythrocyte concentration thereby increasing hematocrit and hemoglobin concentrations (Bagley et al., 1990). A robust meta-analysis utilizing data from 37,000 broilers provided evidence that mycotoxins can depress hematocrit (Scanes, 2015). Broiler diets supplemented with $NaHCO_3$ have been shown to significantly increase blood hematocrit and hemoglobin levels (Ahmad et al., 2005).

Hb parameter results from the i-STAT®1 are derived from calculations rather than direct measurement (i-STAT®1, 2006). Hemoglobin is the most abundant protein in avian erythrocytes with domestic chickens having a lower concentration that their wild bird counterparts (Scanes, 2015). Even the nuclei of avian erythrocytes have been found to contain hemoglobin molecules (Scanes, 2015). Hb is the molecule responsible for oxygen binding at the level of the lungs, and subsequent release into peripheral tissues for oxygenation and cellular metabolism (Scanes, 2015). Just like mammalians, avian hemoglobin is a tetrameric protein comprised of four protein subunits, each in close

association with a heme unit containing ferrous iron (Scanes, 2015). Chemical bonds to heme units tie up most of the O_2 in circulation (Powell, 2015). As expected, low dietary iron as well as copper deficiencies levels have been shown to negatively impact hemoglobin concentrations in avians (Tako et al., 2010; Rettenmund et al., 2014; Baumgartner et al., 1978).

The parameter sO_2 is also calculated by the i-STAT®1 clinical analyzer and is fundamentally a measure of the saturation of oxygen chemical bonds on hemoglobin (i-STAT®1, 2006). A discord between arterial and venous blood concentrations of sO_2 is expected, and fascinatingly this difference has been shown to be constant in avian species regardless of activity level (Scanes, 2015). A study using Gyr falcons as its model species illustrated statistically significant ($P< 0.001$) differences of sO_2 (as well as Na) levels between arterial and venous blood samples (Raghav et al., 2015). Avian embryos have a markedly higher oxygen affinity of hemoglobin than adult birds (Scanes, 2015). Birds that fly at high altitudes or dive deep underwater have increased affinity for oxygen on hemoglobin (Scanes, 2015).

Concerning the impact of genetics on sO_2, Cobb broiler breeders were shown to have a significantly higher sO_2 and pO_2 than Ross, a competitive counterpart (Martin et al., 2010). Perhaps the aforementioned differences between can be correlated to production trait and performance differences between the two strains of broiler birds. Gene expression of organic phosphates, like inositol hexakisphosphate and inositol-P_5, have been correlated to alterations of both chicken and pigeon hemoglobin affinity for oxygen (Vandecasserie et al., 1971). This differential gene expression elucidates sO_2 hertiability, although recently this parameter was not found to be strongly heritable

(Rowland et al., 2019). Seven genes are also implicated with hemoglobin production in chickens (Scanes, 2015). Perhaps gene expression of other molecules similar to inositol hexakisphosphate can be correlated to higher sO_2 and pO_2, and subsequently contrasted between genetically divergent varieties of production birds to aid in the selection of more robust poultry in modern production schemes. Nonetheless, further research is necessary to accurately attribute genetic components to the differences experienced in these parameters.

Acid-Base and Blood Gas Parameters

Although all 13 blood gas and biochemistry parameters can be correlated to physiologic acid-base status in some form, this section is primarily dedicated to six primary acid-base parameters: pH, $pvCO_2$, pvO_2, bicarbonate (HCO_3), total concentration of carbon dioxide (TCO_2) and base excess (BE). The normal physiologic pH of chickens is approximately 7.40, and deviations from this expected value can provide a great starting point when investigating alterations in acid-base homeostasis (Scanes, 2015). The analyte pH is subject to a negative change of 0.01 units every 10 minutes following sampling from a specimen due to continued hematologic cellular metabolism (Raghav et al., 2015). Due to its rapid pen-side analysis within 2-3 minutes, fresh whole blood samples should never be subject to artificial changes in pH due to timing with the i-STAT®1 clinical analyzer, thus illustrating yet another advantage of such technology (i-STAT®1, 2006).

On a daily basis, laying hens showed no statistically significant differences in blood pH values during egg formation timepoints (Venne et al., 2019). By altering scope to cover a much larger timeframe, rapidly growing broiler-type birds have been

documented to have a mean pH of 7.35 where as a mature population of broiler-type birds had a mean pH of 7.42 (Olanrewaju et al., 2010; van As et al., 2010; Martin et al., 2010). Post-peak production broiler breeder hens also were shown to have significantly higher blood pH than pre-peak and peak production birds (Martin et al., 2010). Utilizing swine as a species model, evidence suggests blood pH remains statistically unchanged when measured up to 9 hours post-ingestion of a meal (Dersjant-Li et al., 2002).

In addition to the physiologic differences amongst birds themselves, their environment has been shown to significantly impact parameters such as pH as listed in several publications describing changes in heat stress (Van Goor et al, 2017). Heat stress is a very common condition affecting birds worldwide, and is responsible for perhaps some of the most substantial losses in productivity in the poultry industry (Rowland et al., 2019). Birds of variable ages, genetic backgrounds, etc. are likely able to respond to such heat stress differently (Wang et al., 2018; Van Goor et al., 2016; Van Goor et al., 2017; Rowland et al., 2019). In general, chickens under heat stress will fan out their wings, decrease feed and water intake, shunt blood to extremities and pant (Powell, 2015). When the respiratory rate a bird increases, the blood pH of the individual increases, and other blood gas and biochemical abnormalities can be readily appreciated too (Powell, 2015).

The blood gas parameters $pvCO_2$ and pvO_2 are certainly also heavily correlated with the acid-base status, and can help at least partially explain variations in blood pH (Scanes, 2015). Regarding venous versus arterial blood samples, $pvCO_2$ in a growing chicken is approximately 56 mm Hg whereas the $paCO_2$ in a mature hen can be anticipated to be around 25 mm Hg (Gleeson and Brackenbury, 1984; Christensen et al., 2012). In general, $pvCO_2$ levels have been shown to increase during the growth phase of

broiler-type birds (van As et al., 2010). Interestingly, $pvCO_2$ levels of such birds have been documented to increase from 48 to 69 mm Hg over the time-interval of 11-47 days of age, yet mature broilers were documented to have a mean $pvCO_2$ value of 38 mm Hg (vas As et al., 2010; Martin et al., 2010). The disparity between growing and mature birds is most likely due to increased demand, activity and subsequent cellular respiration during growth (Scanes, 2015). Although significant changes in the parameter are documented between wide age intervals and growth phases, PCO_2 values did not vary statistically in actively laying birds sampled during various timepoints during cyclical daily egg production (Venne et al., 2019). Nutritional fasting has been shown to decrease $pvCO_2$ in growing birds (Christensen et al., 2012). A bird's $pvCO_2$ has also been shown to be dramatically influenced via respiratory pattern, with panting subsequent to heat stress decreasing blood $pvCO_2$ (Scanes, 2015). Excessive exercise can also induce hyperventilation in chickens, induce respiratory alkalosis, and thus lower $pvCO_2$ (Scanes, 2015). Hypoventilation from upper and lower airway obstructions, sedation, etc. can lower a birds pCO_2 level <35 mmHg by inducing respiratory acidosis (Raghav et al., 2015). Arterial pCO_2 is more greatly impacted by respiration patterns, whereas venous pCO_2 is a better reflection of host metabolism (Scanes, 2015).

The pvO_2 parameter shows a strongly positive correlation to pH, although it is inversely correlated to $pvCO_2$. As expected, arterial versus venous pO_2 values collected from specimens have dramatic differences (Scanes, 2015). An expected mean value of paO_2 in adult *Gallus gallus* hens is reportedly 99 mm Hg whereas a pvO_2 value of approximately 44 mm Hg can be anticipated in growing birds (Gleeson and Brackenbury, 1984; Christensen et al., 2012). Bird age, in addition to sample type can also impact

anticipated pO_2 levels (Scanes, 2015). Rapid growth phases of broiler chickens have been associated with declines in pvO_2 (Scanes, 2015). For example, a study involving broiler birds demonstrated 11-day old broilers to have a mean pvO_2 level of 58 mm Hg contrasted with 47-day old broilers having a mean pvO_2 value of 35 mm Hg (van As et al., 2010). Chicken and Japanese quail models have demonstrated low pvO_2 will be coupled with an experienced increase in circulating erythrocyte concentrations and hematocrit (Scanes, 2015). Although short term flight in avian species has not been proven to increase paO_2, exercising chickens on treadmills revealed a paradoxical increase in arterial pO_2 most likely due to increased respiration (Butler et al., 1977; Gleeson and Brackenbury, 1984). Ambient temperature has been shown to affect pvCO2 and pvO2 values generated by the i-STAT®1 clinical analyzer (Raghav et al., 2015). The CD8+ cartridges do, however, have temperature incorporation mathematics to obtain both raw and temperature corrected values (Raghav et al., 2015).

The final three parameters (HCO_3, TCO_2 and BE) in the set of acid-base parameters are all derived from calculations via the i-STAT®1 clinical analyzer (i-STAT®1, 2006). Bicarbonate is an integral parameter in the determination of a bird's acid-base status, and acts as the major buffer in systemic circulation (Reece et al., 2015). TCO_2 levels have previously been shown to be highly correlated to HCO_3 levels with correlation values of >0.90 (Reece et al., 2015; Schaal et al., 2016). The two parameters are considered essentially analogous as very large proportion of TCO_2 values are derived from calculations of HCO_3 by the i-STAT®1 clinical analyzer (Reece et al., 2015; Schaal et al., 2016). The bird approaches an alkalotic state as circulating HCO_3 increases, whereas decreases often indicates a physiologic shift toward acidosis (Harris, 2009).

Gene expression of the molecule carbonic anhydrase is implicated with appreciable fluctuations in bicarbonate values (Scanes, 2015). More specifically, carbonic anhydrase is responsible for the conversion of readily available carbon dioxide and water in circulation to yield H_2CO_3, a molecule formally named carbonic acid (Scanes, 2015). Carbonic acid (a weak acid) molecules can then dissociate thereby forming bicarbonate (HCO_3-) molecules (conjugate base) and hydrogen ions (H+) in a reversible fashion contingent upon the acid-base status of an individual (Scanes, 2015). The activity of carbonic anhydrase has been shown to increase when an individual experiences states of hypoxia, subject to beta-adrenergic agonists and increased blood ferrous iron levels (Igbo et al., 1994; Glombitza et al., 1996; Wu et al., 2007). Once in systemic circulation, bicarbonate is thought to be conserved via renal filtration mechanisms similar to mammalian species, and are believed to be just as efficient (Montesinos and Ardiaca, 2013).

Bicarbonate levels are also implicated with egg production as the molecule $CaCO_3$ is a principle component of avian eggshells from the combination of HCO_3^- and Ca^{++} (Dacke et al., 2015). Conversion of calcium phosphate to calcium carbonate has been cited as a critical reaction in the formation of eggshells (Venne et al., 2019; Dacke et al., 2015). HCO_3 has been witnessed to peak at approximately the 12 and 16 hour timepoints during egg formation post-oviposition, a timepoint in which the calcium carbonate shell nears the completion of formation (Venne et al., 2019). Conversely, the lowest values of circulating bicarbonate can be expected at oviposition (Venne et al., 2019). Supplementation of dietary $KHCO_3$ was shown to significantly increase blood

bicarbonate levels, thereby also significantly increasing BE values in a trial involving broiler chickens (Ahmad et al., 2005).

The determination was made by Steinmetz et al., that BE results should be very carefully interpreted for *Gallus gallus* as the results obtained from the i-STAT®1 analyzer had no correlation to results obtained from a benchtop analyzer (2007). This early publication utilized EG7+ cartridges rather than CG8+ cartridges, but nonetheless utilized the same clinical analyzer as witnessed in chapters 3-5. Schaal et al. also found BE to be an unremarkable parameter when evaluating the Hy-Line W-36 CV (2015). BE values have, however, been witnessed to variably increase in Lohmann commercial leghorns proximal to the time period of eggshell formation (Venne et al., 2019). Overall, the BE parameter seems to have limited applicability and utility when compared against the 12 other blood gas and biochemistry parameters reported by the i-STAT®1 clinical analyzer and associated CG8+ cartridge.

Practical Implementation

To date, the i-STAT®1 clinical analyzer has been utilized in companion animals, food-animals like bovine and swine, equine, small mammals, aquatic species and wildlife/exotic species models (West et al., 2014; Lebonte et al., 2015; Peiró et al., 2010; Bleul and Gotz, 2014; Yildirim et al., 2015; Silverman et al., 2002; Kutter et al., 2012; Beisser et al., 2011; Ardiaca et al., 2013; Harter et al., 2015; Montesinos and Ardiaca, 2012; Valéria et al., 2008). Currently, the i-STAT®1 user guide includes reference ranges for feline, canine and equine which comes with the purchase of the analyzer (i-STAT®1, 2006). Of the many species with documented i-STAT®1 utilization, implementation in poultry species is relatively novel in its scope. Specific and usable information such as

reference ranges available for *Gallus gallus*, the domestic chicken, are limited. This section aims to summarize some of the most influential, recent and relevant publications of i-STAT®1 implementation with emphasis on chickens as the primary model species.

One of the very first publications of i-STAT®1 in a commercial poultry species utilized Lohmann leghorn layer chickens to validate the technology against conventional serum chemistry analyzers (Steinmetz et al., 2007). Using a chicken model, Steinmetz et al. found the i-STAT®1 analyzer and EG7+ cartridges to reliably measure pH, pO2, pCO2, Na, iCa, PCV, and accurately calculate HCO3, tCO2, Hb, sO2 when compared to benchtop analyzers (2007). K and BE were the only two parameters shown not to have consistent results with benchtop analyzers (Steinmetz et al., 2007). Other studies comparing the i-STAT®1 to classical benchtop analyzers have witnessed discrepancies too (Edling et al., 2001; Heatley et al., 2005; Howard and Wack, 2002). The parameter iCa has been shown to be inaccurately low in the i-STAT®1 compared to a classical benchtop analyzer (Howard and Wack, 2002). In a raptor study, the i-STAT®1 reported lower Na, K and Hct values statistically than a classical benchtop analyzer (Heatley et al., 2005). In addition to statistical comparison between analyzation methods, Steinmetz et al. also provided individual parameter means for the 11 parameters included on a EG7+ cartridge: pH, pO2, pCO_2, Na, iCa, PCV, HCO_3, TCO2, Hb, sO_2, K and BE (2007). As mentioned in an earlier section of this chapter, much of the research in poultry has utilized the CG8+ cartridge which in addition to the 11 aforementioned parameters, also includes Glu and Hct.

Selective breeding of the species *Gallus gallus* is principally divided between commercial breeds and varieties used for egg and meat production. Due to their

extensively divergent genetics, extrapolation of reference intervals between broilers and layers should be used with caution. Several years after the publication by Steinmetz et al. which used laying-type chickens as a model, reference ranges for one Cobb and two Ross broiler strains of breeder hens were established covering an age interval of 25 – 36 weeks (Martin et al., 2010). This time interval was reportedly specifically targeted as metabolic diseases in broiler breeder hens have the propensity to occur proximal to the time period of peak production (Martin et al., 2010). Wide variations in reference intervals were witnessed for the pO_2 sO_2 and BE parameters (Martin et al., 2010). Statistically significant differences were noted between the Cobb and Ross strains of broiler birds (Martin et al., 2010). More specifically, the Cobb strain of hens was found to have higher pO_2 and sO_2, and lower Glu levels than the two Ross strains (Martin et al., 2010). These findings provide evidence that differences in blood gas and biochemistry parameters can indeed vary widely between subpopulations of *Gallus gallus*, even between birds bred for the same principle purposes.

In addition to sampling three different strains of broiler breeders, the study also incorporated sampling of birds from four different integrators, and reported significant differences in several blood parameters between integrators (Martin et al., 2010). Na, iCa and pH were found to be consistent between integrator (Martin et al., 2010). Based on the consistency of these three parameters between integrators, Martin et al. hypothesized they are less likely to be affected by nutritional differences and management practices (2010). By diversifying the integrators included in the study, the authors hoped the reference intervals established would be more useful when interpretations are made in future works.

The study also reported differences between ages of the production birds sampled with post-peak (33-36 weeks of age) production birds having lower Glu and pCO_2, as well as higher Na, pH and BE levels than pre-peak (25-28 weeks of age) and peak (29-32 weeks of age) production hens (Martin et al., 2010). The authors do mention a low sampling number (n=20) of post-peak production breeders, which may explain some of the apparent variation between age groups (Martin et al., 2010). HCO_3 and TCO_2 were found to be dissimilar between pre-peak and peak production hens (Martin et al., 2010).

There are several studies building on applications of i-STAT®1 with analysis of values of significance to commercial production and pathological conditions. The aforementioned broiler breeder varieties studied by Martin et al. were part of a calcium tetany trial where the metabolically-driven disease was further characterized biochemically (2010). The most important results of this study have previously been discussed in the *Electrolyte Parameter* subsection of this chapter. Beyond specific parameter results previously discussed, Martin et al. concluded a confirmatory diagnosis of calcium tetany should include the identification of low iCa values in tandem with clinical signs of impaired mobility in actively laying broiler breeder hens (2010). A final diagnosis of calcium tetany based on clinical signs alone could be incorrect as the clinical signs of derangements of other blood electrolytes (Na and K) have the propensity to present very similar to acute hypocalcemia clinically (Martin et al., 2010).

A dietary calcium supplementation study utilizing ISA Brown layer pullets also implemented i-STAT®1 technology. The source of the supplemental calcium was calcium carbonate ($CaCO_3$) derived from limestone. In this study, 35 day old pullets were grouped and fed either a normal-calcium (NC) diet (8.5 g Ca/kg) or a high-calcium (HC)

diet (36.3 g Ca/kg). In addition to obtaining blood samples for determination of blood gas and biochemistry parameters, urine was also collected. HC pullets experienced elevated blood iCa, pH, HCO_3, and BE than the NC pullets. BE was reportedly five times higher in the HC group than the NC group. The HC pullets had lower K, PCO_2, PO_2 and sO_2 than the NC pullets, but there was no significant difference in their Na levels. Based on the results of the blood analysis, it was concluded $CaCO_3$ induced a state of metabolic alkalosis in the HC pullets. Concurrent urinalysis of samples obtained from the pullets revealed statistically significant increases in renal excretion of calcium, but lower sodium. (Gou et al., 2008).

Chicken heat stress trials have also been documented in literature with valuable utilization of i-STAT®1 technology (Van Goor et al., 2016; Van Goor et al., 2017; Wang et al., 2018; Rowland et al., 2019). One of the earliest in a series of heat stress trials was conducted at Iowa State University utilizing an advanced intercross line originally developed via the mating of commercial broiler-type (heat susceptible) birds and Egyptian Fayoumi (heat resistant) birds (Van Goor et al., 2016). Seven days post-heat treatment significantly increased pH, BE, HCO_3, TCO_2, iCa, Hct Hb and sO_2 in the intercross line of commercial broiler and Egyptian Fayoumi laying chickens (Van Goor et al., 2016). This study also provided evidence of decreased pCO_2 and blood Glu after 7 days of heat treatment. Heritability estimates were provided for the parameters, and ranged from 0.01-0.23 with measurements collected during application of heat treatment (Van Goor et al., 2016). HCO_3 on the seventh day of heat treatment had the highest heritability of 0.23, but also had relatively large reported standard error of 0.12 (Van Goor et al., 2016). Correlation analysis revealed many parameters to be significantly

correlated on the same day of measurement, but significant correlations between measurement days were scarce (Van Goor et al., 2016). A genome-wide association study was performed, and candidate genes implicated with chicken responses to heat stress were identified (Van Goor et al., 2016). For example, nine quantitative trait loci were identified for the PCO_2 (Van Goor et al., 2016). Van Goor et al. concluded that determining the heritability and gene expression of these candidate genes could lead to enhanced heat tolerance in commercial poultry.

Van Goor et al. soon followed up with another publication in 2017 evaluating splenic RNA-sequencing data from two individual lines of chickens, broiler-type birds and Egpytian Fayoumi birds. Again, birds were subject to short-term heat stress, but also included a lipopolysaccharide (LPS) challenge in the experimental design (Van Goor et al., 2017). Stimulation by LPS expressed genes differentially between the two lines of birds, with the broiler line having more robust gene expression levels than the Fayoumi line (Van Goor et al., 2017). Conversely, the Fayoumi line was found to have increased differentially expressed genes over the broiler line with application of ambient heat (Van Goor et al., 2017).

This study by Van Goor et al. also incorporated blood gas and biochemistry analysis in its experimental design (2017). Blood samples collected on day 20 (pre-heat) and day 22 (post-treatment), and were analyzed using the i-STAT®1 clinical analyzer and CG8+ cartridges. Results of these blood collections were reported as percent change between the two collection timepoints. Statistical analysis of differences between line alone revealed very few differences of blood gas and biochemistry components, potentially explained due to high sample variability (Van Goor et al., 2017). Regarding

response to heat treatment independent from LPS challenge, broilers had a significantly higher mean iCa value than the Fayoumi (Van Goor et al., 2017). This finding provides evidence of the effect of heat stress on iCa values in avian serum. Broilers were found to have significantly lower pH and BE from the Fayoumi when evaluating the response to LPS independently (Van Goor et al., 2017). Such differential mean values of pH and BE can potentially one day help to explain positive and negative host responses to LPS on a genetic basis. Interestingly, most statistical differences between line were witnessed in the bird populations stimulated by both LPS and ambient heat (Van Goor et al., 2017). With LPS and heat treatments in combination, broilers had significantly higher mean pH, Na, Hct and Hb as well as lower mean BE, HCO_3, TCO_2 and K compared to the Fayoumis (Van Goor et al., 2017).

Another recent publication provides evidence that two genetically distinct inbred lines of chickens (Leghorn and Fayoumi) varied in their acid-base balance and resultant metabolic disorders (Wang et al., 2018). In this study, the Fayoumi was shown to maintain its heat resilience further elucidating that upon the identification of genes responsible, breeding strategies can be implemented in commercial poultry production. The incorporation of i-STAT®1 technology in this trial resulted in the identification of PO_2, TCO_2, HCO_3 and BE as the strongest biomarkers associated with heat tolerance (Wang et al., 2018).

Rowland et al., recently published work characterizing quantitative trait loci (QTL) of the blood chemistry components associated with physiological heat stress response using i-STAT®1 technology (2019). The Hy-Line W-36 birds enrolled in the study had blood collected before and during application of heat stress. All thirteen

parameters associated with the CG8+ cartridge were found to have statistically significant changes at the five hour post-heat stress application (Rowland et al., 2019). The parameters BE, Glu, Hb, Hct, HCO_3, K, pCO_2 and TCO_2 were found to have heritability estimates ranging between 0.21 to 0.45 thereby suggesting their potential use as biomarkers when correlated with economic traits of importance (Rowland et al., 2019). Perhaps identification of additional genes, genetic pathways and genetic markers in different commercial varieties of birds can provide insight to breeding strategies which could in turn lead to hybridization and elaboration of positive traits (Rowland et al, 2019). If successfully incorporated, commercial poultry companies could potentially target CVs of laying hens to countries according to different climates.

Scientific work in this thesis fundamentally build upon research efforts of Schaal et al., which established reference intervals for Hy-Line W-36 pullets and laying hens (2015). Ages of these birds ranged between 4 to 110 weeks of age. Birds were further classified based on production time points as pullets (4 to 15 weeks), first cycle layers (20 to 68 weeks) and second cycle layers (70 to 110 weeks). Age of birds at sampling have been shown to significantly impact all 13 of the analytes provided by CG8+ cartridges, as seen by the difference between Hy-Line W-36 pullets and laying hens. Additionally, K, iCa, Hct, pH, TCO_2, HCO_3, BE, sO_2 and Hb were significantly different between first and second cycle laying hens of the same CV (Schaal et al., 2015). Schaal et al. hypothesized other commercial White Leghorn varieties (such as the Hy-Line W-80 and Hy-Line 80+) would likely have similar blood gas and biochemistry profiles to the Hy-Line W-36. It was also hypothesized colored egg layer CVs produced by Hy-Line International would

likely differ in such profiling from their white egg laying counterparts as they are genetically comprised of dissimilar breeds of laying chickens (Schaal et al., 2015).

Conclusion

This chapter has provided a background on the iSTAT®1 clinical technology as well as provided examples of its application to the poultry industry. Early on in the chapter, the i-STAT®1 and CG8+ cartridge mechanics and general information were reviewed to allow the reader to better understand the primary clinical technology to be incorporated in subsequent chapters. Next, a literature review covering the 13 parameters of the CG8+ cartridge have revealed key parameters of collective interest based on prior publications. This section was divided into three subsections based on parameter relationships. Although some of the information in this section cited specific works involving the i-STAT®1 clinical analyzer, the section was primarily created to give the reader a more thorough background of applicability of the specific parameters themselves in avian species.

The four principle blood electrolytes in these works include iCa, Glu, Na and K. The parameters iCa and Glu perhaps have been the two most investigated and targeted parameters historically, especially when compared to the Na and K parameters. The K parameter has historically been a parameter very labile to sample handing, and has had limited utility in the poultry industry. Na has also had little utility in poultry research. As mentioned, calcium is perhaps one of the most heavily cited and researched blood parameters in poultry, especially in commercial layers due to this parameter's heavy implication with eggshell formation and metabolic bone disease. Calcium has been investigated in a number of specific trials such as the determination of effect of

nutritional supplementation of dietary calcium and other classical feed additives. Calcium has also been investigated in specific disease etiologies like broiler calcium tetany and caged-layer fatigue. Overall, the applicability of iCa to modern layer production is very robust and promising.

The three parameters (sO_2, Hct and Hb) are most heavily related to erythrocytes and their functionality. Effect of age, environment and genetics were elucidated for these parameters. The remainder of the parameters (pH, $pvCO_2$, pvO_2, HCO_3, TCO_2, BE) which were grouped together based on their relationship to acid-base status and regulation. Although BE has historically had very limited utility, the effects of other parameters have been well cited especially in heat stress trials.

Several studies have been conducted with i-STAT®1 using a chicken model. Several of the 13 blood gas and biochemistry parameters have been shown to differ between genetically dissimilar subpopulations of poultry. These differences based on genetic factors have brought forth the need to establish reference intervals for specific populations of poultry such as different commercial varieties of laying hens. Despite this, very few commercial layer and broiler CVs have established reference intervals for blood gas and biochemistry parameters. This literature review has also revealed the importance of result validation between clinical devices as to eliminate potential discrepancies in blood gas and chemistry data as well as to determine the specific reliability of the i-STAT®1.

With the above in mind, significant gaps in the literature exist when applying the blood gas and chemistry parameters to clinical disease states and production data too. Nonetheless, heat stress, osteoporosis and calcium tetany serve as examples of clinical

disease states and challenges already targeted in chickens. As witnessed, applications and inferences made from differences in blood gas and biochemistry markers are far from exhausted. This chapter has acknowledged strengths as well as gaps in the literature for the selected blood gas and biochemistry markers obtained from the i-STAT®1.

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CHAPTER 2. COMPARISON OF CHICKEN BLOOD CHEMISTRY AND ELECTROLYTE PARAMETERS BETWEEN THE PORTABLE I-STAT®1 CLINICAL ANALYZER AND VETSCAN VS2® SERUM BIOCHEMISTRY PANEL USING HY-LINE COMMERCIAL WHITE-EGG LAYING HENS.

Modified from a manuscript submitted to the *Journal of Poultry Science*

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Abstract

The i-STAT®1 clinical analyzer has become an increasingly popular tool in clinical production animal medicine due to its ability to report pen-side results in a cost effective and timely manner when compared to a benchtop serum biochemistry blood gas and chemistry analysis. This study compares the results of the portable Abbott i-STAT®1 analyzer and the Abaxis VetScan VS2® for the glucose (Glu mg/dl), ionized calcium (iCa mmol/L), sodium (Na mmol/L) and potassium (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-STAT®1 portable device. A subset of 22 blood samples were reserved, and serum 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 (p-value <0.001) in means between the two methods for each of the parameters. Parameters with significant mean differences were then subject to correlation and regression analysis to further evaluate relationships between results of the two methods. Significant differences between means were found for glucose, sodium and potassium. Calcium was found to be not directly comparable by the two analyzation methods. 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-STAT®1

Introduction

The i-STAT®1 clinical analyzer allows for pen-side results in a cost effective and timely manner when compared to standard benchtop serum biochemistry blood gas and chemistry analysis. As a result, the i-STAT®1 has become an increasingly popular tool in poultry medicine due to its ability to report fast and accurate results (Schaal et al., 2016). In recent years, the device has been implemented in various investigations including but not limited to reference interval establishment, calcium tetany in broiler-type birds and various heat-stress studies (Steinmetz et al., 2007; Martin et al., 2010; Martin et al., 2011 Schaal et al., 2016; Van Goor et al., 2016; Van Goor et al., 2017; Wang et al., 2018; Rowland et al., 2019). Reference interval variability are observed between commercial types and varieties of chickens making accurate inferences on blood gas and chemistry data difficult for clinicians and field veterinarians thus evaluating analysis method as a potential confounding factor adding to the observed variability is of practical importance (Sauer et al., 2019). The i-STAT®1 has been compared to other analyzation methods, with one of the first such comparisons utilizing a chicken model being published in 2007 by Steinmetz et al. This study utilized Lohmann leghorn layer-type birds to validate results of the i-STAT®1 against an unspecified conventional serum chemistry analyzer. This study also utilized the EG7+ cartridge, rather than the CG8+ cartridge more commonly used today. Nonetheless, it was concluded the i-STAT®1 can reliably measure pH, pO2, pCO2, Na, iCa, PCV, and accurately calculate HCO3, tCO2, Hb, sO2 compared to the unspecified benchtop analyzation method (Steinmetz et al., 2007). This short study provides a comparison of the results of the Abbott i-STAT®1 portable analyzer and the Abaxis VetScan VS2® benchtop clinical analyzer with a *Gallus gallus* species model. The specific parameters used for comparison are glucose (Glu), calcium

(Ca), sodium (Na) and potassium (K) as they are analytes shared between the two specific analyzation methods.

Materials and Methods

Bird Husbandry, 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. 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 on June 28, 2017 (mid-summer). Hens were 66 weeks (461 days) of age at the time of sampling. A minimum sampling size of 20 healthy reference individuals was determined following recommendations of the American Society for Veterinary Clinical Pathology for direct validation (Friedrichs et al., 2012). Thirty blood samples (n=10 per CV) were obtained via venipuncture of the brachial wing vein using 1 ml syringes (with no anti-coagulant) and needles. Randomization (allocation concealment, implementation) as well as blinding of samples and personnel were not performed.

Each sample of fresh, uncoagulated blood was directly loaded into a CG8+ cartridge, and immediately analyzed by an i-STAT®1 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 analyzation 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 two methods 4 were shared: glucose (Glu mg/dL), calcium (ionized Ca mmol/L from i-STAT®1 and total Ca mg/dL from VetScan VS2®), sodium (Na in mmol/L from i-STAT®1 and in mEq/L from VetScan VS2®) and potassium (K in mmol/L from i-STAT®1 and in mEq/L from VetScan VS2®).

Statistical Analysis

Results from all CVs of laying hens were pooled as the number sampled per CV was too insignificant to detect potentially confounding or bias. Statistical analysis was performed via paired T-test for the data included in this study to test for statistical differences (p-value <0.001) in means for each of the applicable parameters between the two methods. Apparent bias in the represented T-values could be affected by the smaller sample size of this data. Parameters found to have statistically significant mean differences were then subject to correlation and regression analysis.

Results and discussion

Of the 30 serum samples submitted to the Iowa State University Department of Veterinary Pathology for VetScan VS2® analyzation, a subset of successful results for calcium (n=22), glucose (n=22), sodium (n=21) and potassium (n=17). The submission failures were reportedly due to insufficient samples or hemolysis, neither of which were readily visually appreciable at the time of consultation and submission to pathology staff. When analyzing the effect of analyzation method, results indicate significant differences in means between analyzation methods for glucose and sodium (Table 1). Glu and Na were found to be significantly correlated (p-value <0.05), but with correlation coefficient significantly different from 1 (95% confidence interval not including 1). Regression analysis showed the i STAT®1 had a positive proportional analytic bias for Glu and Na relative to the VetScan VS2®. A change of 1 mg/dL of Glu in i STAT®1 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 STAT®1 is equivalent to 0.73 (±0.13) mEq/L in VetScan VS2®. Statistically significant differences between the two analyzation methods for Glu and Na illustrate the importance of cautious inference when comparing scientific research utilizing different analyzation methods. There was no significant linear relationship between values of K for the two methods.

Concerning the calcium parameter, the VetScan VS2® analytical range is for 4-16 mg/dL. This set of blood samples collected from actively laying hens consistently yielded a reported total calcium value of >20.0 by the VetScan VS2®, a result not applicable for basic statistical comparison between analyzation methods. Nonetheless, the iSTAT®1 clinical analyzer did yield a mean ionized calcium value of 1.69 mmol/L (SD=0.11), a value within the analytical range of 0.25-2.50 for the i-STAT®1 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 x 0.2495 = mmol/L] (Tully et al., 2009; Stockham and Scott, 2008). Blood iCa comprises approximately 50% of the total Ca in a blood sample in a mammalian host, however this fractional value was approximately 35% in a study utilizing a turkey model (Goff et al., 2015; McHurtry et al., 1984). Utilizing the more conservative value 35% from an avian model rather than a mammalian estimation, the mean estimated total Ca from the iSTAT®1 clinical analyzer was estimated to be 19.35 mg/dL [Est total Ca = iCa x (1/0.2495) x (1/0.35)]. The estimated values of 19.35 mg/dL falls out of the previously indicated VetScan VS2® dynamic range and makes accurate value comparisons in actively laying hens for blood iCa challenging.

Conclusions

This study has provided statistical evidence that mean Glu and Na were different between analyzation methods; the i-STAT®1 had a relative positive proportional analytic bias but the instruments remained correlated. Nonetheless, no relationship was shown for K between the two analyzation methods. Additionally, the K parameter lacked the minimally sufficient number of samples yielding 20 viable results (n=17). The calcium parameter was found to be incomparable between the two devices. Nonetheless, the iSTAT®1 was not only able to report a continuous numerical value for calcium, it was also able to provide the ionized calcium blood concentrations. Ionized calcium is the metabolically active form of calcium in systemic circulation, thus serving as an applicable and precise method of reporting blood calcium when conducting research with laying hens, which will inevitably have blood calcium levels consistently out of the reference interval in benchtop analyzers. A major limitation of this study is its applicability to other analyzation methods beyond the iSTAT®1 clinical analyzer and the VetScan VS2® benchtop analyzer. As neither the i-STAT®1 and VS2® are technically considered official standardized instruments this comparison could be considered problematic if either of the two analyzation methods are indeed imprecise. In general, the Abbott i-STAT®1 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

The authors do not declare any conflict of interest.

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Tables

Table 1. Results of the paired T-test analysis between i-STAT®1 clinical analyzer and the VetScan VS2® including means and standard deviations (SD) for calcium, glucose, sodium and potassium parameters.

	Calcium		**Glucose***		**Sodium***		**Potassium**	
	i-STAT	**VetScan**	**i-STAT**	**VetScan**	**i-STAT**	**VetScan**	**i-STAT**	**VetScan**
Units	mmol/L	mg/dl	mg/dl	mg/dl	mmol/L	mEq/L	mmol/L	mEq/L
Mean	1.69	>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

*p-value significance <0.001

CHAPTER 3. ESTABLISHMENT OF HY-LINE COMMERCIAL LAYING HEN WHOLE BLOOD GAS AND BIOCHEMISTRY REFERNCE INTERVALS UTILIZING PORTABLE i-STAT®1 CLINICAL ANALYZER

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Abstract

Blood gas and biochemistry reference intervals were established for three genetically distinct commercial varieties (CV) of Hy-Line laying hens; two brown egg layers (Hy-Line Brown, Hy-Line Silver Brown) and a tint-egg layer (Hy-Line Sonia) utilizing the i-STAT®1 analyzer. Each respective variety of laying hen was sampled on a replicate cycle of two weeks for six total replicates (35-46 weeks of age). Blood samples were obtained in the production house from the brachial vein, and subsequently analyzed by the i-STAT®1 portable device i-STAT®1 clinical analyzer reports blood gas and biochemistry values for the 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 1800 individual hen i-STAT®1 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, physiologic abnormalities and production performance.

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 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). 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_2decreases 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 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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Tables

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	PCO2 ($pvCO_2$ mm Hg)	PO2 (pvO_2 mm Hg)	HCO3 (mmol/L)	BE (mmol/L)	sO2 (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**	**^{A} 7.33**	**^{B} 50.5**	**^{B} 39.0**	**^{B} 26.2**	**^{C} 0.2**	**^{A} 67.7**	**^{A} 243.4**	**^{A} 150.7**	**^{A} 5.2**	**^{B} 27.7**	**^{AB} 1.7**	**^{C} 22.9**	**^{C} 7.8**
	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**	**^{B} 7.32**	**^{B} 51.1**	**^{A} 41.0**	**^{B} 26.1**	**^{B} -0.1**	**^{A} 69.9**	**^{B} 241.6**	**^{B} 148.9**	**^{B} 5.1**	**^{B} 27.6**	**^{A} 1.7**	**^{B} 23.4**	**^{B} 8.0**
	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**	**^{AB} 7.33**	**^{A} 52.7**	**^{B} 38.3**	**^{A} 27.4**	**^{A} 1.4**	**^{A} 66.5**	**^{B} 241.9**	**^{A} 150.5**	**^{A} 5.2**	**^{A} 29.0**	**^{B} 1.7**	**^{A} 23.7**	**^{A} 8.1**
	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 (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 (**TCO_2** mmol/L), ionized calcium (**iCa** mmol/L), hematocrit (**Hct** % Packed Cell Volume [PCV]), hemoglobin (**Hb** g/dl) utilizing combined data from two brown layer (Hy-Line Brown, Hy-Line Silver Brown) and one tint-egg layer (Hy-Line Sonia) varieties.

Variable	N	Mean	Std Dev	Minimum	Maximum
pH	1613	**7.32**	**0.06**	7.10	7.52
PCO2 ($pvCO_2$ mm Hg)	1600	**51.3**	**7.3**	29.4	76.3
PO2 (pvO_2 mm Hg)	1611	**39.5**	**5.6**	26	66
HCO3 (mmol/L)	1614	**26.6**	**1.9**	20.1	33.5
BE (mmol/L)	1614	**0.5**	**2.4**	-8	7
sO2 (sO_2 %)	1605	**68.0**	**7.8**	42	94
Glu (mg/dl)	1613	**242.3**	**9.5**	208	279
Na (mmol/L)	1615	**150.0**	**2.7**	141	159
K (mmol/L)	1615	**5.1**	**0.4**	3.9	6.5
TCO2 (mmol/L)	1614	**28.1**	**2.0**	21	36
iCa (mmol/L)	1617	**1.7**	**0.1**	1.38	2.16
Hct (% PCV)	1610	**23.33**	**2.23**	14	34
Hb (g/dl)	1610	**7.93**	**0.76**	4.8	11.6

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 two brown layer (Hy-Line Brown, Hy-Line Silver Brown) and one tint-egg layer (Hy-Line Sonia) varieties (above diagonal) and their corresponding p-values (below diagonal).

	pH	**PCO2** ($pvCO_2$ mm Hg)	**PO2** (pvO_2 mm Hg)	**HCO3** (mmol/L)	**BE** (mmol/L)	**sO2** (sO_2 %)	**Glu** (mg/dl)	**Na** (mmol/L)	**K** (mmol/L)	**TCO2** (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
PCO2 (pvCO2 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
PO2 (pvO2 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
HCO3 (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 (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
TCO2 (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	

CHAPTER 4. GENERAL CONCLUSIONS

A background of i-STAT®1 clinical technology and listing of its applications in the scope of the poultry industry have been previously described. The i-STAT®1 clinical analyzer has many advantages and some limitations compared to other in-house analyzation methods. The portable analyzer has the ability to not only be used in a laboratory setting, but also in-field allowing for very diverse utility. Despite its portability, the device can become sensitive to high ambient temperatures resulting in a higher rate of analyzation failures, and the subsequent need for additional blood collection. Classical benchtop analyzers (like the VetScan VS2®) are generally stored and operated in more temperature-neutral environments, but again this convenience comes with the disadvantage of a lack of portability by such devices. If anticoagulant is not utilized in the i-STAT®1 system, the operator of the device essentially has a matter of seconds to pre-load the CG8+ cartridge before fresh whole-blood samples begin to coagulate. Conversely, the VetScan VS2® utilizes blood serum samples thus eliminating the concern of sample clotting.

Through personal experience with this technology, extremely smooth and precise blood draws from the brachial wing vein result in the highest rate of analyzation success for all parameters, especially potassium. Although a CG8+ cartridge only requires approximately two drops of whole blood for successful analyzation, the most successful analyzations in the various portions of this study were from blood draws of 0.3 – 0.5 ml. Perhaps this is at least partially due to the dilution of activated coagulation factors induced by the initial venipuncture of the needle into the brachial wing vein. Nonetheless, blood draws resulting in <0.1 due to operator error or generalized difficulty in collection

resulted in the highest analyzation failure rate in this study. In the event <0.1 ml of fresh whole blood was collected; such samples were discarded due to the high incidence of analyzation failure, and blood was drawn from the bird's other wing vein. The VetScan VS2® generally requires a whole blood sample of at least 1 mL for adequate serum harvest and subsequent analyzation by the device in order to prevent "insufficient sample" results. This volume of blood required by the VS2® generally requires syringes greater than 1 mL volume capacity. The large negative pressure gradient created by syringes of such size (for example 3 mL) compared to 1 mL syringes resulted in increased incidence of brachial wing vein collapse, collection redraws and generally prolonged individual collection times. Blood samples collected utilizing 1 mL syringes i-STAT®1 resulted in the most successful results.

In terms of timeliness of the i-STAT®1 system, results are obtained very quickly upon successful blood collection, and insertion of loaded CG8+ cartridges into the i-STAT®1 for analyzation. The device has the capability to store a very large number of sample analyzation results to, allowing for heightened convenience of result storage while out in the field. For these reasons, this technology can be a suitable option for robust sampling populations and studies requiring large data sets for statistical analysis.

Of the 13 blood gas and biochemistry parameters of interest, iCa and Glu were perhaps the most distinctly marked in the literature and targeted for practical inferences in modern poultry production. Evidence of significant differences of the 13 parameters when bird genetics, age, health, environmental conditions were factored into consideration. Applications of iSTAT®1 technology to disease and nutrition trials have been very limited although several studies have been conducted on heat stress recently

(Gou et al., 2008; Schaal et al., 2016; Van Goor et al., 2016; Van Goor et al., 2017; Wang et al., 2018; Rowland et al., 2019). Additionally, work investigating broiler breeder reference intervals and a subsequent calcium tetany disease trial in broilers has implemented the technology (Martin et al., 2010; Martin et al., 2011). Pertinent to the layer industry, hen osteoporosis and caged layer fatigue are well documented in the literature, yet research directly involving practical implementation of iSTAT®1 technology is still lacking. Associations between egg quality and other layer production parameters such as the persistency of lay are also scarce in regard to application of the results obtained from the i-STAT®1.

Again, one of the great advantages of the iSTAT®1 clinical analyzer is its portability. Several studies have illuminated the differences in results reported between the iSTAT®1 and classical benchtop analyzation methods (Steinmetz et al., 2007; Edling et al., 2001; Heatley et al., 2005; Howard and Wack, 2002). Some of these studies lacked information on the benchtop analyzation method by which the i-STAT®1 was compared to, and the scientific reasoning for the choices of other analyzation methods were not readily apparent. The VetScan VS2® was selected as a benchtop analyzer to assess the reliability and consistency of the i-STAT®1. It is important to note that the VetScan VS2® is not necessarily a "gold standard" analyzation method. In fact, the term "gold standard" should always be interpreted carefully when cited in literature, as ultimately no one device holds this title in the realm of clinical pathology. Perhaps a meta-analysis of the results of side to side comparisons between the i-STAT®1 and various benchtop analyzers could prove useful.

In the work simply comparing the i-STAT®1 and VS2®, specific results from the portable iSTAT®1 were both directly (Glu, Na, K) and indirectly (iCa) compared to the VetScan VS2® benchtop clinical analyzer. This work revealed significant differences between the two devices for Glu, Na and K. Although statistically different in mean values, results for Glu and Na were also found to be correlated between the two devices. The iSTAT®1 clinical analyzer reports ionized calcium values whereas the VetScan VS2® reports total calcium values. Although not directly comparable, calculations were made to make tentative conclusions comparing the two devices. The iSTAT®1 was able to provide calcium results more directly applicable to laying hens in production. Results generated from the two devices can be found to be correlated depending on parameter, but careful interpretation must always be employed when directly generalizing results. Caution should also be employed when applying results for specific parameters to established reference intervals for specific devices.

Reference intervals representing physiologic normal values for blood gas and biochemistry parameters have been previously shown to vary contingent upon genetic influence (Martin et al., 2010; Van Goor et al., 2016; Van Goor et al., 2017; Wang et al., 2018). Such genetic variation elucidated the need to investigate potential differences in modern, commercially available varieties of laying hens. Blood gas and biochemistry reference intervals were subsequently for three CVs of brown and tint egg laying hens (Hy-Line Brown, Hy-Line Silver Brown and Hy-Line Sonia) amidst full egg production. The three aforementioned CVs of laying hens are amongst the world's most popular colored egg laying hens.

All 13 parameters obtained from CG8+ cartridges were found to have statistical differences between the three colored-egg laying CVs. Ionized calcium was found to be tightly regulated between CVs, with other parameters illustrating stronger differences between CVs. Nonetheless, confounding between CVs was likely, and was potentially influenced by many factors including age, stage of production, genetic selection, and nutrition among other factors. Statistically significant differences were also likely to be affected by the large sample sizes in this particular manuscript. Such miniscule yet statistically significant differences are likely not appreciable in the field. Therefore, these results should be repeated to ensure future reproducibility of these established reference intervals, and to further examine differences between CVs. Careful interpretation of published reference intervals must always be employed as many of the potential confounding factors are certainly not ubiquitous amongst flocks in modern poultry production.

In continuation of research efforts not specifically included in this thesis, the author aims to establish of pre-production and production blood gas and biochemistry reference intervals for the white-egg laying Hy-Line W-36, Hy-Line W-80 and Hy-Line W-80+ CVs thereby expanding upon work already published on the Hy-Line W-36. This work will thereby expand Hy-Line CV reference intervals from the three colored-egg laying CVs (Hy-Line Brown, Hy-Line Silver Brown and Hy-Line Sonia) and previously established Hy-Line W-36 to include a total of six of the world's most popular commercially available egg layers. Future work also aims to provide evidence of blood gas and chemistry values varying between the different age groups of Hy-Line W-36, Hy-Line W-80 and Hy-Line W-80+. To the author's knowledge, documenting the population

of birds' blood gas and biochemistry data over such a large timeframe is novel, especially for these CVs of laying hens. Correlations with production data are also possible, especially within the data collected during the blood collections of the three colored-egg laying CVs (Hy-Line Brown, Hy-Line Silver Brown and Hy-Line Sonia).

The scope of implementation of i-STAT1 technology in the laying industry will hopefully now be broadened due to the provided mean values and reference intervals provided by this work. The birds were clinically normal and housed on highly bio-secure research farms, similar timepoints, under the same management and nutrition when establishing these reference intervals thereby limiting their clinical applicability to birds under those specific conditions. Thus such reference intervals may be rendered more useful in clinical disease trials when sampled under more diverse set of sampling time points, flock locations, ages, management schemes and nutritional standards. Such application of blood gas and chemistry data to clinical disease states as well as production data are assuredly lacking in literature, and need to be explored in future research efforts.

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