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# Venous blood gas and chemistry components are moderately heritable in commercial white egg-laying hens under acute or chronic heat exposure

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# Venous blood gas and chemistry components are moderately heritable in commercial white egg-laying hens under acute or chronic heat exposure

## Abstract

Heat stress has a large negative impact on poultry around the world in both intensive and small-scale production systems. Better understanding of genetic factors contributing to response to high ambient temperatures would provide a basis to develop strategies for alleviating negative impacts of heat on poultry production. The objective of this work was to characterize the genetic control (heritability estimate and quantitative trait loci (QTL)) of blood chemistry components before and after exposure to acute and chronic high ambient temperature in a commercial egg laying line Hy-Line W-36 female parent line mature hens were exposed to 4 wk of daily cyclic heat exposure. Blood was collected pre-heat, on the first day of heat, and 2 and 4 wk post heat initiation and analyzed immediately using an i-STAT® hand-held blood analyzer. Thirteen blood components were quantified at the 4 time points: pH, pCO<sub>2</sub>, pO<sub>2</sub>, HCO<sub>3</sub>, TCO<sub>2</sub>, sO<sub>2</sub>, iCa, Na, K, base excess, glucose, “hematocrit” (estimated from blood electrical conductivity, BEC), and “hemoglobin” (calculated from BEC). Heritabilities were estimated using genomic relationship information obtained from 600k SNP chip data. All 13 parameters exhibited a significant change after 5 h of heat exposure and most did not return to pre-heat levels throughout the duration of the study. Eight parameters (base excess, glucose, hemoglobin, HCO<sub>3</sub>, hematocrit, K, pCO<sub>2</sub>, TCO<sub>2</sub>) had heritability estimates differing from zero at one or more time points (0.21 to 0.45). The traits with significant heritability would be good candidates for use as biomarkers in a selection program if they are correlated with traits of economic importance that are more difficult to measure. QTL were identified for nine of the traits at one or more time point. These nine traits, however, did not have significant heritability estimates suggesting that while some QTL have been identified their effects are generally small.

## Keywords

chronic heat stress, acute heat stress, i-STAT, GWAS, laying hen

## Disciplines

Agriculture | Animal Sciences | Genetics | Poultry or Avian Science

## Comments

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# Venous blood gas and chemistry components are moderately heritable in commercial white egg-laying hens under acute or chronic heat exposure

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**ABSTRACT** Heat stress has a large negative impact on poultry around the world in both intensive and small-scale production systems. Better understanding of genetic factors contributing to response to high ambient temperatures would provide a basis to develop strategies for alleviating negative impacts of heat on poultry production. The objective of this work was to characterize the genetic control (heritability estimate and quantitative trait loci (QTL)) of blood chemistry components before and after exposure to acute and chronic high ambient temperature in a commercial egg laying line Hy-Line W-36 female parent line mature hens were exposed to 4 wk of daily cyclic heat exposure. Blood was collected pre-heat, on the first day of heat, and 2 and 4 wk post heat initiation and analyzed immediately using an i-STAT<sup>®</sup> hand-held blood analyzer. Thirteen blood components were quantified at the 4 time points: pH, pCO<sub>2</sub>, pO<sub>2</sub>, HCO<sub>3</sub>, TCO<sub>2</sub>, sO<sub>2</sub>, iCa, Na, K, base excess, glucose, “hematocrit”

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**Key words:** chronic heat stress, acute heat stress, i-STAT<sup>®</sup>, GWAS, laying hen

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## INTRODUCTION

Heat stress has a large negative impact on poultry around the world in both intensive and small-scale production systems (Lara and Rostagno, 2013; Nyoni et al., 2018). It is well documented that heat stress results in decreased egg production (Daniel and Balnave, 1981; Deng et al., 2012), decreased egg quality (Bollengier-Lee et al., 1999; Franco-Jimenez et al., 2007), and eventual mortality (Wolc et al., 2018) in laying hens. A better understanding of physiological changes and iden-

tification of genetic factors contributing to response to high ambient temperatures would provide a basis for alleviating the negative impact of heat on poultry production by management or genetic selection strategies.

Hand-held blood analyzers, initially developed for human bedside diagnostics, provide a quick and convenient method for measuring multiple blood gas and chemistry components. This is an emerging technology for quantifying health and welfare status of poultry because of its accuracy, speed, and ease of use. Previously the i-STAT<sup>®</sup> device (Abbott Labs) has been used in other animal species (Tinkey et al., 2006; Harter et al., 2014; Rettenmund et al., 2014; Yildirim et al., 2015) and to establish reference ranges for broiler breeders and laying hens (Steinmetz et al., 2007; Martin et al., 2010; Schaal et al., 2015). Van Goor et al. was the first report using the i-STAT<sup>®</sup> blood analyzer to evaluate chickens under high ambient temperature (Van Goor et al., 2016).

The objectives of the current work was to characterize the genetic control of blood chemistry parameters

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**Table 1.** Heritability estimates and standard errors for 13 blood gas and chemistry components across 4 time points. Bold indicates estimate differs from 0.

Trait	pre	5 h	2 wk	4 wk
Base Excess (BE)	0.01 (0.09)	<b>0.21 (0.10)</b>	0.17 (0.11)	<b>0.26 (0.12)</b>
Glucose (Glu)	0.08 (0.11)	NC <sup>1</sup>	<b>0.37 (0.12)</b>	0.10 (0.10)
Hemoglobin (Hb)	<b>0.44 (0.11)</b>	0.04 (0.09)	0.22 (0.11)	<b>0.31 (0.11)</b>
Bicarbonate (HCO <sub>3</sub> )	0.01 (0.09)	0.19 (0.10)	<b>0.29 (0.12)</b>	<b>0.42 (0.12)</b>
Hematocrit (Hct)	<b>0.45 (0.11)</b>	0.05 (0.09)	0.20 (0.11)	<b>0.32 (0.11)</b>
Ionized calcium (iCa)	0.01 (0.09)	NC	0.13 (0.11)	0.05 (0.10)
Potassium (K)	0.04 (0.10)	0.07 (0.10)	0.06 (0.09)	<b>0.29 (0.12)</b>
Sodium (Na)	NC	NC	0.12 (0.11)	0.003 (0.08)
Partial pressure of CO <sub>2</sub> (PCO <sub>2</sub> )	0.03 (0.09)	0.19 (0.11)	NC	<b>0.26 (0.11)</b>
pH	0.05 (0.09)	0.09 (0.10)	0.001 (0.08)	0.12 (0.11)
Partial pressure of O <sub>2</sub> (PO <sub>2</sub> )	0.02 (0.08)	NC	NC	0.01 (0.09)
Oxygen saturation (sO <sub>2</sub> )	0.05 (0.09)	0.05 (0.10)	NC	0.02 (0.09)
Total carbon dioxide (TCO <sub>2</sub> )	NC	0.13 (0.10)	<b>0.34 (0.12)</b>	<b>0.33 (0.12)</b>

<sup>1</sup>Does not converge

before and after exposure to acute and chronic high ambient temperature in mature hens of a commercial egg laying line. Blood components measured rapidly with a pen-side device would be ideal phenotypes to use as biomarkers for genetic improvement. This is the first report of pen-side blood component genetic analysis in a commercial egg laying line under heat stress.

## MATERIALS AND METHODS

### Animals, Husbandry, and Heat Treatment

Hy-Line W-36 female parent line chicks were reared at Hy-Line International (Dallas Center, IA). At 18 wk of age, 400 pullets were transferred to Virginia Tech (Blacksburg, VA) and placed in individual cages for a 6-wk acclimation period within a climate-controlled room that was maintained at approximately 23°C. Heat treatment began at 24 wk of age, lasting 4 wk and continued until 28 wk of age. The daily cyclic heat cycle, beginning at 9:00 a.m., was 7 h at 35°C and then 30°C for the remaining 17 h. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Virginia Tech (log # 14-093).

### Phenotypes

Blood was collected at four time points. Collection occurred one day prior to heat initiation, and at 4 to 6 h post heat initiation during the first day of heat exposure (hereafter referred to as the 5-h time point), and 2 and 4 wk post heat initiation. Blood was collected from the ulnar vein with syringe and needle, then immediately transferred to the chamber of an i-STAT<sup>®</sup> hand-held blood analyzer (Abbott Laboratories, San Diego, CA). Thirteen blood components were quantified using the CG8+ cartridge: pH, pCO<sub>2</sub>, pO<sub>2</sub>, HCO<sub>3</sub>, TCO<sub>2</sub>, sO<sub>2</sub>, iCa, Na, K, BE, glucose, “hematocrit”, and “hemoglobin”. The i-STAT<sup>®</sup> analyzer and cartridges are designed and optimized for use in human medicine. Because of this, there are some relevant concerns for the direct application of this technology to avian biology, as discussed in the results and discussion section.

Other phenotypes including egg production, egg weight, Haugh units, yolk weight, shell quality, feed intake, digestibility, and 6 more were also recorded pre and post heat exposure. These traits are discussed in a companion manuscript to be submitted to *Genomics Selection and Evolution*.

### Genotyping and Quality Control

Genomic DNA was isolated by GeneSeek, Neogen Genomics (Lincoln, NE, United States) from whole blood collected at 17 wk of age. Genotyping was done using the Axiom Chicken 600k Genotyping Array (Kranis et al., 2013) and genotyping array annotation files were based on galGal genome version 5.0 (Thermo Fisher Scientific). Quality filtering requirements included: CR  $\geq 95$ , MinorAlleleFrequency  $\geq 0.01$ , FLD  $\geq 4$ , HomRO  $\geq -0.6$ , BB.varX  $\leq 0.9$ , BB.varY  $\leq 0.45$ , AB.varX  $\leq 0.55$ , AB.varY  $\leq 0.5$ , AA.varX  $\leq 0.6$ , HomFLD  $\geq 9$ , HetSO  $\geq -0.2$ , ConversionType  $\neq$  “OTV”. Quality metrics are described in the Axiom Analysis Suite User Guide obtained from Thermo Fisher Scientific (Applied Biosystems, 2017). After filtering, 261,509 SNPs and 374 animals remained for analysis.

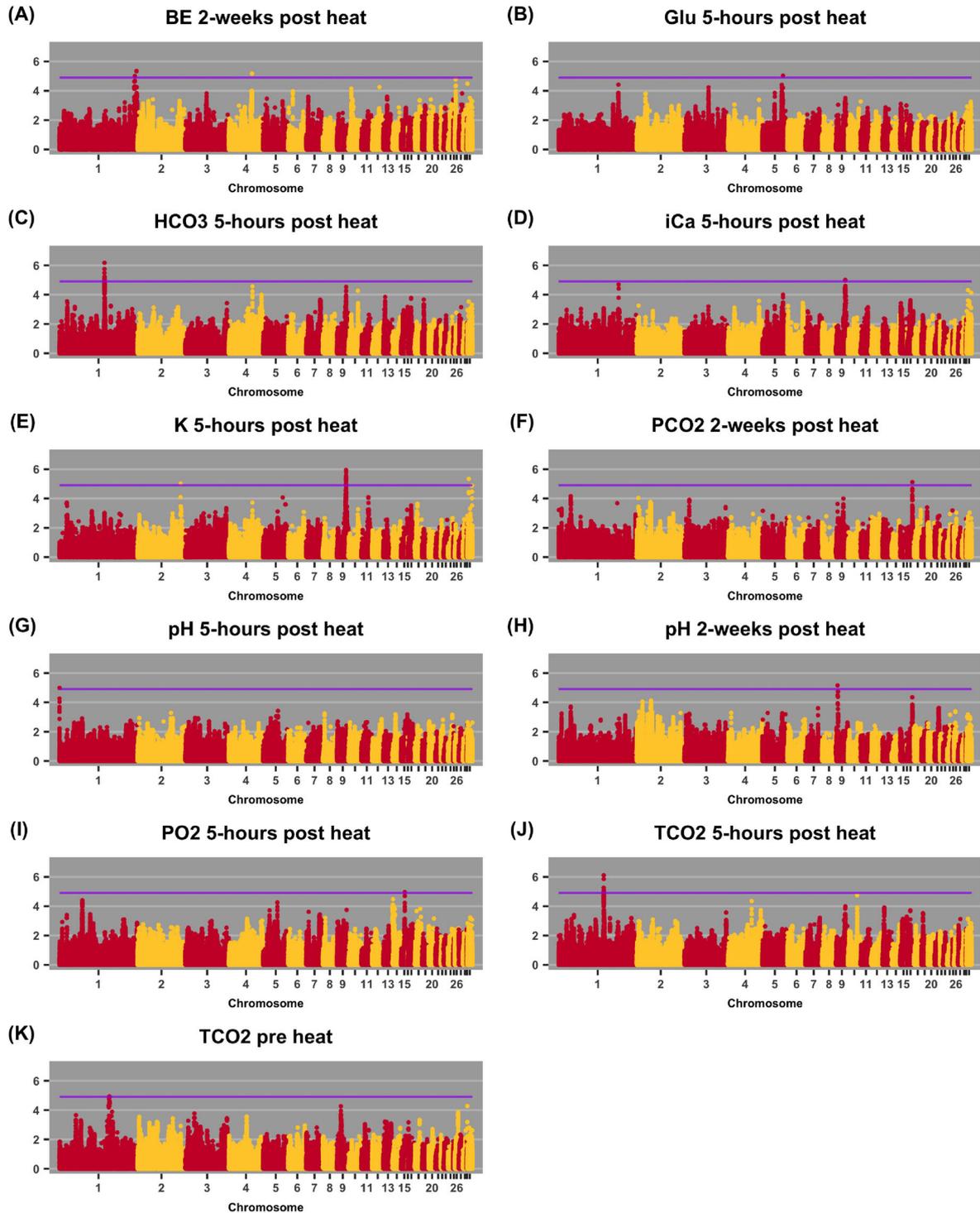
### Data analysis

Heritabilities and variance components were estimated using ASReml 4.0 (Gilmour et al., 2015) with a univariate animal model:

$$Y_{ij} = \mu + FE_i + A_j + e_{ij}$$

Y is the dependent variable of phenotype. Fixed effect was cage row within room (FE<sub>i</sub>). Animal genetic effects (A<sub>j</sub>) with a genomic relationship matrix computed from SNP genotypes, as described by (VanRaden, 2008), and residuals (e<sub>ij</sub>) were the two random effects.

Quantitative trait loci (QTL) identification was performed using a hierarchical generalized linear model (Rönnegård et al., 2010), with the same effects



**Figure 1.** Manhattan plots for traits with significant QTL. The purple line indicates a 20% genome wide threshold of  $1.2 \times 10^{-5}$ . The y-axis represents the  $-\log_{10}(P\text{-value})$  of SNP association.

described for heritability estimation, in R—GenABEL (Aulchenko, 2015). The association analysis method used in GenABEL, *polygenic.hglm*, and *mmscore*, functions similarly to the FASTA method for related individuals described by (Chen and Abecasis, 2007). A modified Bonferroni multiple test correction was used to determine the number of independent tests, as previously described (Rowland et al., 2018). The number of independent tests was determined to be 16,085

and a 20% genomewide threshold was calculated to be  $1.2 \times 10^{-5}$ .

## RESULTS AND DISCUSSION

### Use of *i-STAT*<sup>®</sup> Technology in Chickens

The data generated by *i-STAT*<sup>®</sup> technology has not generally been validated against “gold standard”

**Table 2.** Details of significant QTL: position, *P*-value of association, and number of SNPs in the QTL.

Trait	Time	Position (Chr:Mb)	<i>P</i> -value	Number of SNPs
TCO <sub>2</sub>	pre heat	1:129	$1.19 \times 10^{-5}$	4
	5 h	1:116	$7.73 \times 10^{-7}$	8
PO <sub>2</sub>	5 h	15:7.5	$1.10 \times 10^{-5}$	2
pH	2 wk	9:3.6	$6.91 \times 10^{-6}$	1
	5 h	1:0.2	$1.02 \times 10^{-5}$	2
pCO <sub>2</sub>	2 wk	17:8	$7.62 \times 10^{-6}$	1
iCa	5 h	9:19	$9.79 \times 10^{-6}$	3
HCO <sub>3</sub>	5 h	1:116	$6.71 \times 10^{-7}$	12
Glu	5 h	5:49	$9.25 \times 10^{-6}$	1
BE	2 wk	1:193	$4.53 \times 10^{-6}$	5
		4:63	$6.73 \times 10^{-6}$	1
K	5 h	9:19	$1.14 \times 10^{-6}$	71
		2:137	$9.60 \times 10^{-6}$	1

techniques for measuring blood chemistry and other components in chickens. Of special concern about using the i-STAT<sup>®</sup> for measuring blood chemistry components in chickens are the values reported for “hematocrit” and “hemoglobin”. Because the structure of chicken erythrocytes (elongated and nucleated) differs greatly from that of most mammalian cells, the blood electrical conductance assay and the algorithms used by the i-STAT<sup>®</sup> to generate “hematocrit” and “hemoglobin” values from chicken blood samples may not be equivalent to the standard packed-cell volume method of measuring hematocrit. However, even though the i-STAT<sup>®</sup>-generated numbers for “hematocrit” and “hemoglobin” do not represent the same biologically relevant measurements as reported from standard assays, they do represent heritable measurements as shown in Table 1 and therefore are of potential value in a selection program.

An additional consideration for this study is that the measurements of the blood chemistries were done on venous blood. Composition of venous and arterial blood differ greatly, and blood gas and pH balances are regulated at a systemic level by the arterial, not venous, blood composition (Wideman et al., 2003). For the purposes of the current study, however, the researchers chose to draw venous blood, which presents fewer difficulties in staunching blood leakage from the site, an important consideration in testing an assay for potential application in the field. Also, other reports on use of the iSTAT in chickens used venous blood (Schaal et al., 2015; Van Goor et al., 2016). Thus, there was interest in determining the genetics of venous blood chemistries.

## Heritability

Eight of the blood parameters had heritability estimates differing from zero (defined as an estimate greater than two times the standard error) at one or more time point (0.21 to 0.45, Table 1). The traits with significant heritability would be good candidates for use as indicator traits in a selection program if they are correlated with traits of economic importance that may be more difficult to measure.

In the first and only other report of  $h^2$  for traits measured using an i-STAT<sup>®</sup> device, Van Goor, et al. measured blood parameters in a heat stressed research chicken line (Van Goor et al., 2016). From pre and post heat exposure measurements, only one parameter, HCO<sub>3</sub> post heat, was found to have non-zero heritability in both studies. The estimates were similar between the two studies, 0.23 reported by Van Goor, et al. and 0.29 reported here. Closter et al. reported  $h^2$  for HCO<sub>3</sub> to be 0.19 in cold-stressed broilers (Closter et al., 2009). All 3 studies report moderate  $h^2$  for HCO<sub>3</sub> in different lines and under different environmental conditions. Closter et al. used the GEM Premier 3000 system to quantify pH, *p*vCO<sub>2</sub>, *p*vO<sub>2</sub>, HCO<sub>3</sub>, TCO<sub>2</sub>, and sO<sub>2</sub> in cold stressed broilers at 22 d of age. The GEM Premier is a comparable technology to the i-STAT<sup>®</sup> system. Closter, et al. additionally estimated significant  $h^2$  for pCO<sub>2</sub> and TCO<sub>2</sub>. Our estimate of pCO<sub>2</sub> after 4 wk of heat exposure was 0.26, higher than that of Closter, et al. in cold stressed broilers (0.15). Our  $h^2$  estimates of TCO<sub>2</sub> (0.34 after 2 wk of heat and 0.33 after 4 wk of heat) were also higher than that of Closter et al. (0.19).

## QTL Associations

QTL were identified for nine of the blood components at one or more time point (Figure 1, Table 2), although it is important to add a word of caution—these 9 traits did not have significant heritability estimates. Heritability not differing from 0 indicates a lack of detectable genetic control due to insufficient statistical power or a lack of genetic variation overall in the trait. Therefore, identification of QTL for a trait without significant  $h^2$  suggests that some genomic regions are significant but overall the effects are very small. Many association studies are reported without presenting the estimated heritability of analyzed traits, so interpretation of the reported QTL should be guarded. Heritability estimation is an important first step for genetic/genomic studies. Determining the existence of significant total genetic variation to phenotypic variability indicates that genes are involved in the phenotypic expression and therefore can be interrogated by, for example, association analyses. Reports of QTL in studies that do not present evidence of significant  $h^2$  should be viewed with caution, as while individual genomic regions may be identified the effect size is likely small.

In summary, all 13 parameters exhibited a significant change due to high ambient temperature exposure at one or more time point. Eight of the blood parameters (base excess, glucose, hemoglobin, HCO<sub>3</sub>, hematocrit, K, pCO<sub>2</sub>, TCO<sub>2</sub>) had heritability estimates differing from zero at one or more time point (0.21 to 0.45). The traits with significant heritability would be good candidates for use as indicator traits in a selection program if they are correlated with traits of economic importance

that may be more difficult to measure. QTL were identified for nine of the traits at one or more time point, however, these nine traits did not have significant heritability estimates.

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