

QTL for body composition traits during heat stress revealed in an advanced intercross line of chickens

A. Bjorkquist¹, C. Ashwell², M. Persia¹, M. F. Rothschild¹, C. Schmidt³ and S. J. Lamont¹

¹Iowa State University, ²North Carolina State University, ³University of Delaware

ABSTRACT: Breeding chickens for heat tolerance is desirable. The F18 and F19 generations of a broiler by Fayoumi advanced intercross line (AIL) were used to facilitate fine mapping of QTL. Three major phenotype collection phases were: pre-heat, acute heat, and chronic heat, with birds being exposed to daily heat cycles from 22 to 28 days of age. Body temperature was measured at all three phases; body weight at pre-heat and chronic heat phases. Breast muscle yield (as percentage of body weight) was measured after necropsy at d 28. Feed digestibility was assayed from ileal content collected at d 28. Birds were genotyped using a 600K Affymetrix chicken SNP array. Using Genome Wide Association Studies (GWAS) significant QTL were found for each of the measured traits, indicating the feasibility of improving performance under hot conditions by genomic selection. This is the first GWAS using this novel AIL of chickens under heat stress.

Keywords: chickens; heat stress; GWAS

Introduction

The modern broiler chicken has been bred for increased muscle mass. Heat stress causes an increase in body temperature, a decrease in growth rate, and increased mortality. Poultry production will likely continue to expand in geographical regions with hot climates. Heat stress causes an estimated annual economic loss of \$125-165 million in the U.S. poultry industry alone (St-Pierre et al. (2003)).

The objective of our study was to identify QTL for body temperature, body composition traits, and digestibility in a chicken AIL using Genome Wide Association Studies (GWAS). Our experimental design gives insight into the genomic regions associated with response to heat stress in chickens. This information may be used for breeding birds more resilient to heat stress in the changing climate.

An advanced intercross line (AIL) was created from diverse genetic lines of chickens. The AIL is a unique resource population that has undergone many rounds of recombination, facilitating fine mapping of QTL. The F18 and F19 AIL generations were used in this study. Body weight, body temperature, breast weight, and digestibility under heat stress were traits used for GWAS. This is the first study in chicken to estimate heritabilities and perform a GWAS of these traits during heat stress in the chicken. Significant QTL were identified for all traits.

Materials and Methods

Animals. The broiler line was previously bred for body weight while the Fayoumi line was imported to the United States from Egypt because of reported resistance to avian leucosis. One broiler sire was mated to 6 highly inbred (>50 generations sib-mating) Fayoumi hens. This created the founder line for the AIL. The animals used in this study are from the F18 and F19 generations.

Experimental design. Birds were raised in four replicates in floor pens under standard feed and rearing conditions. At 17 days of age, the birds were transferred to six environmental chambers per replicate, and acclimated for 3 days. From day 22 to 28 of age, the chambers heated to 35°C for 7 hours per day. The DNA isolated from 468 AIL, 6 broiler, and 6 Fayoumi chickens was genotyped on the Affymetrix 600K chicken SNP axiom array.

Phenotypic traits. Body weight (g) was measured at day 7, 14, 21, and 28 of age. Body temperature (°C) was measured rectally on day 20, 22, 28 of age. Breast yield was calculated by dividing the pectoralis muscle weight by the total body weight on day 28 of age, and multiplying by 100. Digestibility was calculated by:

$$\frac{\text{Avg DM}\% - \text{DM}\% * \left(\frac{\text{Avg Ti}\%}{\text{Ti}\%}\right)}{\text{Avg DM}\%}$$

where Avg DM% is the average dry matter percent of the ileal content collected at necropsy at day 28 across all AIL in the study, DM% is an average of 3 samples of dry matter for a particular animal, Avg Ti% is the average amount of a titanium marker in the ileal content across all AIL in the study, and Ti% is an average of 3 individual measurements and is a percentage of a titanium marker in samples of ileal content from an individual animal.

Statistical analyses. Parameters for inclusion of SNP genotypes in analysis included SNP call rate $\geq 95\%$ and minor allele frequency $\geq 5\%$.

Testing for phenotypic correlations, normal distribution of phenotypic traits, and fixed effects for each trait, and calculation of heritabilities were done using JMP statistical software (JMP® (2012)). Sire heritabilities were estimated using the JMP EMS (expected mean square) traditional ANOVA method. Fixed effects for each trait were

determined based on ANOVA estimates with significant terms included as fixed effects with a P value ≤ 0.05 (JMP® (2012)).

The GWAS of phenotypic traits with SNP genotypes was done using GenSel software (Fernando and Garrick (2012)). Bayes B, which fits all SNPs simultaneously as random effects, was used for the analysis. The mixed model used for the GWAS:

$$y = Xb + \sum_j^k z_j \alpha_j \delta_j + \varepsilon$$

Where y = vector form of phenotypes, X = incidence matrix to account for fixed effects on phenotypes, b = vector of fixed effects, z_j = vector of genotypes for SNP j based on the number of B alleles (0,1, or 2), or the average of the genotypes at SNP j , α_j = allele substitution effect for SNP j , δ_j = whether SNP j was included in the Markov chain Monte Carlo (MCMC) chain, and ε is the error associated with the analysis.

The SNPs were grouped into ~1000 non-overlapping 1 Mb windows across the genome. Analyses were run on all SNPs. 41,000 MCMC iterations were run for each analysis and the first 1000 iterations were discarded. δ_j was set so that $\pi = 0.9978$.

Results

All 6 broilers and 6 Fayoumis and 456 of the 468 AIL that were genotyped, passed the sample QC. A total of 210,117 SNPs had a minor allele frequency $\geq 5\%$ and were used for subsequent analyses.

Phenotypic means are listed in Table 1. It is interesting to note that the change in body temperature between day 22 and day 20, as well as day 28 and day 20, was not statistically different than zero.

Table 1. Phenotypic mean with standard deviation (mean(STDev)), heritability (h^2), largest percent of genetic variation (%GV) explained, and chromosome where %GV was revealed for traits measured in an advanced intercross line during pre-heat, acute-heat, and chronic-heat phases, and their changes

Trait [‡]	Mean (STDev)	h^2	%GV	Chromosome
BW d7	64.7 (8.08)	4%	3.5%	7
BW d14	140.4 (18.34)	22%	3.5%	7
BW d21	253.6 (34.24)	25%	1.48%	2
BW d28	402.5 (55.68)	36%	2.27%	6
Change in	149.2	21%	1.79%	6

BW d28-21	(33.91)			
BT d20	42.33 (0.30)	27%	0.96%	14
BT d22	42.44 (0.37)	17%	0.56%	15
Change in BT d22-20	0.09 (0.44)	6%	0.44%	11
BT d28	42.28 (0.31)	20%	1.89%	15
Change in BT d28-20	-0.016 (0.41)	10%	0.41%	22
Breast weight	4.41 (3.62)	19%	8.6%	1
Digestibility	4.52 (0.05)	12%	0.59%	21

[‡]BW: Body weight measured in grams at days 7, 14, 21, 28 and the change between day 28 and day 21

BT: Body temperature measured in °C at days 20, 22, 28, the changes between day 22 and 20, and the change between day 28 and day 20

Breast weight: measured in grams at day 28 and expressed as a percentage of body weight at day 28

Digestibility: Measured as the log of digestibility and expressed as a percentage

Many 1 Mb SNP windows explained a large percentage of genetic variation (Table 1). The highest amount was for breast weight percentage with 8.6% of genetic variation explained by a 1 Mb window. Many genetic regions were confirmed as supporting the locations in the chickenQTLdb (Hu et al. (2013)).

The largest percent of genetic variation explained by one MB window for each trait and the QTL chromosomal location is located in Table 1.

Discussion

The use of this unique resource population of chickens and a high density SNP genotyping array allows for fine mapping of QTL. Most estimated heritabilities were comparable to those found in literature. Body weight at day 7, however, was much lower than expected at 4% heritability. This may be due to calculating the heritability based on the sire variance component and large maternal effects on early body size. Body weights from day 14-28 were moderately heritable, ranging from 22-36%. The body weight gain during heat stress (day 28-21) is a novel phenotypic trait and was moderately heritable at 21%.

Our heritability estimation for body temperature is larger than that estimated for a broiler line, 5% (el-Gendy and Washburn (1995)). Body temperature pre-heat (day 20) was moderately heritable at 27%. The heritability of body temperature under acute and chronic heat conditions were 17% and 20%, respectively. This suggests greater influence of genetics on body temperature when birds are exposed to heat for a longer period of time. The heritability for change from pre-heat body temperature during acute and chronic heat was low at 6% and 10% respectively. This suggests absolute body temperature is under greater genetic control

than the heat-induced change in body temperature. The differential change in body temperature could be used as a factor for breeding more heat tolerant birds. Heat-tolerant birds would be able to resist increases in body temperature during heat stress. The low heritabilities of temperature change suggest that it will be challenging to genetically select for resistance to body temperature change.

Breast weight percentage was moderately heritable at 19%. This is similar to a study estimating percent breast weight under non-heat conditions in broilers at 24% (Gaya et al. (2006)). The study of digestibility under heat challenge is a novel trait. We estimated a moderate heritability of 12% for the log percent digestibility.

Breast yield is a major economic trait in broilers. Significant QTL were found for breast yield before and during heat stress using our AIL. The largest QTL revealed was for breast weight which explained 8.6% of the genetic variance.

Body composition traits were measured on an AIL during pre-heat, acute-heat, and chronic-heat phases. QTL were revealed that may be useful for breeding chickens that are more robust to heat stress.

Acknowledgments

The authors thank the Iowa State University Poultry Research Center staff for animal care; members of the coPDs' labs for technical assistance throughout the animal experiments and sample collection; Jack Dekkers, and Dorian Garrick and their labs for GWAS advice. This research was funded by: USDA-NIFA-AFRI Climate Change Award, #2011-67003-30228 and AB is a USDA National Needs Fellow, Award #2011-38420-20050. Support also comes from the Hatch and State of Iowa Funds.

References

- Chen X., Wei P., Xu S., et al. (2013). *Anim. Sci. J.* Nov;84(11):737-9.
- Dikmen S., Cole J., Null D., et al. (2013). *PLoS One.* Jul 23;8(7): el-Gendy, E., Washburn, K. (1995). *Poult. Sci.* Feb;74(2):225-30.
- Fernando R. and Garrick D. 2009 2nd edition *GenSel.* Iowa State University.
<http://big.ansci.iastate.edu/bigsgui/help.html/> Accessed on February 10, 2014.
- Gaya, L., Ferraz, J., Rezende, F., et al. (2006). *Poult. Sci.* May;85(5):837-43.
- Hu, Z., Park, C., Wu, X., Reecy, J. (2013). *Nucleic Acids Res.* 2013 Jan;41(Database issue). Epub 24 Nov. 2012.
- JMP®, Version 10.0.0. (2012). SAS Institute Inc., Cary, NC, 1989-2007.
- St-Pierre, N., Cobanov, B., Schnitkey, G. (2003). *J. Dairy Sci.* Vol. 86 Supp. E52-E77.