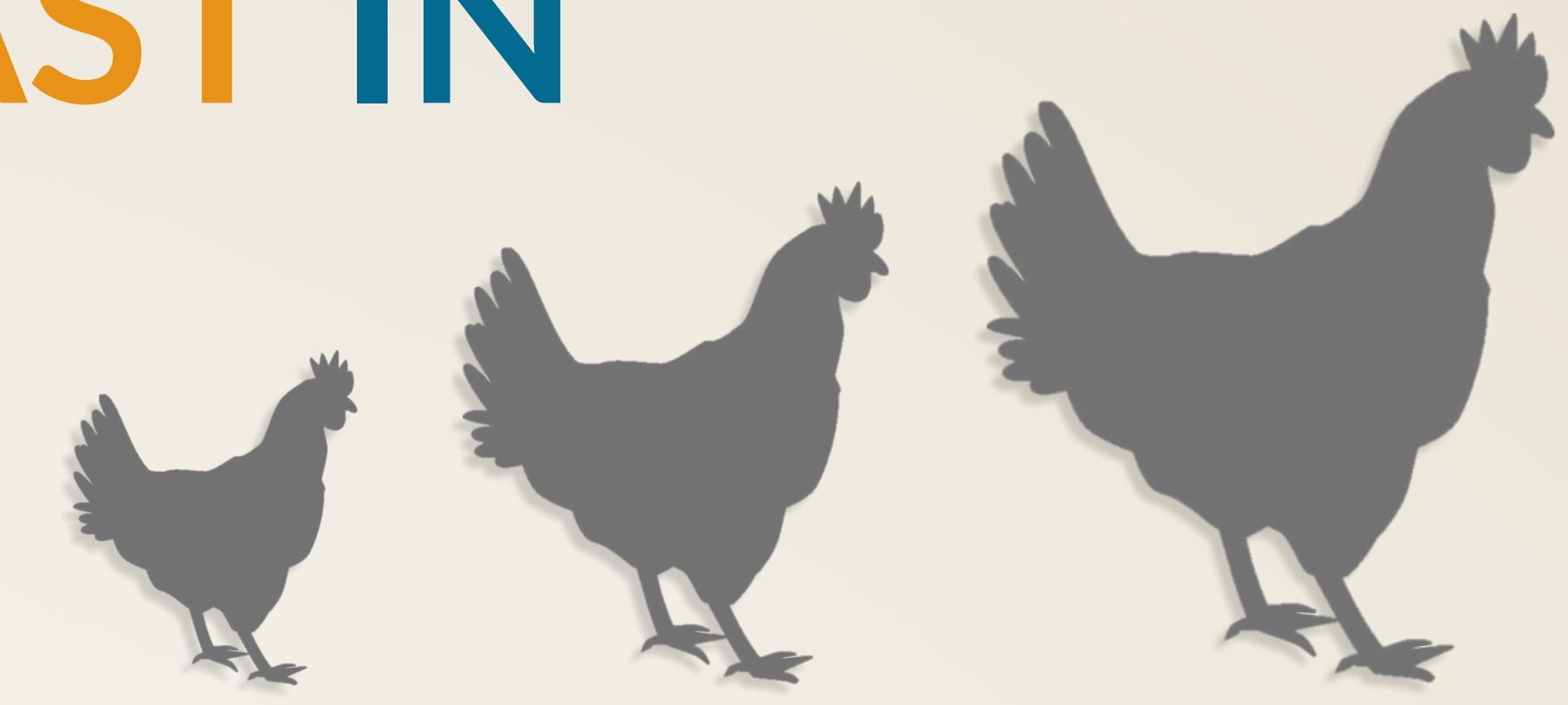


GENOME WIDE IDENTIFICATION AND FUNCTIONAL VALIDATION OF GENES CAUSING SUSCEPTIBILITY TO WOODEN BREAST IN COMMERCIAL BROILER CHICKENS



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BACKGROUND

Wooden Breast or "woody breast" is one of several muscle disorders of commercial broiler chickens that have manifested alongside intense genetic selection for production traits such as high muscle yield, rapid growth, and high feed efficiency. It was first described in the literature in 2014, but was seen in production chicken houses prior to that time. This project aims to elucidate the genetic basis of Wooden Breast and consequently to provide greater insight into its currently unknown etiology.

DISEASE EFFECTS

The characteristic and often diagnostic symptom of Wooden Breast is a palpably hard breast muscle caused by myodegeneration and necrosis of the pectoralis major accompanied by phlebitis (inflammation of veins), fibrosis and the accumulation of interstitial connective tissue. Affected breast muscles often also exhibit subcutaneous and fascial edema, petechial hemorrhages, as can be seen in the figure below (Papah *et al.* 2017).

At a molecular level, the disease is associated with hypoxia, oxidative stress, higher intracellular calcium build-up, and altered carbohydrate metabolism.



ECONOMIC IMPACT

Roughly 10% of all commercial broilers show severe effects of Wooden Breast, and individual flocks have reported incidence rates as high as 50%. The substantial economic impact of the disease stems from its effect on meat quality. Severely affected breast muscles cannot be sold as fillets and are either rendered or downgraded for use in lower-value products such as mechanically separated chicken, soups, or lunchmeat. For premium chicken brands, Wooden Breast is a major concern in terms of customer satisfaction and retention.

PROGRESS

Nearly 2000 chickens were raised to 7 weeks of age and the following data was collected:

- Wooden breast score
- Blood chemistry (i-STAT)
- Body weight at 13 days and 7 weeks
- Tissue weights (pectoralis major, pectoralis minor, wing, external oblique, heart, lungs, liver, spleen)
- Back-to-back wing contact
- Sex

Phenotypic data analysis was performed to explore the relationships among disease state, body weight, tissue weights, sex, and blood chemistry.

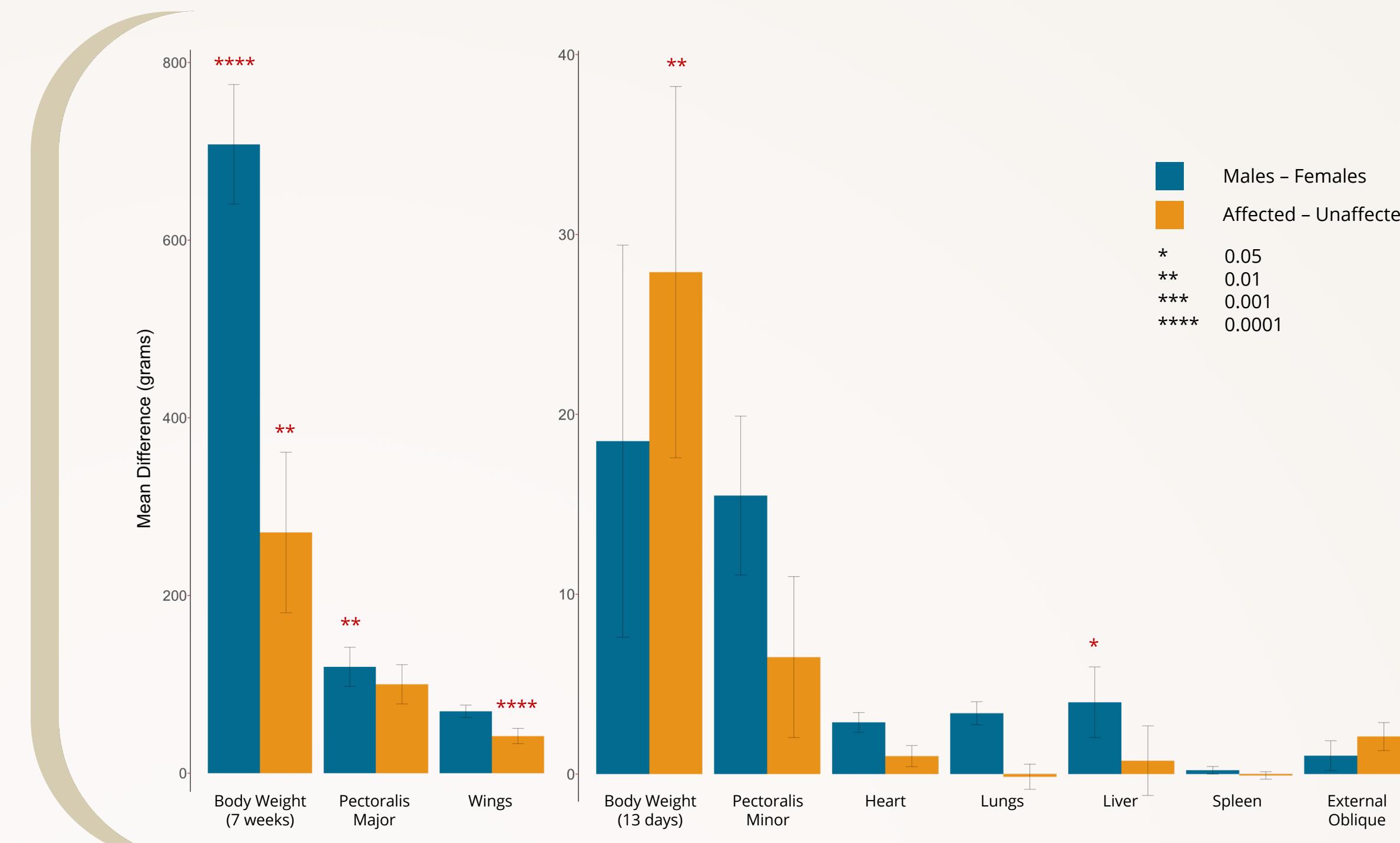
Preliminary research was conducted to determine the best method for genotyping the approximately 1200 chickens that will be used for the genome wide association study.

REFERENCES

- Michael B. Papah, Erin M. Brannick, Carl J. Schmidt & Behnam Abasht (2017): Evidence and role of phlebitis and lipid infiltration in the onset and pathogenesis of Wooden Breast Disease in modern broiler chickens, Avian Pathology, DOI: 10.1080/03079457.2017.1339346

INITIAL FINDINGS

Preliminary results of the phenotypic data analysis support the connection between body weight and Wooden Breast score (0-3 scale with 0 have no signs of Wooden Breast and 3 being severe). However, there is less striking support for the belief that males are more susceptible after controlling for body weight, at least for the range of body weights that could be reasonably compared in this dataset. As might be expected, the pectoralis major muscle is generally heavier in affected birds, but it is not yet clear why the wings are also heavier in affected birds after controlling for overall body weight.



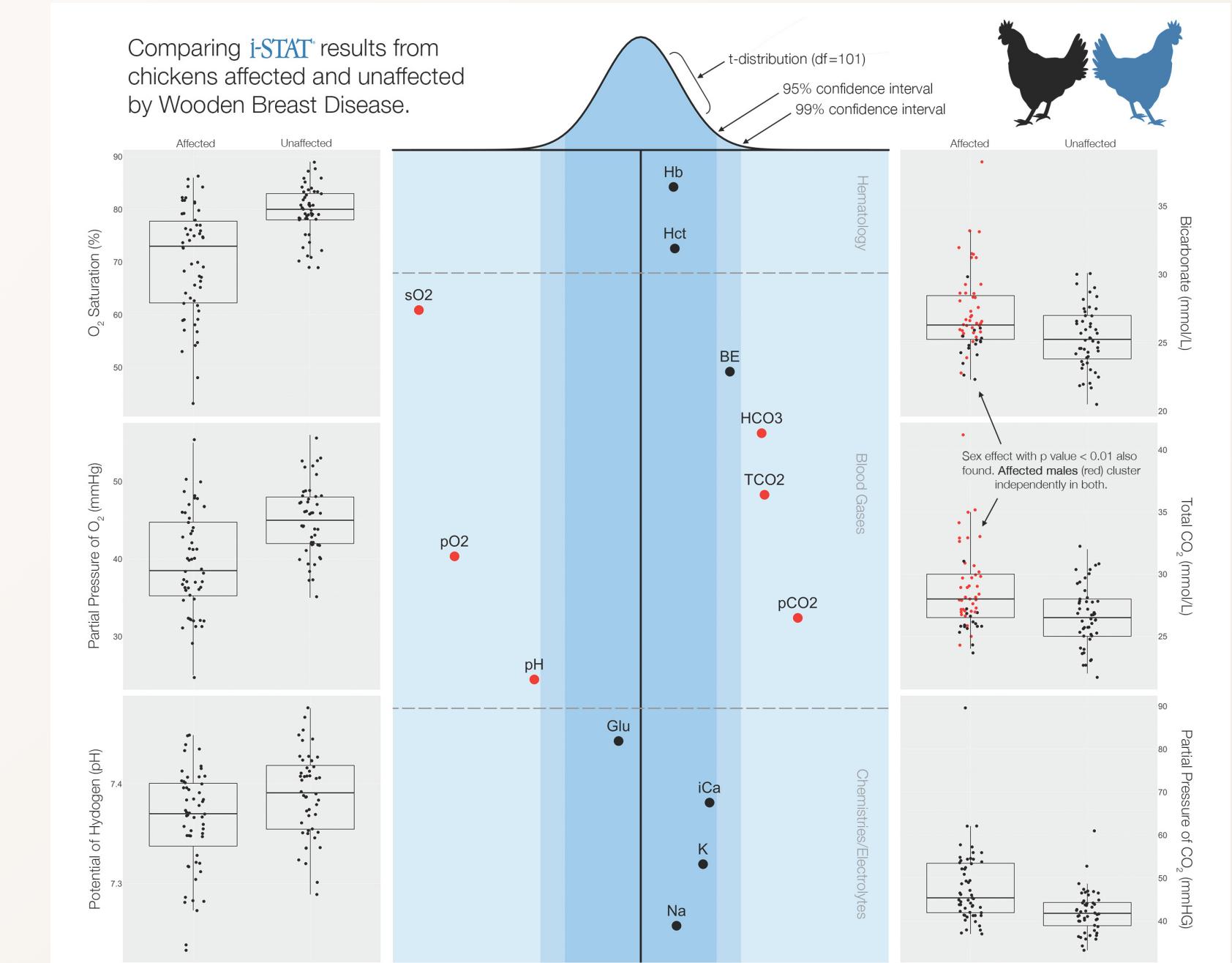
BODY & TISSUE WEIGHTS

- Body weight at 7 weeks of age is **higher** in affected birds even after controlling for sex
- Weight of the pectoralis major and wing are **higher** in affected birds after controlling for overall body weight

There is no published data on the differences in blood chemistry between affected and unaffected birds. The i-STAT handheld blood analyzer was used to measure hemoglobin (Hb), hematocrit (Hct), O₂ saturation (sO₂), base excess (BE), bicarbonate (HCO₃), total CO₂ (TCO₂), partial pressure of O₂ (pO₂), partial pressure of CO₂ (pCO₂), pH, glucose (Glu), ionized calcium (iCa), potassium (K), and sodium (Na). Six of these measurements (see below) showed a significant difference between affected and unaffected birds with a p-value of 0.01. These results are consistent with previously published reports that Wooden Breast is associated with hypoxia and oxidative stress.

BLOOD CHEMISTRY WITH i-STAT

- O₂ saturation, partial pressure of O₂ and pH are **lower** in affected birds
- Bicarbonate, total CO₂, and partial pressure of CO₂ are **higher** in affected birds
- Bicarbonate and total CO₂ also show a sex effect with affected males clustering independently, higher than all other groups



FUTURE WORK

Isolate DNA from blood samples of approximately 1200 birds and send out for genotyping-by-sequencing (GBS), which will return roughly 500K SNPs at 2-3X coverage.

Estimate the heritability of Wooden Breast using genomic and phenotypic data.

Conduct a genome wide association analysis and identify candidate genes.

- Use both Wooden Breast state (affected/unaffected) and score (0-3) as phenotype
- Integrate gene expression and metabolomics data

Determine the effects of candidate genes on pectoralis major muscle satellite cell function by manipulation of gene expression.

ACKNOWLEDGEMENTS

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