Increasing the value of Florida beef through genomics

Raluca Mateescu, Mauricio Elzo and Dwain Johnson
Department of Animal Sciences, University of Florida

FINDINGS

The objective of this research was to genotype 800 animals from a resource multibreed population (cattle spanning the range from 100% Angus to 100% Brahman) developed at the University of Florida Beef Research Unit (BRU) to generate a training and discovery population of adequate size for genomic studies. One important benefit provided by UF population is the availability of phenotypes for several economically important, but difficult and costly to measure traits describing meat quality. Warner-Bratzler shear force (WBSF) as an objective measure of tenderness and several palatability phenotypes were the focus for this preliminary study.

1) The availability of high-density genotypes on this multibreed population allowed us to develop methods to predict breed composition. Knowledge of breed composition can be useful in multiple aspects of cattle production, and can be critical for analyzing the results of whole genome-wide association studies. We examined the feasibility and accuracy of using genotype data to estimate breed composition and subsequently identify the minimum number of markers needed to determine breed composition with adequate accuracy. Our results show that SNPs could be effectively used to estimate breed composition of individual animals. This technology can be very useful in situations where pedigree is unknown (e.g., multiple-sire natural service matings, non-source-verified animals in feedlots or at slaughter). Further, this approach can be used to determine the breed composition of an animal more precisely than the value obtained using the breed composition of its parents based on pedigree. Accurate knowledge of breed composition, including identification and adjustment of breed-based population stratification, is critical when performing genome-wide association studies or assessing the predictability of genomic evaluations in target populations different from training.

2) The high-density genotypes were used in a genome-wide association study and identified several regions throughout the genome associated with tenderness measured by WBSF. Several genomic regions harboring these QTL coincided with those previously reported in Angus cattle genomic studies. However, it is important to note that many other QTLs and pathways where genes from these regions act appear to differ from those identified in taurine cattle for meat quality traits. These results will guide future QTL mapping studies and the development of models for the prediction of genetic merit to implement genomic selection and genomic management for meat quality in Brahman influenced cattle.

RESULTS

Phenotypic data

A phenotypic database has been created to include all animals from the UF multibreed Brahman-Angus population with meat quality data (Warner-Bratzler Shear force and sensory attributes). The phenotypic database includes:

- Pedigree information, including birth year, sex, age of dam, breed composition.
- Growth traits (birth, weaning, yearling, feedlot)
- Carcass traits (HCW, marbling score, REA, yield grade, quality grade)
- Meat quality traits (Warner-Bratzler shear force, tenderness, juiciness, flavor)

Means and standard deviations for several carcass and palatability traits stratified by breed composition are presented in **Table 1**. It is clear just by evaluating these averages that, as percentage Brahman increases, marbling decreases and WBSF increases indicating tougher meat. The sensory attributes as measured by testing panel also indicate a substantial decline in tenderness but relatively uniform juiciness and flavor across breed groups.

Table 1. Means and Standard deviation for carcass and palatability traits by breed groups

Traits	Calf breed composition (A=Angus, B=Brahman)			
WBSF	0.90**A** : 0.10**B**	0.67**A** : 0.33**B**	0.50**A** : 0.50**B**	0.33**A** : 0.67**B**
Marbling	460.8 (98.8)	436.1 (87.6)	393.5 (72.9)	358.6 (49.3)
Fat	0.56 (0.18)	0.54 (0.19)	0.54 (0.19)	0.47 (0.16)
REA	12.29 (1.43)	12.17 (1.58)	11.93 (1.44)	11.39 (1.16)
HCW	733.8 (94.8)	730.1 (91.2)	719.9 (93.5)	667.3 (64.8)
WBSF	4.17 (1.27)	4.05 (1.07)	4.50 (1.18)	4.62 (1.05)
Juiciness	5.41 (0.82)	5.41 (0.75)	5.39 (0.72)	5.36 (0.74)
Flavor	5.70 (0.47)	5.81 (0.47)	5.73 (0.42)	5.60 (0.44)
Tenderness	5.81 (0.73)	5.67 (0.76)	5.51 (0.86)	5.12 (0.92)
Connective	6.33 (0.75)	6.16 (0.82)	6.01 (0.91)	5.87 (0.95)

To disentangle tenderness from marbling, we estimated the effect of breed composition groups on WBSF correcting for marbling. The least square means of WBSF adjusted for marbling for the four breed composition groups were 4.29, 4.11, 4.45 and 4.46, respectively, with the last two groups significantly higher ($P < 0.05$) than the first two, confirming the general perception that inadequate tenderness is a problem in Brahman cattle even after adjusting for marbling. The second argument for addressing tenderness in Brahman-influenced cattle populations is the impact it has on the demand for beef. This is shown in **Table 2**, as the proportion of carcasses with tender meat decreases from 46.15% to 16.56% and proportion of carcasses with tough meat increases from 22.49% to 45.86% as the percentage of Brahman increase from 10% to 67%. The

Table 2. Number of observations, mean WBSF, and percentage of carcasses with tender meat (WBSF ≤ 3.3), average tenderness (3.3 < WBSF ≤ 4.2), and tough meat (WBSF > 4.2) for cattle with different breed composition in the UF multibreed population.

Trait	Calf breed composition (A=Angus, B=Brahman)			
WBSF	0.90**A** : 0.10**B**	0.67**A** : 0.33**B**	0.50**A** : 0.50**B**	0.33**A** : 0.67**B**
N. observations	169	333	279	314
Mean WBSF	3.70	3.88	4.03	4.33
% Tender (WBSF ≤ 3.3)	46.15%	35.14%	28.67%	16.56%
% Average (3.3 < WBSF ≤ 4.2)	31.36%	34.83%	35.13%	37.58%
% Tough (WBSF > 4.2)	22.49%	30.03%	36.20%	45.86%

project outlined in this proposal was designed to address these issues.

Breed Composition Prediction

We examined the feasibility and accuracy of using genotype data to estimate breed composition. Two different methods were used to predict breed composition: Principle Component Analysis (PCA) and model based clustering implemented in STRUCTURE software. The PCA generated 10 principle components with the top two explaining 45.63% and 43.69% of the variation in the 250K genotypes, respectively. Therefore, the top 2 principle components were used to then predict breed composition which is illustrated in **Figure 1**. Individuals deviating from the line are individuals for which expected breed composition predicted from pedigree deviates from genomic breed composition. Model based clustering analysis using STRUCTURE software generated a similar result, thus prediction of breed compositing using the two methods was almost identical. We subsequently identified the minimum number of markers needed to determine breed composition with adequate accuracy. It was clear that a relatively small number of markers (< 100) were needed (data not shown).

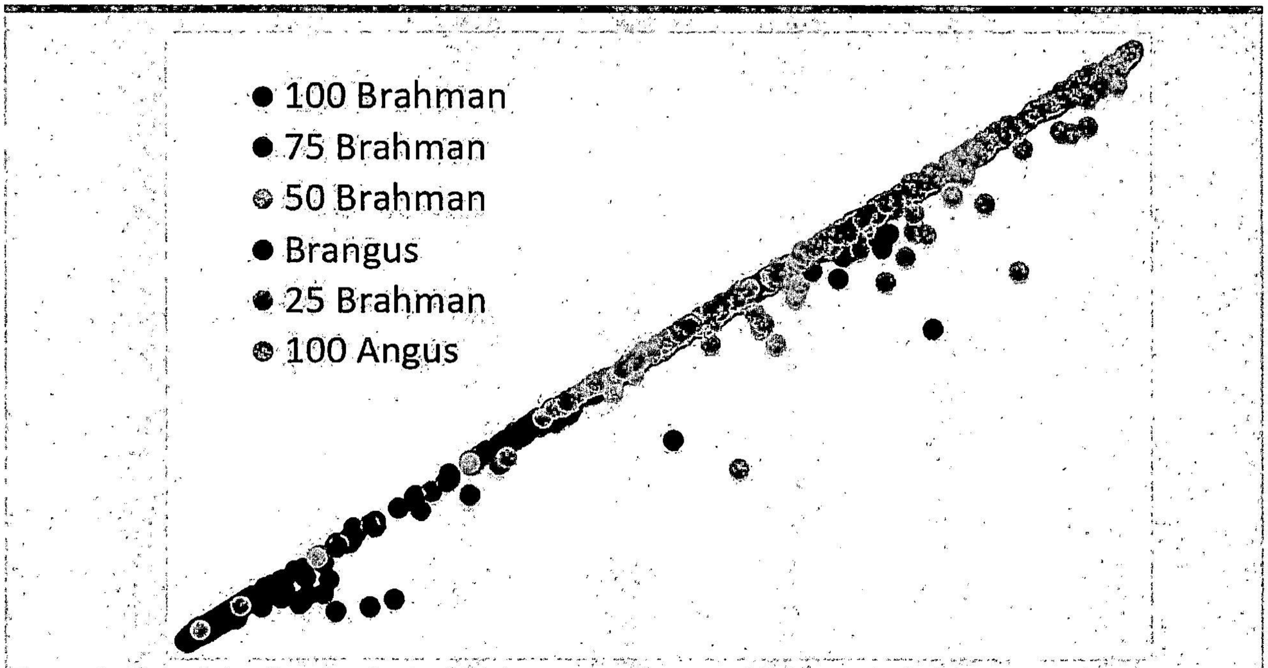

Figure 1. Plot of the first 2 principal components generated from a PCA analysis on 250K genotypes. The resulting plot was color-labeled with the known breed composition from our pedigree (100% Brahman to 100% Angus). The graph indicates a great ability of the PCA to predict breed composition based solely on genotypic data.

Genome Wide Association Study

Genomic DNA was extracted from blood or semen samples and was genotyped with the Bovine 250K SNP BeadChip for collection of high-density SNP genotypes. Contemporary groups were defined based on cross-classifications of year, sex, age of dam and Brahman fraction.

Contemporary groups were used as covariates in all analyses. The SVS (SNP Variation Suite, Golden Helix) software was used to perform a genome-wide association study on this dataset for tenderness and other meat quality traits in multibreed animals. Tenderness assessed by WBSF was the focus trait for this initial study. The genomic heritability for this trait was 0.36. Twelve chromosomes were found to have SNPs with statistically significant association with WBSF, and several more SNPs on other chromosomes were close to reaching significance (**Figure 2**).

An investigation of chromosomal locations with strong associations with WBSF revealed several genes already established by previous research as being associated and having an impact on beef tenderness, such as calpain-1 (Chromosome 29) and calpastatin (chromosome 10). Other regions also associated with WBSF not previously investigated proved to contain genes which, based on their biological role, are ideal candidate genes for this study: FHOD3 (formin) which encodes a protein playing a role in actin filament polymerization, ITPR1 gene encodes the inositol 1,4,5-triphosphate (IP3) receptor, an intracellular IP3-gated calcium channel that modulates intracellular calcium signaling, and laminin which encodes one of the two B-type lamin proteins and is a component of the nuclear lamina.

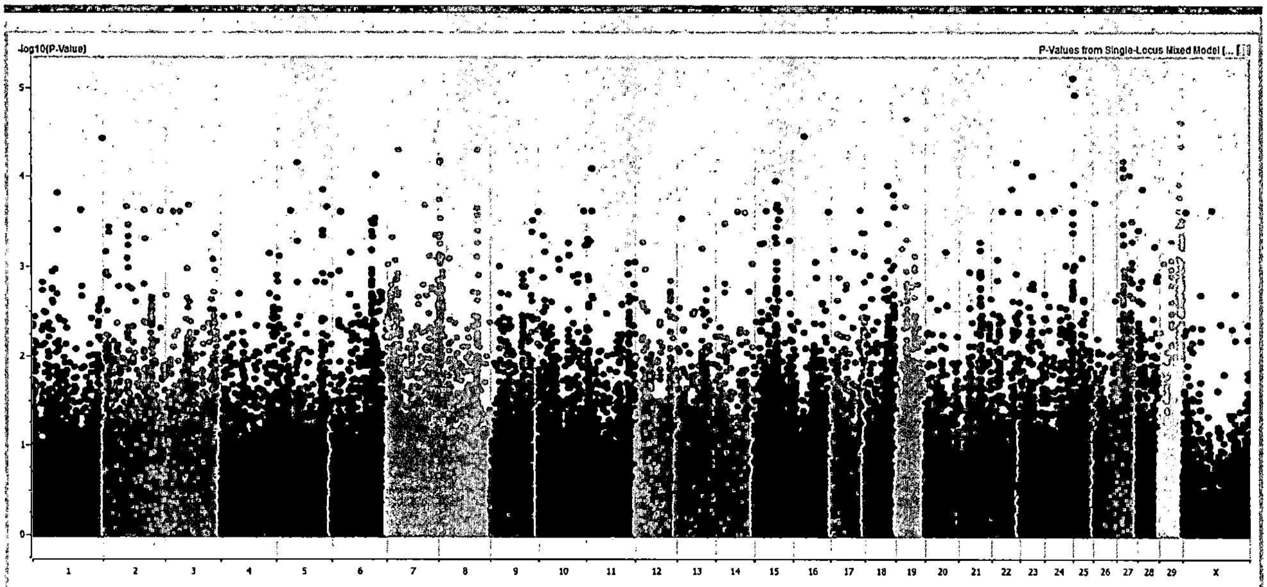

Figure 2. Genome-wide association results for WBSF on Brahman-Angus multibreed animals with 250K genotypes. Genomic location of each SNP across all 29 bovine chromosomes is displayed along the X-axis, with the negative logarithm of the association P-value for each SNP displayed on the Y-axis (each dot represents a SNP). The highest SNPs on each chromosome represent the SNPs more strongly associated with WBSF.

It is important to point out that tenderness evaluated by WBSF was primarily influenced by QTLs of small effect and that no genes of large effect such as attributed to CAPN1 and CAST in *Bos Taurus* cattle were detected. Although we identified SNPs in CAPN1, CAPN2, CAPN5, and CAST genes associated with WBSF measures in this population, they had smaller effects than other QTL candidates and their effects were much smaller than the effects estimated in taurine populations. These results may reflect differences between *Bos Taurus* and *Bos Indicus* cattle for allele frequencies at the CAPN1 and CAST causal mutations or differences between populations in the extent of LD between SNPs and these causal variants that could result in different marker effects in different breeds.

TRAINING NEXT GENERATION BEEF CATTLE SCIENTISTS

Two graduate students were assigned to this project and contributed in all research aspects outlined above. The graduate students were also required to read scientific literature related to topics relevant for this proposal and other current beef industry issues. In addition, the graduate students attended and prepared short presentations for the Animal Science seminar series, the Genetics and Genomics Seminar series, the Animal and Molecular Biology Annual Symposium and the UF Beef Cattle Short Course. Both students will continue their studies on this project and will continue to generate reports, peer-reviewed articles, seminar and conference presentations, greatly enhancing the impact of this project.

In addition to the graduate students, a number of undergraduate students were involved in different aspects of this project. Their activities, although minimal from the point of view of the overall project, allowed us to reach and recruit possible new students into our program, exposed the undergraduate students to cutting-edge genomic research and also familiarize them with issues of importance for the beef industry. At the same time, the undergraduate students worked closely under the guidance of the graduate students, therefore providing a good teaching opportunity for the graduate students. Several undergraduate students who were able to commit more time to the project and were assigned small projects, will be expected to present their research experience at the Animal Science Symposium in October 21-22, 2016.

FUTURE NEEDS

Single nucleotide polymorphisms (SNPs) significantly associated with tenderness are indicative of genome regions likely to harbor genes controlling this trait. Future research is needed to analyze in detail these genomic regions containing significant SNPs in order to identify causal mutations underlying trait variation, and facilitate the selection process to improve meat tenderness. Additional work on mapping markers to their closest causal genes followed by enrichment analysis, where ontology terms can provide insight into genetic mechanisms will greatly increase our understanding of genetic control of tenderness in Brahman influenced cattle and will allow the development of genomic prediction equations that can be used in selection and management to improve tenderness in these populations.

CONCLUSIONS

This study provides the first step toward applying genomic selection for meat quality traits in Brahman influenced crossbred cattle. Like other economically important traits for the beef industry, beef tenderness can be improved through genetics. Beef tenderness is objectively measured by WBSF, the force required to shear a cooked steak after postmortem aging, for which lower values imply more tender beef. Collection of tenderness data on a routine basis is difficult and expensive because it requires collecting rib-steak samples, ageing, cooking, coring, and shearing steaks. This makes improving this trait through traditional selection more difficult and marker–assisted selection appears the only practical route to make significant genetic improvement in WBSF. Our group has the advantage of an extensive dataset which includes this expensive phenotype on a sufficiently large number of cattle which will be used in this project.

The current study and the initial results show that several genomic regions are associated with tenderness of beef in an Angus-Brahman population. Investigation of several regions revealed strong candidate genes with possible direct relations to the meat tenderization process. More detailed analyses of the data collected (phenotypes and genotypes available from the current project) using a systems biology approach for genetic dissection of complex traits is being performed. Gene network theory applied to the results from the genome-wide association study will allow us to detect genetic drivers and key regulators of the tenderness phenotype. A higher-density set of SNP markers in a larger population should provide additional evidence and pinpoint which of these signals are biologically significant.