Contributed paper

REVEALING PHENOTYPIC AND GENETIC RELATIONSHIPS UNDERLYING THE THERMOTOLERANCE-PRODUCTION COMPLEX IN BEEF CATTLE

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SUMMARY

Heat stress is a principal factor limiting production of animal protein in subtropical and tropical regions, and its impact is expected to increase dramatically. Development of effective strategies to improve the ability to cope with heat stress is imperative to enhance productivity of the livestock industry and secure global food supplies. However, selection focused on production and ignoring adaptability results in beef animals with higher metabolic heat production and increased sensitivity to heat stress. The heritabilities estimated in this study in an Angus-Brahman multibreed population demonstrate genetic variation, which supports the hypothesis that selection for improved thermal tolerance is possible. Moreover, the estimated genetic correlations are favorable and indicate the opportunity to develop genomic tools for simultaneous improvement of tolerance to heat stress as well as production.

INTRODUCTION

In tropical and subtropical regions where more than half of the world cattle are maintained, climatic stress is a major limiting factor of production efficiency. This stress is expected to increase due to predicted changes in climate. Beef cattle when exposed to environmental high temperature and humidity, exhibit significant declines in feed intake, growth, fertility and welfare. Selection to increase productivity disregarding the genotype x environment interaction is likely to increase susceptibility to climatic stress. This makes the quest for heat-tolerant cattle with increased efficiency of production and reproduction increasingly important. *Bos indicus* cattle exhibit increased resistance to environmental stressors but they also have slower growth, are less fertile and have poorer meat quality relative to *Bos taurus* cattle. Beef producers in tropical and sub-tropical environments are incorporating a certain proportion of 'indicus' genes in their herds but, without knowledge of genes associated with thermotolerance, this also brings along negative aspects of indicus cattle. Research is needed to uncover the phenotypic and genetic relationships underlying this thermotolerance-production complex and subsequently identify the functional variants for thermotolerance without an antagonistic pleiotropy on production and reproduction. This will allow the incorporation of the GxE interaction in genomic selection programs for improvement of economically important traits in a predicted hotter world.

Animals vary in their ability to dissipate heat and, therefore, in their ability to cope with heat stress, and this variability has a genetic component. The goal of this research is to describe novel traits which can be used to characterize genetic pathways for thermotolerance which are independent or positively associated with production performance. This will allow the incorporation of the GxE interaction in genomic selection programs for improvement of economically important traits in a predicted hotter world.

MATERIALS AND METHODS

Animal population. The University of Florida Institutional Care and Use Committee approved the research protocol (Approval no. 201203578). The population consisted of 330 heifers from the University of Florida multibreed herd (Elzo and Wakeman 1998; Elzo *et al*. 2016, 2017) over 2

years in 2017 and 2018. For mating purposes, animals in the multibreed herd are assigned to 6 breed groups based on breed composition: 100% Angus = 100% to 80% Angus; 75% Angus = 79% to 60% Angus; Brangus = 62.5% Angus; 50% Angus = 59% to 40% Angus; 25% Angus = 39% to 20% Angus; and 100% Brahman = 19% to 0% Angus. Heifers were managed similarly across both years. DNA was extracted from blood samples from all animals and genotyped with the Bovine GGP F250 array (Illumina Inc., San Diego, CA, United States).

Skin biopsies. Skin samples were taken during summer (July 17, 2017 and August 7, 2018) between 0700 and 1100 h. Skin samples were collected from the back, 4 inches down from spine and halfway along horizontal axis. The skin was cleaned and disinfected with 70% ethanol and chlorhexidine (Clorhexidine 2%; VetOne, Boise, ID). A skin biopsy sample was collected using a 0.6 cm diameter punch biopsy instrument (Biopsy Punch, Miltex Inc., PA) and fixed in 10% formalin for approximately 24 h. Samples were dehydrated in 70% ethanol and infiltrated in liquid paraffin and stored until sectioned and stained at the UF Molecular Pathology Core. Sections were cut on a microtome with a thickness of 7 um, and sections were placed on slides, then stained with Harros-Eosin Hematoxylin. All histological sections were analyzed from digitized images obtained from a Nikon T3000 inverted phase microscope equipped image capture equipment (DMZ1200F with NIS Image Elements software). Images were obtained with the microscope in 40 X, and analyzed with ImageJ software. Sweat gland area (mm^2) and sweat gland depth as the distance from the top of the sweat glands to the skin surface (mm) were determined from a constant 4.6 mm^2 cropped image area.

Hair samples. Hair samples were collected from the shoulder, 4 inches down from spine and halfway along horizontal axis of each animal, as described in Hamblen *et al*. (2018). Hair samples were measured for length using ImageJ software. Five long and 5 short hairs from each individual were measured to evaluate the length of the topcoat and undercoat, respectively. The averages of the 5 short and long hairs were used in the statistical analysis.

Body temperature. Core body temperature was measured as vaginal temperature at 15-min intervals for 5 d using an iButton data logger (Dikmen *et al*. 2014) inserted into a blank CIDR device and then into the vagina of each animal. Each iButton was calibrated before the study started and pre-programmed to record body temperature at 15-min intervals on a 24-h cycle. Ambient environmental conditions were monitored using HOBO data loggers which continuously record temperature, humidity dew-point temperature with HOBO-U23 data logger (Onset Computer Corp., Bourne, MA), and black globe temperature by using HOBO-U22 data logger. The temperature humidity index (THI) was calculated as:

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)],$$

where T = air temperature (°C) and RH = relative humidity (%). This equation has been shown to be a good indicator of heat stress (Dikmen and Hansen 2009). Only body temperatures from the 3 continuous days when cattle were on pasture undisturbed were analyzed, as described in Sarlo Davila *et al*. (2019). Based on the thresholds defined by the livestock weather hazard guide and the THI level encountered during our experiment, THI conditions between 84 and 86 were considered high THI. Body temperatures at high THI for each individual were calculated by averaging all the body temperature measurements collected during the time that the THI windows occurred. This was accomplished for each heifer by averaging the body temperature from all 15-minute windows when the heifer was exposed to a high THI interval.

Carcass traits. A certified technician recorded ultrasound images from yearling calves using an Aloka 500 ultrasound system (Hitachi Aloka Medical, Ltd., Wallingford, Connecticut, USA). Analysis of the ultrasonic images with UICS Scanning Software by Walter and Associates, LLC (Ames, 106 Iowa, USA) yielded yearling ultrasound backfat (UFAT, cm) and yearling ultrasound percent intramuscular fat (UPIMF, %) phenotypes.

Statistical analyses. Average information restricted maximum likelihood (AIREML) variance

components, heritabilities, additive genetic correlations, and phenotypic correlations were estimated using single-trait and pairwise two-trait animal linear mixed models. The statistical model for both analyses included the direct additive genetic and residual as random effects, breed group (based on genomic breed composition) and group of data collection as class effect, except for short hair length and skin biopsy records, where group was not significant, and age at measurement as a covariate. The pedigree file consisted of 2,327 individuals, 715 sires and 1,286 dams. All analyses were performed using the airemlf90 package from BLUPF90 software (Misztal *et al*. 2002).

RESULTS AND DISCUSSION

Heritability estimates for skin histology characteristics, hair characteristics, body temperature under high THI conditions, and ultrasound carcass traits are provided in Table 1. A high heritability of 0.69 was estimated for the sweat gland area while the sweat gland depth had a low heritability estimate of 0.09. Heritability was estimated to be 0.33 for short hair length (undercoat) and 0.16 for long hair length (top coat). Heritability for coat score has been estimated to be 0.6, (Turner and Schleger 1960) and McEwan Jenkinson *et al*. (1975) estimated the heritability of hair follicle measurements to range from 0.15 to 0.76. The heritability for body temperature under high THI conditions was estimated to be 0.13 which is similar the heritability estimated reported for rectal temperature in a Brahman x Angus crossbred population (0.19; Riley *et al*. 2012) and dairy cattle (0.17; Dikmen *et al*. 2012). Both studies utilized cattle located in Florida. High heritability estimates were obtained for backfat (0.76) and intramuscular fat (0.37) ultrasound measures.

Table 1. Additive genetic variance (σ^2_a), residual variance (σ^2_e), and heritability (h^2) estimates for skin histology characteristics (sweat gland area and depth), hair characteristics (short and long hair length), core body temperature under high THI conditions, and ultrasound carcass traits (backfat thickness and intramuscular fat) with approximate sampling errors (in parentheses)

Trait[1]	σ^2_a	σ^2_e	h^2
Sweat gland area (mm^2)	2.03 (0.62)	0.89 (0.49)	0.69 (0.18)
Sweat gland depth (mm)	0.002 (0.004)	0.02 (0.004)	0.09 (0.15)
Short hair length (mm)	1.95 (1.07)	3.97 (0.99)	0.33 (0.18)
Long hair length (mm)	3.21 (3.39)	16.82 (3.42)	0.16 (0.17)
Temperature at high THI (°C)	0.02 (0.02)	0.10 (0.018)	0.13 (0.15)
UFAT (cm)	0.001 (0.0003)	0.0003 (0.0002)	0.76 (0.19)
UPIMF (%)	0.22 (0.12)	0.38495 (0.11)	0.37 (0.19)

[1]*UFAT, ultrasound backfat (cm); UPIMF, ultrasound intramuscular fat (%).*

Two-trait AIREML estimates of direct additive genetic and phenotypic correlations between skin histology characteristics, hair characteristics, body temperature under high THI conditions, and ultrasound carcass traits are presented in Table 2. Sweat gland area had a negative genetic correlation with sweat gland depth (-0.49), short and long hair length (-0.45 and -0.28, respectively), and body temperature under high THI conditions (-0.65). These negative correlations suggest a similarity in the genetic control underlying these traits which would allow for selection of animals with large sweat glands, short hair (both topcoat and under coat), and able to maintain a lower body temperature under high THI conditions. More importantly, although weak, the genetic correlations between sweat gland area and the two production traits (backfat and intramuscular fat) were favorable (0.22 and 0.20, respectively). Similarly, there was a medium negative genetic correlation between the body temperature under high THI and the two ultrasound carcass traits, suggesting animals able to maintain a lower body temperature would be more

productive.

Table 2. Two-trait AIREML estimates of phenotypic (above diagonal) and direct additive genetic (below diagonal) correlations between skin histology properties, hair characteristics, and carcass traits

Trait[1]	SWGA	SWGD	SHL	LHL	THighTHI	UFAT	UPIMF
SWGA	0.69	-0.18	-0.22	0.02	-0.23	-0.05	-0.13
SWGD	-0.49	0.10	0.32	0.26	0.12	0.08	0.22
SHL	-0.45	0.27	0.33	0.75	0.23	0.07	0.17
LHL	-0.28	0.02	1.00	0.16	0.23	0.04	0.11
THighTHI	-0.65	-0.61	-0.28	-0.45	0.13	-0.17	0.04
UFAT	0.22	-0.57	-0.34	-0.60	-0.38	0.76	0.23
UPIMF	0.20	0.49	0.08	0.09	-0.33	0.42	0.37

[1]SWGA, sweat gland area (mm2); SWGD, sweat gland depth (mm); SHL, short hair length (mm); LHL, long hair length (mm); THighTHI, temperature at high THI (°C); UFAT, ultrasound backfat (cm); UPIMF, ultrasound intramuscular fat (%).

CONCLUSIONS

The values of heritability estimated in this study indicate a large, exploitable genetic variance which can be used in selection programs to improve heat tolerance in cattle. Novel traits describing the thermotolerance phenotype such as sweat gland area, short hair length and body temperature under high THI conditions had medium to high heritabilities. More importantly, the genetic correlations estimated in this population are encouraging, indicating favorable relationships between the thermotolerance phenotypes and the production traits. This would suggest that genetic programs to improve resilience to environmental stress could be successful and opportunities exists for simultaneous improvement of production related traits.

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