Eye pigmentation quantification and lesion occurrence in white faced cattle

D. G. Riley[1], K. M. Davis[1], T. Smith[2], B. Bolt[3], S. Meadows[3], J. G. Powell[4], M. D. Garcia[5], J. D. Arthington[6], N. DiLorenzo[7], D. L. Lalman[8], R. C. Vann[9], F. M. Rouquette[10], G. R. Hansen[11], A. J. Cooper[12], J. E. Cloud[1], A. D. Herring[1], D. S. Hale[1], J. O. Sanders[1], and T. J. DeWitt[13]

[1]Texas A&M University, Department of Animal Science, Texas AgriLife Research, College Station
[2]Mississippi State University, Department of Animal Science, Starkville
[3]Clemson University, Department of Animal Science
[4]University of Arkansas, Department of Animal Science, Fayetteville
[5]Louisiana State University, Department of Animal Sciences, LSU AgCenter, Baton Rouge
[6]University of Florida, Range Cattle Research and Education Center, Ona
[7]University of Florida, North Florida Research and Education Center Marianna
[8]Oklahoma State University, Department of Animal Science
[9]Mississippi State University, MAFES-Brown Loam Research Center, Raymond
[10]Texas AgriLife Research, Overton
[11]North Carolina State University, Tidewater Research Station, Plymouth
[12]Texas A&M University, Commerce
[13]Texas A&M University, Department of Plant Pathology and Microbiology, Texas AgriLife Research

Hereford cattle are widely used in a variety of production schemes and the breed has the reputation for high levels of heterosis in combination with many other breeds. Production losses in cattle with Hereford background due to bovine ocular squamous cell carcinoma (commonly called "cancer eye") are serious in the U.S. beef industry, due to condemned carcasses at slaughter and especially, shortened productive life of affected animals. Anderson et al. (1957) and Anderson (1960, 1963, 1991) reported the lower incidence of bovine ocular squamous cell carcinoma in white faced cattle with increased pigmentation around the eyes. Areas of corneoscleral pigmentation may form as a response to the occurrence of pre-cancerous lesions. Updated characterization of eye pigmentation in Hereford straightbreds and crossbreds would facilitate identification of responsible genomic regions. The objective of this study was to assess variation in eyelid and corneoscleral pigmentation and lesion occurrence in Hereford × *Bos taurus* and Hereford × *Bos indicus* cross cattle with white faces.

Cattle at multiple locations across the Southern United States contributed records to this project (Table 1). Digital images were taken of both eyes on mature animals and calves that had white faces at those locations in 2012 (Figure 1). Using those images, a procedure was developed using image analysis software to calculate the proportion of non-white pixels in the image area. These were quantified distinctly for eyelid and corneoscleral pigmentation. Analyses were completed using ASReml (Gilmour et al., 2009). Fixed effects investigated included location, animal age (2 levels: mature animal and calf), and breed type of animal (Hereford, *Bos taurus* cross, *Bos indicus* cross [including Braford]). Animal was a random effect. Lesion presence (1) or absence (0) was obtained by visual appraisal of images. Lesion presence was modeled as binomially-distributed, and a logit link function was applied to data. Records of mature bulls (n = 9) were excluded from analyses, as they were represented only in the Hereford and *Bos indicus* breed types, they all had complete eyelid pigmentation and were likely selected in part for that reason.

Eyelid pigmentation proportion was lower in both eyes for Hereford than for *Bos taurus* or *Bos indicus* crosses (Table 2); this was the case when upper and lower eyelids were analyzed distinctly. As expected, there was no detected age difference for eyelid pigmentation ($P > 0.2$); however, a highly significant interaction of age category with breed type was detected (Table 2) for proportion of corneoscleral pigmentation. Cows had higher ($P < 0.001$) proportions of corneoscleral pigmentation (both eyes) than calves and yearlings in *Bos taurus* and *Bos indicus* breed types. No difference was detected ($P = 0.91$) between cows and calves for straightbred Herefords. *Bos taurus* and *Bos indicus* cross cows had greater proportions of corneoscleral pigmentation (either eye) than Hereford cows ($P < 0.001$); all breed types differed ($P < 0.02$) for proportion of corneoscleral pigmentation of the left eye, with *Bos indicus* cows intermediate. Corneoscleral pigmentation proportions were larger ($P < 0.05$) for right eye of *Bos taurus* crossbred calves and yearlings than for Hereford calves. Anderson (1991) noted the increased occurrence of corneoscleral pigmentation with increasing age in mature animals.

Lesion presence was confirmed for 34 of the 1,083 animals. The majority of those (32) were observed in mature cows. The proportion of lesions were greater ($P < 0.001$) for Hereford (0.08 ± 0.03) than for *Bos indicus* or *Bos taurus* crosses, which were both 0.01 ± 0.004. Twenty-nine of the 34 occurrences were located outside of pigmented areas.

These data and analyses are preliminary and subordinate to the ultimate goal of this work: to identify gene(s) responsible for these pigmentation traits. It would be of great benefit, for example to genomically identify bulls that would produce white faced progeny that would consistently have pigmented eyelids. Not enough is understood of corneoscleral pigmentation—its responsive role to lesion formation on the eyeball has not been extensively characterized.

We target 2000 animals with records in this project. We are adding multivariate analyses for use in characterization of these data. Acquisition of chip-based high density SNP genotypes (e.g., Bovine SNP50 or Bovine HD; Illumina, Inc. San Diego, CA) to use with image values will be a critical part of associating differences in pigmentation with areas in the bovine genome. We will get annual images for mature animals that remain in these herds in attempt to confirm changing corneoscleral pigmentation.

References

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Table 1. Distribution of animals by breed type and location

Location	Hereford	*Bos taurus*	*Bos indicus*	Total
University of Arkansas, Fayetteville		35		35
University of Florida				
Marianna			51	51
Ona			90	90
Louisiana State University, Baton Rouge			107	107
Mississippi State University				
Raymond	1	24	17	42
Starkville	29	40		69
North Carolina State University, Plymouth		71		71
Oklahoma State University, Stillwater		102		102
Clemson University, Clemson, SC	68	108		176
Texas A&M Univ., Commerce	17	43		60
Texas AgriLife Research				
College Station	4	73	5	82
McGregor	1	2	81	84
Overton			102	102
Texas commercial producer, Rule		12		12
Total	120	510	453	1,083

Table 2. Breed type means for proportion of eyelid pigmentation by eye and eyelid and breed type-age category means for proportion of corneoscleral pigmentation[1]

	Eyelid pigmentation	Corneoscleral pigmentation	
Eyelid	Overall	Calves and yearlings	Cows
Left eye			
Bos indicus	0.93 ± 0.02^{a}	0.25 ± 0.04^{x}	$0.38 \pm 0.05^{a,y}$
Bos taurus	0.91 ± 0.02^{a}	0.29 ± 0.04^{x}	$0.48 \pm 0.04^{a,y}$
Hereford	0.66 ± 0.02^{b}	0.16 ± 0.07^{c}	0.17 ± 0.06^{b}
Right eye			
Bos indicus	0.95 ± 0.02^{a}	$0.26 \pm 0.04^{ab,x}$	$0.37 \pm 0.05^{b,y}$
Bos taurus	0.88 ± 0.02^{b}	$0.32 \pm 0.04^{a,x}$	$0.53 \pm 0.04^{a,y}$
Hereford	0.67 ± 0.03^{c}	0.16 ± 0.07^{b}	0.17 ± 0.06^{c}

[a-c] Within a column and eye, means that do not share a common superscript differ ($P < 0.05$).
[x-y]Corneoscleral pigmentation only: within a row, means that do not share a common superscript differ ($P < 0.05$).
[1]Other differences not indicated by superscripts for both eyes include: 1) *Bos indicus* and Hereford calves had lower means than mature *Bos taurus* and *Bos indicus* cows 2) *Bos taurus* calves had larger means than Hereford cows.

Figure 1. Examples of images: Top—complete eyelid pigmentation; Middle—incomplete eyelid pigmentation, substantial corneoscleral pigmentation; Bottom-- incomplete eyelid pigmentation