Published July 17, 2015

Technical note: Digital quantification of eye pigmentation of cattle with white faces

K. M. Davis,* T. Smith,† B. Bolt,‡ S. Meadows,‡ J. G. Powell,§ R. C. Vann,# J. D. Arthington,‖ N. DiLorenzo,¶ D. L. Lalman, F. M. Rouquette Jr.,†† G. R. Hansen,§§ A. J. Cooper,## J. E. Cloud,* M. D. Garcia,‖‖ A. D. Herring,* D. S. Hale,* J. O. Sanders,* T. B. Hairgrove,¶¶ T. J. DeWitt,*** and D. G. Riley*[1]**

*Department of Animal Science, Texas A&M University, College Station 77843; †Department of Animal and Dairy Sciences, Mississippi State University, Starkville 39762; ‡Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC 29634; §Department of Animal Science, Division of Agriculture, University of Arkansas, Fayetteville 72701; #MAFES-Brown Loam Experiment Station, Mississippi State University, Raymond 39154; ‖Range Cattle Research and Education Center, University of Florida, Ona 33865; ¶Department of Animal Sciences, North Florida Research and Education Center, University of Florida, Marianna 32446; **Department of Animal Science, Oklahoma State University, Stillwater 74078; ††Texas A&M Agrilife Research and Extension Center, Overton, 75684; §§Department of Animal Science, North Carolina State University, Plymouth 27962; ##Department of Agricultural Sciences, Texas A&M University, Commerce 75429; ‖‖School of Animal Sciences, Louisiana State University AgCenter, Baton Rouge 70803; ¶¶Department of Animal Science, Texas A&M AgriLife Extension Service, Texas A&M University, College Station 77843; and ***Department of Wildlife and Fisheries Science, Texas A&M University, College Station 77843

ABSTRACT: Cancer of the eye in cattle with white faces occurs less frequently in cattle with pigmented eyelids. Corneoscleral pigmentation is related to eyelid pigmentation and occurrence of lesions that may precede cancer. Objectives of this study were to assess 1) variation in the proportion of eyelid and corneoscleral pigmentation in Hereford, *Bos taurus,* and *Bos indicus* crossbreds and 2) the occurrence of lesions with the presence of pigmentation in those areas. Hereford and *Bos indicus* crosses (Brahman or Nellore with Angus and Hereford and straightbred Brafords) and *Bos taurus* crosses (Angus-Hereford) were included in the study ($n = 1{,}083$). Eyelid pigmentation proportions were estimated by pixel quantification and were evaluated as total proportions and for upper and lower eyelids distinctly for each eye. Fixed effects included breed type, age categories, and sex of the animal. Lesion presence (1) or absence (0) was obtained by visual appraisal of image and was assumed to be binomially distributed. Eyelid pigmentation proportions (overall, upper, and lower eyelids) for Hereford ranged from 0.65 ± 0.03 to 0.68 ± 0.03 and were significantly lower than *Bos indicus* (range from 0.93 ± 0.02 to 0.95 ± 0.02) or *Bos taurus* (ranged from 0.88 ± 0.02 to 0.92 ± 0.02) crosses. Corneoscleral pigmentation in Hereford cows (0.17 ± 0.06) did not differ ($P = 0.91$) from Hereford calves and yearlings (0.16 ± 0.07). *Bos indicus* and *Bos taurus* crossbred cows had larger corneoscleral pigmentation (0.38 ± 0.05 and 0.48 ± 0.04 for left eyes and 0.37 ± 0.05 and 0.53 ± 0.04 for right eyes, respectively) than all calves ($P < 0.001$), and their corneoscleral pigmentations were greater than that of Hereford cows ($P < 0.003$). *Bos indicus* and *Bos taurus* cows had greater proportions of left eye corneoscleral pigmentation (0.38 ± 0.05 and 0.48 ± 0.04, respectively) than Hereford cows (0.17 ± 0.06) and all young animal breed types ($P < 0.05$). Right eye proportions differed for all cow groups ($P < 0.05$; 0.53 ± 0.04, 0.37 ± 0.05, and 0.17 ± 0.06). Among calves and yearlings, Hereford had a lower right eye corneoscleral pigmentation proportion (0.16 ± 0.07) than *Bos taurus* ($P = 0.02$). The lesion proportion for Hereford (0.08 ± 0.03) was significantly greater than that of either *Bos indicus* (0.01 ± 0.005) or *Bos taurus* (0.01 ± 0.003). Crossbreeding with *Bos taurus* or *Bos indicus* animals appears to increase eye pigmentation, which may help reduce the occurrence of cancer in eyes of cattle with white faces.

Key words: corneoscleral pigmentation, eye cancer, eye pigmentation, Hereford, white spotting

J. Anim. Sci. 2015.93:3654–3660
doi:10.2527/jas2014-8786

[1]Corresponding author: david-riley@tamu.edu

Received December 5, 2014.
Accepted May 1, 2015.

Figure 1. Corneoscleral pigmentation on the sclera, which is often white.

Table 1. Distribution of animals by breed type and location

Location	Hereford	*Bos taurus* crosses	*Bos indicus* crosses	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 University, 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

INTRODUCTION

Production losses such as condemned carcasses at slaughter and shortened productive lives in cattle with white faces due to bovine ocular squamous cell carcinoma (cancer of the eye) affect the choice of breed for mating programs of cow-calf producers. Classic work reported lower incidence of such cancer in Hereford cattle associated with increased pigmentation around the eyes (Anderson et al., 1957; Anderson, 1960, 1963, 1991). That group quantified the proportion of eye pigmentation using a manual process (counting squares within grids). Modern technology would permit a more refined characterization of eyelid pigmentation and perhaps other parts of eye anatomy. There appears to be increased eye pigmentation in crossbred Hereford cattle, but this has not been characterized. The objectives of this study were to assess 1) variation in the proportion of eyelid and corneoscleral pigmentation (pigmented sclera, typically white; Fig. 1) in Hereford, *Bos taurus,* and *Bos indicus* crossbreds with white faces using a modern protocol and 2) the occurrence of eyelid or eyeball lesions with the presence of pigmentation in those areas.

MATERIALS AND METHODS

All procedures involving animals were approved by the Texas A&M University Institutional Animal Care and Use Committee.

Data were collected from cows, calves, and bulls from research and producer herds across the southern United States (Table 1). Cattle with Hereford background were *Bos taurus* crosses (Angus-Hereford), *Bos indicus* crosses (Brahman or Nellore with Angus and Hereford and straightbred Brafords), and straightbred Hereford; cattle with any white at all on the face were included. Digital photographs of each eye were taken while cattle were restrained with heads caught in a working chute. Multiple images were often taken to ensure at least one adequate image of each eyelid. The camera used for most images was an Olympus Camedia C-740 digital (Olympus Company of the Americas, Center Valley, PA) with 3.2 megapixels and 10× optical zoom. Other cameras used included Kodak CX7530 Zoom Digital Camera (Eastman Kodak Company, Rochester, NY) and the Canon EOS Rebel T3i (Canon Company, Ota, Tokyo, Japan) at 2 locations. Images were taken on 1 or 2 d per location.

Images of eyes with partial pigmentation were then processed with Adobe Photoshop Elements 2.0 (Adobe Systems Incorporated, San Jose, CA), cropping separately for eyelid and corneoscleral regions. Images for eyelids were limited to the region surrounding the eyelid edges that did not have hair (Fig. 2). Position of the iris was not constant in all images; corneoscleral pigment was quantified only on visible sclera within each image. Some coloration of parts of a few images was conducted to manage shadows, eyelashes, or obstruction that obscured the observed pigmentation of the eyelid or corneoscleral regions (when those were apparent from additional images on the same eye). Edited images were converted to 8-bit gray-scale images to quantify pixel intensities in whole numbers from 0 (completely black) to 255 (pure white) and were transferred to a pure white background using Image J software (Java, Bethesda, MD).

Figure 2. Example of editing of original image (top panel) to crop non-eyelid area (bottom panel) for subsequent pixel quantification methodology.

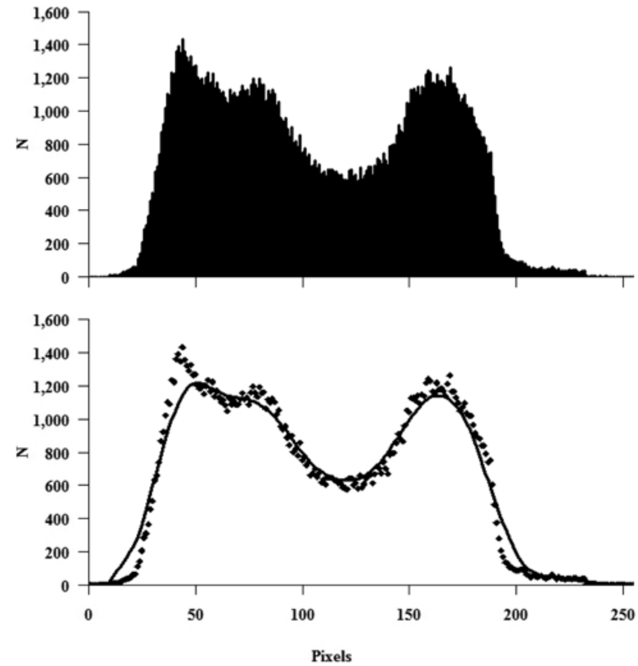

Figure 3. Histogram plot of pixel color intensity (top panel) and plot of the Loess curve fitted to pixel intensity values (bottom panel) for the cropped image in Fig. 2 (bottom panel). The low point (trough) between the two peaks was used to separate pixels into dark values (left peak) and lighter, nonpigmented values (the right peak). These were then used to calculate pigmented area as a proportion of the area.

Histograms of those pixels for images were generated, and a best fit line graph was created with Loess regression of the number of pixels at given intensity (y) on the pixel intensity (x) coordinates (Fig. 3). This bimodal line over these points delineated (in most cases) 2 peaks and a trough in the plotted image data. The peaks represented the highest 2 groups of pixel intensities, that is, essentially a pigmented (dark) peak and an unpigmented (light) peak, and the trough (generally 1 per image) delineated pixel intensities into the 2 categories (dark and light). First and second derivatives of this line were examined to help establish delineation. Some images required minimal refinements to more closely delineate the 2 peaks and trough.

Some degree of shading was accomplished by eyelashes, making lower eyelid pigmentation a potentially more critical component of a protection system (J. Ellis, Ellis Farms, Chrisman, IL, personal communication); therefore, proportion of pigmentation was separated into upper and lower eyelids. The x and y coordinates were rotated objectively using eigenvalues from decomposition of the covariance matrix of pixel values and intensities. Image coordinates were rotated to the major principle component axis, which was always along the long dimension of the eyelid, then oriented over the x axis, effectively separating upper and lower eyelid areas as pixels with positive and negative y values. That rotation angle was approximately 43° for most images. Proportion of pigmented area was then calculated as a ratio of the pigmented pixels to total pixels. Eyes with complete or no pigmentation were not subjected to this procedure and were assigned values of 1 and 0, respectively.

Dependent variables were therefore proportions of eyelid pigmentation and were evaluated as total, upper, and lower eyelids for each eye per animal. In the same manner as described above, corneoscleral pigmentation was evaluated on observable sclera in images for each eye. Potential lesions were confirmed by a veterinarian who examined images (not live animals). Eyes with the presence of a confirmed lesion were assigned values of 1; those with no lesion were assigned a value of 0.

Statistical Analyses

Traits were analyzed using mixed linear models with ASReml (Gilmour et al., 2009). Fixed effects investigated included breed type (Hereford, *Bos taurus* crosses, *Bos indicus* crosses and composites), sex of animal (male, female), and age category. In preliminary analyses, multiple constructs of age were investigated, and the ultimate parameterization of age was as a 2-level fixed effect: animals 2 yr or older and animals 1 yr or younger, including calves. *Bos taurus* crosses were all Angus-Hereford crosses. *Bos indicus* crosses included Brahman or Nellore with Angus and

Table 2. Breed type means for proportion of eyelid pigmentation by eye and eyelid

	Eyelid		
Eye	Upper	Lower	Overall
Left eye			
Bos indicus crosses	0.94 ± 0.017[a]	0.94 ± 0.016[a]	0.94 ± 0.016[a]
Bos taurus crosses	0.90 ± 0.019[a]	0.92 ± 0.017[a]	0.91 ± 0.018[a]
Hereford	0.67 ± 0.061[b]	0.69 ± 0.060[b]	0.68 ± 0.060[b]
Right eye			
Bos indicus crosses	0.94 ± 0.015[a]	0.95 ± 0.015[a]	0.94 ± 0.014[a]
Bos taurus crosses	0.89 ± 0.020[b]	0.89 ± 0.019[b]	0.89 ± 0.019[b]
Hereford	0.70 ± 0.057[c]	0.68 ± 0.058[c]	0.69 ± 0.056[c]

[a–c]Within a column and eye, means that do not share a common superscript differ ($P < 0.05$).

Table 3. Breed type-age category means for proportion of corneoscleral pigmentation[1]

	Age category	
Eye	Calves and yearlings	Cows
Left eye		
Bos indicus crosses	0.24 ± 0.035[x]	0.37 ± 0.052[a,y]
Bos taurus crosses	0.28 ± 0.037[x]	0.49 ± 0.048[a,y]
Hereford	0.10 ± 0.048	0.11 ± 0.035[b]
Right eye		
Bos indicus crosses	0.25 ± 0.040[a,b,x]	0.36 ± 0.055[b,y]
Bos taurus crosses	0.31 ± 0.042[a,x]	0.53 ± 0.052[a,y]
Hereford	0.09 ± 0.045[b]	0.10 ± 0.034[c]

[a–c]Within a column and eye, means that do not share a common superscript differ ($P < 0.05$).

[x,y]Within a row, means that do not share a common superscript differ ($P < 0.05$).

[1]Other differences not indicated by superscripts include the following: 1) *Bos indicus* cross and Hereford calves had lower ($P < 0.01$) means than mature *Bos taurus* and *Bos indicus* cross cows for both eyes, and 2) *Bos taurus* cross calves had larger ($P < 0.05$) means than Hereford cows for both eyes, and *Bos indicus* cross calves had larger left eye proportion of pigmentation than Hereford cows.

Hereford and straightbred Brafords. In preliminary analyses, there was weak support ($0.1 < P < 0.25$) for the interaction of sex with age category. A small number of records of mature bulls ($n = 5$ Hereford and $n = 4$ Braford) with complete eyelid pigmentation appeared to be responsible for that interaction; because that age-sex combination proportion appeared to be a consequence of selection for complete eye pigmentation and the others less so, those records were removed from final analyses, and sex was nested within age category. Location was included as a random effect; breed types were not represented at each location. All traits were analyzed assuming a binomial distribution, and a logit link function was applied for analysis and comparison of adjusted means. Pearson correlation coefficients of dependent variables were calculated using the CORR procedures of SAS (SAS Inst. Inc., Cary, NC). Subsequent analyses of all pigmentation proportions were conducted to evaluate red vs. black base coat color as a main effect with 2 levels and as components of interaction with the age effect within 2 of the 3 breed type categories (*Bos indicus* crosses and *Bos taurus* crosses) as subsets but with the same model parameterizations as the overall analyses.

RESULTS AND DISCUSSION

A large number of animals had complete eyelid pigmentation and were not subjected to the pixel quantification methodology; just over ¼ of the animals ($n = 299$ and 309 for left and right eyes) were assessed with that procedure. Corresponding groups ($n = 509$ and 494 for left and right eyes) were evaluated with pixel quantification methodology for proportion of corneoscleral pigmentation.

Upper and lower eyelid pigmentation proportions were highly correlated ($r = 0.89$ for both eyelids; $P < 0.001$). Eyelid pigmentation amounts on the right and left eyes were strongly associated ($r > 0.65$; $P < 0.001$). Corneoscleral pigmentation proportion was less strongly associated with eyelid pigmentation proportions ($r = 0.26$; $P < 0.001$), which is consistent with phenotypic correlations reported in Hereford cattle (Vogt et al., 1963; Anderson, 1991). Vogt et al. (1963) also reported a high genetic correlation of pigmentation in the 2 areas.

Neither age category nor sex (within age category) effects were influential in analyses of proportion of eyelid pigmentation ($P > 0.21$). *Bos taurus* and *Bos indicus* crossbred breed types had greater ($P < 0.05$) means for all quantifications of left eyelid pigment proportions than Herefords (Table 2). However, right eyelid pigmentation proportions differed for all 3 breed types ($P < 0.05$), with *Bos taurus* crosses intermediate to the other groups.

A highly significant interaction of age category with breed type was detected (Table 3) for proportion of corneoscleral pigmentation. Cows had greater ($P < 0.01$) proportions of pigmentation (both eyes) than calves and yearlings in *Bos taurus* and *Bos indicus* breed types. No difference in proportion of corneoscleral pigmentation was detected ($P > 0.86$) between cows and calves for straightbred Herefords. As with eyelid pigmentation, *Bos taurus* and *Bos indicus* cross cows had greater left eye proportions of corneoscleral pigmentation than Hereford cows ($P < 0.03$), but *Bos indicus* cross cows did not differ from Hereford cows ($P = 0.11$) for proportion of corneoscleral pigmentation of the right eye. Corneoscleral pigmentation proportions were larger ($P < 0.05$) for the right eye of *Bos taurus* crossbred calves and yearlings than for Hereford calves and yearlings. The difference between left eye corneoscleral pigmentation portions in *Bos taurus* and

Table 4. Proportions and distribution of lesions by breed type, age category, location on eye, and pigmentation of area (n = 34)

Item	Hereford	*Bos indicus* crosses	*Bos taurus* crosses
Proportion	0.08 ± 0.033[b]	0.01 ± 0.005[a]	0.01 ± 0.003[a]
Age category			
Calf	—	—	2
Mature	19	8	5
Location of lesion			
Eyelid	11	3	6
Upper			2
Lower	9[1]	2	2
Both	2	1	2
Eyeball	10[2]	5	1
Caruncle	1	—	—
Pigmentation			
Within pigmented area	—	3	1
Outside pigmented area	19	5	6

[a,b]Within the row of adjusted mean proportions, those that do not share a common superscript differ (P < 0.05).

[1]Three of these Hereford cows had lesions on lower eyelids of both eyes, but they were each included in analyses as single lesion occurrences.

[2]Three of these Hereford cows also had lesions on lower eyelids. Two of those 3 had lesions in both eyes. These were each included in analyses as single lesion occurrences.

Hereford calves approached importance (P = 0.07). It was previously reported that corneoscleral pigmentation increases with age (Vogt et al., 1963; Anderson, 1991), and ultimate measurements may not be sufficiently quantified until after 5 yr of age.

Lesion presence was confirmed for 34 of the 1,083 animals. Lesions were observed on animals in 9 of the 14 herds, with a range within those herds from 1 (0.011 proportion of total animals) to 9 (0.13 proportion of total animals). The overall incidence of 0.03 was greater than that reported (0.017 confirmed squamous cell carcinoma of 1,105 "cow observations") for straight Herefords in Colorado (Russell et al., 1976) and similar to that for Hereford purebreds (0.047 of 1,566 cows; Woodward and Knapp, 1950) but less than those for Hereford purebreds (0.25 of 696) and Hereford crossbreds (0.18 of 261) in the mid-20th century (Anderson, 1970, as cited by Heeney and Valli, 1985). The distribution of lesions by breed type, age category, anatomical location, and pigmentation status is shown in Table 4. There were 5 lesions observed on completely white (n = 47) eyes, and on 7 of 1,552 eyes that were completely pigmented. No sex within age difference was detected (P = 0.8) for lesion occurrence. *Bos indicus* and *Bos taurus* crosses had lower proportions of lesions than Hereford animals (P < 0.05; Table 4). Not surprisingly, young animals (calves and yearlings) had lower (P < 0.05) mean proportion of lesions (0.005 ± 0.0026) than mature cattle (0.071 ± 0.017); 2 of the 34 animals with lesions were young animals. This result was consistent with earlier work (Woodward and Knapp, 1950) and was likely due to the greater quantity of exposure to harsh environmental conditions (particularly sun and possibly other irritating agents, such as flies) across time (Anderson and Skinner, 1961).

Vogt et al. (1963) associated increases in corneoscleral pigmentation with lower lesion frequencies in Hereford cows. Lesions developed in previously unpigmented areas of the eyeball were documented as progressively surrounded by corneoscleral pigmentation, possibly as a defense mechanism (Vogt et al., 1963; Anderson, 1991); that is, lesions seemed to initiate the appearance of corneoscleral pigment. Although older cattle may be more susceptible to bovine ocular squamous cell carcinoma, they may also have increasing amounts of corneoscleral pigmentation.

There was partial confounding of breed type and age of animals with locations in these data. *Bos indicus*–influenced animals were represented in records from only the southernmost herds. Variation among locations could indicate differential solar and ambient influence on these traits. It is likely that the observed proportion of lesions underestimates the actual proportion of lesions that occurred across time, as all locations cull animals with severe lesions.

It is reasonable to consider that pigmented eyes were favored in selection programs, at least informally. The failure to detect an age effect may suggest that such selection was not strong in females. However, the average eyelid pigmentation proportion for the 9 bulls with records was unity and, of course, was larger than the rest of the population. This form of selection may have taken place to varying degrees in many Hereford and Braford lines; the desire to breed Hereford cattle with eye pigment is not a new concept (Pitt, 1920; Russell et al., 1956). Pitt (1920) noted the preference among cattle breeders in Jamaica for cattle with pigment "around the eyes on account of their supposed immunity to the attacks of flies and certain eye diseases." Such selection may have resulted in cattle with fewer white markings. Pitt (1920) believed that increased eyelid pigmentation ("red-eyed") was associated with smaller amounts of white on the body overall, especially in the neck area. Anecdotal review of sale catalogs in the United States shows many individuals with minimal white markings within the Hereford breed.

The characterization of corneoscleral pigmentation in these data is incomplete, and proportions are probably underestimated in many cases. This limitation is due to the inability to see or photograph the entire sclera. Research efforts to associate visible sclera with cattle temperament have employed video of eyes, and still frames were selected in which the iris was centered (Sandem et al., 2002; Core et al., 2009). Labelle et al. (2013) used subjective scores representing differing amounts of corneoscleral pigmentation ("corneal pigmentation") from digital images of eyes of individuals from a specific dog breed (pugs); the ability to handle dogs and photograph eyes would seem to be much easier. Spotting patterns in Holstein-Friesian and Jersey cross cows were evaluated by assignment of subjective scores after inspection of digital images (Liu et al., 2009). Preliminary analyses of a subset of the data from the present study (Davis et al., 2013) utilized subjective scores as an approximation of amount of eye pigmentation, which was somewhat similar to the early characterization of eye pigmentation (Anderson et al., 1957). Another alternative that may permit observation of greater area of the sclera may be images in which animals are looking forward, moving the iris forward and exposing more of the back half of the sclera. Even though this would provide more area of the sclera to subject to the process for pixel quantification, the other side would remain uncharacterized. When animal heads are immobilized in working chutes, it is possible to manipulate the eyelid and skin surrounding the eye to more completely observe the sclera. This method would be less efficient, especially for older animals, as many chutes permit much head movement.

A subset of the total animals with records in the *Bos indicus* breed type category were black ($n = 60$ of 452), and even fewer of the animals in the *Bos taurus* breed type were red ($n = 20$ of 511). In subsequent analyses that evaluated only records of *Bos indicus* breed type animals, there were trends for 5 of the 6 eyelid pigmentation proportions as dependent variables ($0.06 < P < 0.162$) for black cattle to have greater proportions of eye pigmentation. An interaction of color with age category may be important ($P = 0.098$) from analysis of left corneoscleral pigmentation proportion, with means of 0.58 ± 0.08, 0.33 ± 0.04, 0.30 ± 0.07, and 0.23 ± 0.04 for black cows, red cows, black calves and yearlings, and red calves and yearlings, respectively. In analyses of that subset of data, color as a main effect was significant for corneoscleral pigmentation in both eyes (black left and right: 0.44 ± 0.06 and 0.42 ± 0.07; red left and right: 0.28 ± 0.05). Often, red *Bos indicus* cattle are brindled with black pigment, and some have large amounts of black. Others are born red and darken as they age to the extent that they could be confused with animals that are black. Although numbers of records did not support assessment of the effect of color within the *Bos taurus* breed type, such cattle might provide data that better clarify that effect than *Bos indicus*. It is attractive to consider accumulation of black pigment as an adaptive modification in cattle with a red base coat color.

There are alternative possibilities for imaging and the processing of images. A similar processing strategy was employed for assessing black vs. nonblack (recategorized as white) pigment in monarch butterfly larva by Davis et al. (2004), who subsequently automated that process. Digital imaging has been investigated for animal identification, either through algorithms evaluating dynamics and neural networks of face images of cattle (Kim et al., 2005) or retinal imaging (Barry et al., 2011; Rojas-Olivares et al., 2012) with commercial storage, processing, and assistance with the analyses of images. Conservation researchers have employed camera trap systems to obtain images, 3-dimensional surface models, and a combination of algorithms to assess skin surface patterns in various mammals (Kelly, 2001; Karlsson et al., 2005; Karanth et al., 2006; Hiby et al., 2009), primarily for identification of individuals or for comparison to illegally taken skins. A digital single-lens reflex camera adaptor was developed and used to photograph and detail eye vascularity in dogs (Alario et al., 2013); this sensitive procedure required extensive demobilization and sedation.

Conclusion

This work represents an updated assessment of pigmentation in white-faced cattle using digital images, objective image manipulation, and quantification of pixels; previous work was based around somewhat subjective (visual quantification over a grid) determinations of the amount of eyelid pigment. Consistent with earlier work, there was lower incidence of eye lesions in cattle with white faces that had increased pigmentation around the eyes, and mature animals had greater proportions of corneoscleral pigmentation than young animals. There were clear breed type differences in pigmentation of eyelids and corneoscleral pigmentation. Crossbreeding Hereford with dark-pigmented breeds such as Angus or *Bos indicus* breeds such as Brahman or Nellore may increase pigmentation and thereby reduce the probability of cancer in eyes. A subsequent objective in this work will be assessment of variation of these traits within straightbred Hereford with increased numbers of records. Identification of genomic regions responsible for this trait variation could have practical benefit in selection programs. Investigation of the inheritance and lifetime progression of corneoscleral pigmentation may be merited, especially in straightbred Herefords. The interaction of this pigmentation with lesion formation is incompletely understood and may represent an opportunity to enhance health and longevity in cows with white faces.

LITERATURE CITED

Alario, A. F., C. G. Pirie, and S. Pizzirani. 2013. Anterior segment fluorescein angiography of the normal canine eye using a dSLR camera adaptor. Vet. Ophthalmol. 16:10–19. doi:10.1111/j.1463-5224.2012.01007.x.

Anderson, D. E. 1960. Studies on bovine ocular squamous carcinoma (“cancer eye”) V. Genetic Aspects. J. Hered. 51:51–58.

Anderson, D. E. 1963. Genetic aspects of cancer with special reference to cancer of the eye in the bovine. Ann. N. Y. Acad. Sci. 108:948–962. doi:10.1111/j.1749-6632.1963.tb13432.x.

Anderson, D. E. 1970. Cancer eye in cattle. Mod. Vet. Pract. 51:43–47.

Anderson, D. E. 1991. Genetic study of eye cancer in cattle. J. Hered. 82:21–26. doi:10.1093/jhered/82.1.21.

Anderson, D. E., J. L. Lush, and D. Chambers. 1957. Studies on bovine ocular squamous carcinoma (“cancer eye”) II. Relationship between eyelid pigmentation and occurrence of cancer eye lesions. J. Anim. Sci. 16:739–746.

Anderson, D. E., and P. E. Skinner. 1961. Studies on bovine ocular squamous carcinoma (“cancer eye”) XI. Effects of sunlight. J. Anim. Sci. 20:474–477.

Barry, B., U. Gonzales Barron, F. Butler, S. Ward, and K. McDonnell. 2011. Verification of sheep identity by means of a retinal recognition system. Trans. ASABE 54:1161–1167. doi:10.13031/2013.37081.

Core, S., T. Widowski, G. Mason, and S. Miller. 2009. Eye white percentage as a predictor of temperament in beef cattle. J. Anim. Sci. 87:2168–2174. doi:10.2527/jas.2008-1554.

Davis, A. K., B. Farrey, and S. Altizer. 2004. Quantifying monarch butterfly larval pigmentation using digital image analysis. Entomol. Exp. Appl. 113:145–147. doi:10.1111/j.0013-8703.2004.00211.x.

Davis, K., T. Smith, J. D. Arthington, N. DiLorenzo, B. Bolt, M. D. Garcia, J. G. Powell, D. L. Lalman, G. R. Hansen, R. C. Vann, S. Meadows, and D. G. Riley. 2013. Eye pigmentation in Hereford, Hereford × *Bos taurus* or Hereford × *Bos indicus* cows and calves. J. Anim. Sci. 91(Suppl. 2):8. (Abstr.).

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2009. ASReml user guide release 3.0. VSN Int., Hemel Hempstead, UK..

Heeney, J. L., and V. E. O. Valli. 1985. Bovine ocular squamous cell carcinoma: An epidemiological perspective. Can. J. Comp. Med. 49:21–26.

Hiby, L., P. Lovell, N. Patil, N. S. Kumar, A. M. Gopalaswamy, and K. U. Karanth. 2009. A tiger cannot change its stripes: Using a three-dimensional model to match images of living tigers and tiger skins. Biol. Lett. 5:383–386. doi:10.1098/rsbl.2009.0028.

Karanth, K. U., J. D. Nichols, N. S. Kumar, and J. E. Hines. 2006. Assessing tiger population dynamics using photographic capture–recapture sampling. Ecology 87:2925–2937. doi:10.1890/0012-9658(2006)87[2925:ATPDUP]2.0.CO;2.

Karlsson, O., L. Hiby, T. Lundberg, M. Jussi, I. Jussi, and B. Helander. 2005. Photo-identification, site fidelity, and movement of female grey seals (*Halichoerus grypus*) between haul-outs in the Baltic Sea. Ambio 34:628–634.

Kelly, M. J. 2001. Computer-aided photograph matching in studies using individual identification: An example from Serengeti cheetahs. J. Mammal. 82:440–449. doi:10.1093/jmammal/82.2.440.

Kim, H. T., Y. Ikeda, and H. L. Chou. 2005. The identification of Japanese Black cattle by their faces. Asian-Australas. J. Anim. Sci. 18:868–872.

Labelle, A. L., C. B. Dresser, R. E. Hamor, M. C. Allender, and J. L. Disney. 2013. Characteristics of, prevalence of, and risk factors for corneal pigmentation (pigmentary keratopathy) in pugs. J. Am. Vet. Med. Assoc. 243:667–674. doi:10.2460/javma.243.5.667.

Liu, L., B. Harris, M. Keehan, and Y. Zhang. 2009. Genome scan of pigmentation traits in Friesian-Jersey crossbred cattle. J. Genet. Genomics 36:661–666.

Pitt, F. 1920. Notes on the inheritance of colour and markings in pedigree Hereford cattle (with plates VII-X). J. Genet. 9:281–307. doi:10.1007/BF02983274.

Rojas-Olivares, M. A., G. Caja, S. Carné, A. A. K. Salama, N. Adell, and P. Puig. 2012. Determining the optimal age for recording the retinal vascular pattern image of lambs. J. Anim. Sci. 90:1040–1046. doi:10.2527/jas.2010-3648.

Russell, W. C., J. S. Brinks, and R. A. Kainer. 1976. Incidence and heritability of ocular squamous cell tumors in Hereford cattle. J. Anim. Sci. 43:1156–1162.

Russell, W. O., E. S. Wynne, and G. S. Loquvam. 1956. Studies on bovine ocular squamous carcinoma (“cancer eye”) I. Pathological anatomy and historical review. Cancer 9:1–52. doi:10.1002/1097-0142(195601/02)9:1<1::AID-CNCR2820090102>3.0.CO;2-Z.

Sandem, A. I., B. O. Braastad, and K. E. Bøe. 2002. Eye white may indicate emotional state on a frustration-contentedness axis in dairy cows. Appl. Anim. Behav. Sci. 79:1–10. doi:10.1016/S0168-1591(02)00029-1.

Vogt, D. W., D. E. Anderson, and G. T. Easley. 1963. Studies on bovine ocular squamous carcinoma (“cancer eye”) XIV. Heritabilities, phenotypic correlations, and genetic correlations involving corneoscleral and lid pigmentation. J. Anim. Sci. 22:762–766.

Woodward, R. R., and B. Knapp Jr. 1950. The hereditary aspect of eye cancer in Hereford cattle. J. Anim. Sci. 9:578–581.