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Mechanical properties of black, grey, and white hoof material of sheep

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Abstract

Black, grey and white sheep hoof samples were tested to determine whether hoof colour affected resistance to physical insults. Novel methods for abrasion of hoof keratin and resistance to a punch were developed. Hooves (n=72) were obtained at slaughter, and the hoof horn from the abaxial wall of both claws was punched with a 10 mm hollow punch driven by an Instron universal tester to determine peak punching force. Mean force per unit thickness (\pm s.d.) required to punch black (165.6 ± 18.0 kg/mm) and grey (167.4 ± 18.7 kg/mm) hoof horns were not significantly different, but white hoof required significantly less force (160.6 ± 18.1 kg/mm) to punch ($P = 0.007$). However, white hoof horn was thicker ($P < 0.001$) than black (2.22 vs 1.97 mm, respectively) and total force to punch was therefore similar. Circular pieces of hoof removed from the punch (n=144) were weighed and subjected to an abrasion test. During fifteen hours of abrasion, a large amount of keratin was removed from the samples (25%) but there was no significant difference with respect to hoof colour. The lack of differences between hoof colours in resistance to physical insults suggests colour is unlikely to affect hoof disorders.

Keywords: ovine; hoof pigmentation; abrasion testing

Introduction

Sheep producers have long believed that animals with black hooves are less susceptible to hoof disorders. Although disorders like “shelly hoof” (Conington et al., 2010) and overgrown hooves may not be contagious, they can be time-consuming and laborious to treat. Though little scientific research has been undertaken, there are anecdotal reports among sheep husbandry commentators suggesting that black hooves are inherently resistant to disorders: “Generally, black-pigmented hooves are hardier than white-colored hooves.” (Sheep 201 2017). Many breed standards require dark-pigmented hooves, for example Lincoln, and Texel, while the Poll Dorset standard specifies white hooves (New Zealand Flock Book, 2015).

The structure and mechanical properties of bovine and equine hoof have been investigated in some detail (Bertram & Gosline 1987; Vermunt & Greenough 1995; Kasapi & Gosline 1996; Clark & Petrie 2007; Hinterhofer et al. 2007). There are claims that white equine hooves exhibit poor horseshoe nail retention, are less resilient, softer when wet, more brittle when dry, prone to chipping or cracking and thus wear more quickly than coloured hooves (Thomas 2006). However, these claims have been refuted where no difference could be found between white and pigmented horse hooves in strength, structure or nail holding (Runciman et al. 2004). The structure and mechanical properties of ovine hooves have not been investigated. The following experiment was designed to test the hypothesis that black hoof keratin is more resistant to physical insults than white hoof keratin.

Materials and methods

Sample collection and preparation

Whole feet from sheep of mixed age, mixed sex, and

mixed breed groups were collected from two slaughter facilities and stored chilled before initial processing. Hooves from either side, and from both front and hind legs were obtained. A range of hooves were selected from the many available, including black, white, grey and hooves with black, grey and white stripes.

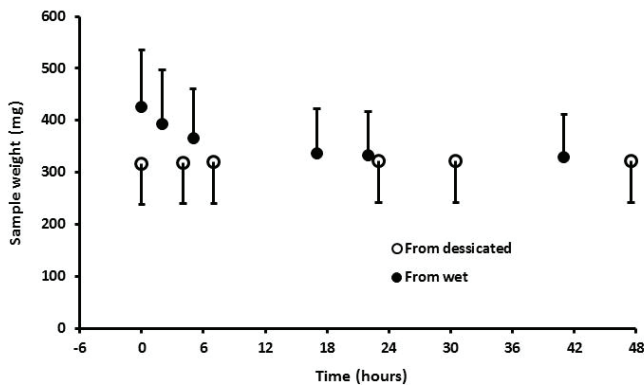
The hoof horn from the abaxial claw wall was separated from the rest of the foot by using hoof snips to incise the horn at the heel, and then a sharp knife to remove the claw horn to the tip. Hoof snips were used to remove the segment of hoof from the claw, the softer heel and the harder curved section at the leading edge of the toe. The abaxial wall of the hoof was retained from both claws and placed in separate bags, labelled, and frozen until further processing. The two claws of a given foot were regarded as replicate samples within animal.

Hoof horn samples were removed from the freezer and thawed in small batches. Adhering tissue was scraped from the flesh side of the hoof horn segment, using a scalpel and without damaging the hoof horn. Much of the white line material remained attached to the hoof horn following scraping. Each sample was then placed in a conditioned room (65% relative humidity, 20°C) for several days, until thoroughly dried.

Equilibration to standard conditions

The time taken for a piece of ovine hoof to equilibrate to 65% relative humidity and 20°C was established using representative samples to establish a protocol for subsequent experiments. Small pieces of black and white hoof keratin (n = 15) were dried in a vacuum desiccator, weighed, and then allowed to equilibrate in an environment of 20°C and 65% relative humidity. The samples were weighed at intervals over the following 48 hours. These samples were then soaked in water overnight and removed

Figure 1 Time taken for small samples of hoof to equilibrate in 65% R.H. and 20°C, when removed from water or from a vacuum desiccator. Error bars indicate the standard deviation of weight of sample, but are only shown in a single direction.



into the controlled environment room, where surface moisture was quickly removed using paper towel before weighing. The samples were allowed to equilibrate and were weighed at intervals over the following 48 hours. The change in weight is shown in Figure 1 and had stabilized at 24 hours while equilibrating from either wet or dry. The error bars in the graph indicate the standard deviation of the weight of sample in one direction only for clarity of the illustration, and readers should note that each individual sample showed a very consistent ranking within the range while either drying or regaining moisture.

Punch testing

Punch testing was conducted in a conditioned room at 65% relative humidity and 20°C using an Instron (Model 4204, Instron Corp., Canton, MA, USA). A circular hollow punch (Groz Engineering Tools, Khekri Daula, National highway 8, Gurgaon 122001 Haryana, India) was mounted in a holder and rested on the hoof horn as close as possible to the leading edge and distal end of the hoof. A range of punch diameters (8, 10 and 12 mm) were used for different experiments outlined below. A 5 kN load cell was set to 500 kg, sampling frequency at 10 Hz, with a full scale deflection of 25 mm. The Instron was set to push the punch through the hoof horn at 10 mm/minute, and kilograms of force required and the distance travelled (Extension) were collected electronically from the Instron using in-house software. The sample was then measured with callipers from four angles to determine thickness. The white line material was very strongly bonded to the hoof horn, so both were tested together. Differential drying of these two materials exaggerated the natural curve of the horn, and the punch pushing down removed this curve before it began to cut through the two materials. The natural curve of the hoof made it very difficult to secure and punch whole hoof pieces with the white line or flesh side down, so a 12 mm punch was used to remove a more manageable section of dried hoof.

Wet vs dry. In order to determine the best conditions for punch testing, prior to testing different-coloured hoof

horn samples, two sample conditions and two sample orientations were examined first. The 12 mm round samples from two claws of 24 sheep ($n = 48$) were allocated to paired tests and punched again with an 8 mm punch. Half of the left claws were randomly allocated to be tested wet and the other half dry, with the right claw allocated to the opposite treatment in each case. Grey hooves were used because these were most readily available. The effect of orientation was examined by positioning half of the samples with the white line uppermost and the punch was pushed through from this side, and the other half of the samples were punched with the white line down. Each variable of interest was then compared between the sample conditions and orientations using analysis of variance (ANOVA), which consisted of two factors: condition (wet or dry) and orientation (up or down), and their interaction. When the interaction was found to be statistically non-significant, the second ANOVA excluding the interaction, was used for group comparisons.

Hoof colour. To test the effect of hoof colour, a randomly selected white sample was punched with the white line up using a 10 mm punch, followed by a grey sample, then a black sample, and this was repeated until 72 claws had been punched. The procedure was repeated with the second claw from each hoof until 144 samples were punched, with 48 samples in each colour. The white and grey samples consisted of equal numbers from front and rear legs (24), while 22 black samples were from the front legs and 26 from the rear. This process required five days, and hence, samples were blocked by these days to ensure that equal numbers of white, grey, and black samples were tested on each day. Comparison between the samples with different hoof colours was made with mixed model ANOVA, which consisted of main effects of three factors: hoof colour, day (testing day) and leg (front or rear leg) as fixed effects, and within-foot correlation (correlation among two claws of the same foot) as random effects. The factors' interaction terms were not included since neither of them was found to be statistically significant. Punched pieces remained in the conditioned room until subsequently used for abrasion testing.

Abrasion testing

Sets of eighteen punched pieces (10 mm) were weighed, then placed in 50 mL Falcon tubes lined with P60 sandpaper (Norton Abrasives, 1 New Bond St, Worcester, MA 01615, USA). These tubes were randomly placed in an eighteen-sample Trident shaker (WRONZ Developments Ltd, Private Bag 4749, Christchurch, New Zealand) and shaken for 15 hours. The Trident shaker oscillated the Falcon tubes along their length. The worn samples were removed from the tubes and allowed to equilibrate for 24 hours at 65% relative humidity and 20°C before being weighed. The abrasion testing (testing all 144 samples) was blocked into eight runs including 6 samples of each colour in each run, with the 72 samples from the first claw being tested in the first four runs and the 72 samples from

the second claw in the remaining four runs. The sandpaper was not renewed between runs. The amount of wear was defined as: (1) the weight of sample eroded during shaking (weight difference between before and after abrasion) and (2) the weight difference as a percentage of the pre-abrasion weight (abrasion %).

The weight differences were compared between the hoof colour groups using mixed model analysis of covariance (ANCOVA). The fixed effects in the ANCOVA were main effects of two factors: hoof colour and run, plus pre-abrasion weight as a single covariate. Interaction terms were not included, since neither of them was found to be statistically significant. The ANCOVA also modelled within-foot correlation as random effects. The abrasion % values were compared between the hoof colour groups with mixed model ANOVA, which consisted of main effects of hoof colour and run factors only as fixed effects, and within-foot correlation as a random effect.

Results

Punch testing conditions

Testing dry versus wet revealed dramatically different behaviour (Figure 2) when punching, but whether the white line material was up or down had less effect on the output from the Instron (Table 1). Dry samples were on average thinner ($P < 0.001$) than paired samples when wet (2.08 vs 2.86 mm). The dry samples were significantly lighter ($P < 0.001$) at 106 mg than those that had been soaked in water for 24 hours (195 mg). There was no significant interaction between hydration and orientation during punch testing ($P = 0.484$). Mean force per unit thickness was statistically significantly larger in dry hooves than wet hooves (Table 1), in both test positions ($P < 0.001$). Mean force per unit thickness was statistically significantly larger when the white line side was uppermost (Table 1) in both dry and wet hooves ($P = 0.003$).

Figure 2 Example force extension curves generated while pushing an 8 mm punch through wet or dry ovine hoof samples, with the white line side of the hoof horn uppermost (Up) or down (Down).

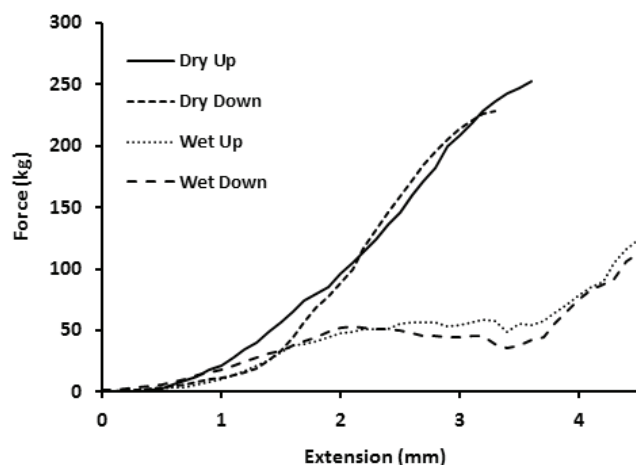


Table 1 Average weight of punched sample (Weight), and mean extension (Extension), thickness (Thickness), and force (Force) recorded when punching grey ovine hoof samples dried at 65% R.H. and 20°C (Dry) or soaked in water for 24 hours (Wet). The mean force per unit thickness to punch the samples when they were tested with the white line side up or down is also shown. (Standard deviation in parentheses).

Parameter	Dry	Wet
Weight (mg)	106 (± 10)	195 (± 27)
Extension (mm)	4.19 (± 0.36)	5.34 (± 0.19)
Thickness (mm)	2.08 (± 0.15)	2.86 (± 0.35)
Force (kgf)	243.7 (± 19.8)	121.5 (± 18.1)
Force/thickness (kgf/mm)		
White line up	121.7 (± 8.5)	46.2 (± 8.7)
White line down	112.6 (± 5.9)	40.4 (± 7.7)

Table 2 Average force (kgf), and average force per unit thickness (kgf/mm) required to punch black, grey, and white hoof samples and average thickness (Thickness) of those samples. (Standard deviation in parentheses)

Hoof Colour	kgf	Thickness (mm)	kgf/mm
Black	325.0 (± 35.4) ^a	1.97 (± 0.15) ^a	165.6 (± 18.0) ^a
Grey	346.5 (± 37.2) ^b	2.08 (± 0.18) ^b	167.4 (± 18.7) ^a
White	354.7 (± 32.7) ^b	2.22 (± 0.21) ^c	160.7 (± 18.1) ^b
P-value	< 0.001	< 0.001	0.007

Comparison of hoof horn colours

White hoof material was thicker than black hoof ($P < 0.001$), and required more force for the Instron to push a 10 mm punch through compared with black hoof material ($P < 0.001$), but white hoof was more easily punched when adjusted for thickness (Table 2). Grey hoof was intermediate in peak force to push a punch through and thickness, but required the highest mean force per unit thickness to push a punch through per unit thickness. The force per unit thickness to punch white and black hooves was significantly different ($P = 0.007$), but there was no difference between black and grey hoof. It is interesting to note that hoof material from the front leg (157.6 ± 1.4 kgf/mm) required significantly less force ($P = 0.048$) to punch through than material from the rear leg (161.4 ± 1.3 kgf/mm).

Abrasion testing

Figure 3 shows the percentage abrasion over the course of abrasion testing all 144 samples. A decrease in the proportion of the initial sample worn away was noticeable, most likely due to loss of abrasiveness and clogging of the sandpaper. This slight reduction in performance of the abrasion test was overcome by blocking between runs and randomisation within hoof colour.

The weight of the punched samples, both pre- and post-abrasion, was associated with their thickness (Figure 4). A regression equation for the initial weight of the sample (y) was calculated as $y = 0.0904x + 0.0045$, where x was thickness in millimetres ($R^2 = 0.925$). When the samples

Figure 3 Percentage abrasion of hoof samples over the course of testing 144 samples, in batches of 18, with two replicates from each hoof randomised within blocks of 72. A slight decline in the effectiveness of the abrasive paper was evident with a large number of tests.

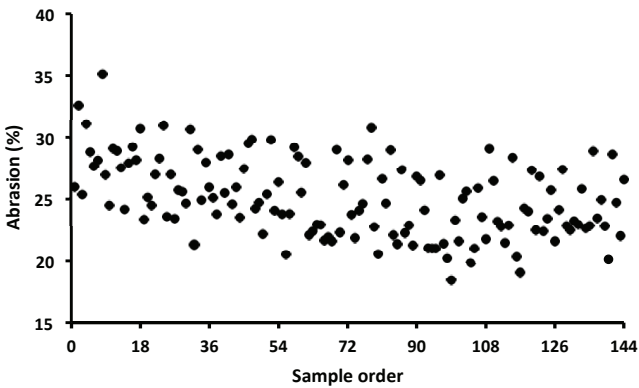
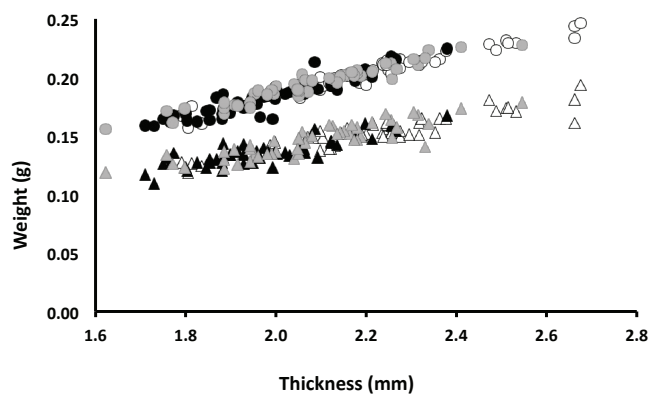


Figure 4 The relationship between thickness of ovine hoof keratin measured before abrasion testing and weight measured before and after abrasion testing. Circles indicate values before abrasion testing and triangles indicate values after abrasion testing. Open circles/triangles indicate white hoof samples, closed black circles/triangles indicate black hoof samples, while grey circles/triangles indicate grey hoof samples.



were removed from the shaker, equilibrated, and weighed again, they had lost around 25% of their weight. Wear occurred in a manner which was related to their thickness, such that their weight post-abrasion (α) was related to their initial thickness ($\alpha = 0.0665x + 0.0059$). Since the samples were allocated randomly to the abrasion test, and the effectiveness of the abrasion test declined between batches, the association between initial thickness and weight post-abrasion became more variable ($R^2 = 0.818$). However, there was no statistically significant difference between the weight removed during abrasion nor the amount of wear as a percentage (Table 3) according to colour of the hoof.

Discussion

Contrary to the hypothesis, black ovine hoof keratin did not abrade more readily than white or grey ovine hoof keratin in the abrasion test ($P = 0.573$). The method was

Table 3 Mean weight difference (Difference) between samples before and after abrasion, and weight difference expressed as a percentage (Abrasion %) for black, grey, and white hoof samples. (Standard error of the mean in parentheses)

Hoof Colour	Difference (mg)	Abrasion %
Black	49.05 (± 0.84)	25.10 (± 0.39)
Grey	47.90 (± 0.77)	24.78 (± 0.39)
White	48.65 (± 0.83)	25.28 (± 0.39)
P-value	0.573	0.653

very effective in producing wear on small hoof samples, and could be utilised in other natural or industrial materials. Although white hooves appeared to require more total force to punch with an Instron, white hoof horn was thicker and significantly higher force per unit of thickness was required to punch through black and grey hoof material than white. To put things in perspective though, hoof material from the rear leg also required more force per unit thickness to punch ($P = 0.048$). Despite being detectable and statistically significant, the very small proportional differences in resistance to the punch between front and rear (2%), or indeed between black, grey and white hoof material (4%) may be of little consequence to the function of the hoof horn or resistance of the whole hoof structure to wear. In contrast, wet hoof material required about half the force for the Instron to push a punch through than when dry, either from the white line side or from the outside of the horn. This difference between wet and dry states may be of greater consequence to insults to the hoof horn than the colour of the hoof.

The relationship between hoof pigmentation and strength or hardness has previously been examined in equine and bovine hoof. Horse hoof colour had no effect on peak load or energy required to extract horseshoe nails (Runciman et al. 2004). Higher lesion scores of the foot sole were associated with less-pigmented feet in dairy cattle, and Jersey cattle were less likely to have claw problems than black-and-white breeds (Chesterton et al. 1989). Logue et al. (1994) found no difference in hardness between Jersey cross and Holstein-Friesian cross cows. Vermunt and Greenough (1995) reviewed reports from a number of authors that were equivocal on the hardness of bovine hoof horn with respect to colour, but suggested that contusions and lameness were more likely in animals with lighter-coloured hooves. Resistance to the punch in the current experiment was only tested on hoof horn and not the sole as in some reports, and differences may be expressed in ovine hoof soles.

Testing wet samples brought complications with respect to how much water should be removed by paper towel prior to testing, and the potential to cause wetting of the instruments and corrosion of the punch or the callipers used to measure thickness. Samples tested with the horn and, thus, the convex curve of the horn uppermost, required less force to punch through (7.5%), though in this orientation the sample could shift dramatically while the curvature was

removed during compression. Proceeding with dry samples with the white-line side uppermost was the most convenient option for testing the differences between hoof colours and gave a more secure positioning of the punch and less erratic recording of the force-extension curve from the Instron.

Numerous reports on hoof material from other species reported possible anisotropic behaviour of samples during mechanical tests (Kasapi & Gosline, 1996; Douglas et al. 1996; Hinterhofer et al. 2007). By using a circular punch, the potential effects of non-uniform behaviour due to the inherent structure of the hoof were largely avoided here. Interestingly, the hoof pieces remained circular following the abrasion test (Figure 5). This suggests that hoof wear is similar regardless of orientation, though of course the continuously growing hoof on the live animal would predominantly be exposed to erosion at only one surface. Lambertz et al. (2014) compared Rhoen and Merinoland sheep, and reported hoof shape differences between these breeds but hoof colour was not mentioned. Although the wear of keratin may not be affected with respect to the direction of growth of the hoof horn in the abrasion test here, the loads placed on the hoof horn by the animal could very well be different in hooves of different shapes. This may produce differences in hoof wear during walking, for example, a lack of wear on the tip of the claw may predispose the hoof to become overly long. It is interesting to speculate that a thicker hoof may be better-adapted to walking long distances over harsh dry ground as expected of Merino sheep in rangelands across the globe, while thin hooves may wear faster, retain shape and be better suited to crossbred sheep in small paddocks on softer wet soils under more intensive grazing conditions. Hoof colour is possibly an unrelated a consequence of breeder preferences.

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Figure 5 A white hoof sample after being punched from the hoof horn with an Instron and a black hoof sample after abrasion testing. The samples were 10 mm across before abrasion, smaller and thinner after abrasion, but they remained circular.



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