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4 authors:

D. Phillip Sponenberg
Virginia Tech (Virginia Polytechnic Institute and State University)

Shosuke Ito
Fujita Health University

Kazumasa Wakamatsu
Institute for Melanin Chemistry Fujita Health University

Ludeman Eng
Virginia Tech (Virginia Polytechnic Institute and State University)

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Pigment Types in Sheep, Goats, and Llamas

D. PHILLIP SPONENBERG,[1] SHOSUKE ITO,[2] KAZUMASA WAKAMATSU,[2] AND LUDEMAN A. ENG[1]

[1]Virginia-Maryland Regional College of Veterinary Medicine, VPI&SU, Blacksburg, Virginia, 24061, [2]Fujita-Gakuen Health University School of Hygiene, Toyoake, Aichi 470-11 Japan.

Pigment types in various colors of fiber from sheep, goats, and llamas were assayed by a method using high performance liquid chromatography. In these three species the black/gray group is due to eumelanin, which is fully intense in all three species. Red phenotypes are due to pheomelanin and fade considerably with age in fiber from sheep and goats, but not in llamas. This phenomenon has implications on the genetic mechanisms used in generating white fiber. Brown phenotypes in sheep are due to eumelanin, in goats these phenotypes are equivocal, and they were not observed in llamas.

Key words: Eumelanin, Pheomelanin, Sheep, Goat, Llama, Coat Color

INTRODUCTION

Humans use the fiber produced by a variety of species for clothing and other textile needs; in fact, this was one of the early technological hallmarks of civilization (Mason, 1984). Early in the development of fiber from these species, whiteness of fiber was selected since such fibers are easily dyed with several substances to give bright colors (Adalsteinsson, 1983). Natural colors, in contrast, are limited to the black, gray, brown, and reddish to yellowish shades of eumelanin and pheomelanin. Recent resurgence of interest in the color variation of fiber-producing animals and in the genetic basis of those colors has revealed some of the genetic basis for the whiteness that has been the goal of most selection in these species. We compare here the presence of eumelanin and pheomelanin in fibers from various colors of sheep, goats, and llamas.

MATERIALS AND METHODS

Eumelanin and pheomelanin in samples of fiber of various colors were analyzed. The fiber samples were collected by D.P.S. over a period of several years. These included all of the common and many of the rarer color phenotypes of sheep, goats, and llamas. Samples from all available phenotypes were analyzed. Analysis for melanin content was by the method of Ito and Fujita (1985) using chemical degradation and high performance liquid chromatography (HPLC). Approximately 30 mg hair were homogenized in water at a concentration of 10 mg/ml. For eumelanin estimations (which were performed in duplicate), 200 μl of homogenate (2 mg hair) were transferred to a screw-capped test tube, mixed with 800 μl of 1 M H_2SO_4 and oxidized with 3% $KMnO_4$. The product, pyrrole-2, 3, 5-tricarboxylic acid (PTCA), was analyzed by HPLC with ultraviolet detection. For pheomelanin estimation, 200 μl of the homogenate were transferred to a screw-capped test tube and hydrolyzed at 130°C for 24 h with 500 μl of 57% hydriodic acid in the presence of H_3PO_2. The product aminohydroxyphenylalanine (AHP), was analyzed by HPLC with electrochemical detection. Contents of PTCA and AHP of 1 ng roughly correspond to a eumelanin content of 50 ng and a pheomelanin content of 5 mg, respectively.

RESULTS AND DISCUSSION

The results of this analysis are presented in Tables 1–3. The advantage of using the present technique to determine melanin content of fiber is that it chemically quantitates both pheomelanin and eumelanin in the same sample. Eumelanin and pheomelanin can sometimes be confused phenotypically. For example, the crossbred sheep 32 (Table 1) is classified as red but is clearly eumelanic. The other common method of determining melanin content rests on the detection of pheomelanin by an electron spin resonance spectroscopy technique (Sealy et al., 1982; Vsevolodov et al., 1987). Whereas the studies of Sealy et al. (1982) indicate that the character of the ESR spectra can predict mixtures, those of Vsevolodov et al. (1987) indicate solely the presence or absence of pheomelanin and could potentially overlook samples in which mixed melanogenesis had occurred. The ESR technique also is limited to determining the ratio of pheomelanin to eumelanin rather than the absolute quantities. The HPLC technique has the advantage of quantitating both melanins.

The results of melanin analysis in Tables 1–3 support the contention that the black of sheep, goats, and llamas is eumelanin, and that the red is pheomelanin. The only intensely pheomelanic sheep and goats were young animals. Older animals of the same genotype fade con-

Address reprint requests to D.P. Sponenberg, Virginia-Maryland Regional College of Veterinary Medicine, VPI&SU, Blacksburg, VA 24061.
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TABLE 1. Eumelanin and Pheomelanin Contents in Sheep Wool

Color	Breed	PTCA[a] (ng/mg hair)	AHP[b] (ng/mg hair)	PTCA/AHP ratio[c]	Melanogenesis type[d]
White (A^{Wh})					
13	Cotswold	4.9	10	0.49	
15	Cotswold	0.6	15	0.040	
52	Sulfolk	2.5	13	0.19	
Fading red (A^{Wh})					
46	Blackbelly cross (lamb)	11	476	0.023	P (most)
30	Crossbred	18	224	0.080	P
29	Crossbred, lamb	16	77	0.21	
32	Crossbred	113	16	7.1	E?
49	Karakul lamb	8.5	70	0.12	
51	Karakul adult	11	123	0.089	P
55	Crossbred adult	23	60	0.38	
31	Crossbred 2 yr	5.7	46	0.12	
54	Crossbred adult	2.9	87	0.033	
Gray					
14	Cotswold	136	13	10	E?
11	Lincoln	165	16	10	E?
Black					
12	Crossbred	495	6.6	75	E
44	Border Leicester	670	30	22	E
56	Finn-Rambouillet	342	23	15	E
58	Finn-Dorset	937	26	36	E (most)
Brown					
16	Crossbred	61	15	4.1	
17	Crossbred	40	10	4.0	
18	Crossbred	46	10	4.6	
19	Montadale	82	14	5.9	
45	Shetland	201	36	5.6	E
47	Crossbred	694	43	16	E
48	Corriedale	207	20	10	E
50	Crossbred	213	60	3.6	E
53	Corriedale	211	33	6.4	E
76	Crossbred	643	35	18	E
77	Crossbred	182	106	1.7	E?

[a]Pyrrole-2,3,5 Tricarboxyic acid, an eumelanin indicator. Values exceeding 100 ng/mg are underlined.
[b]Aminohydroxyphenylalanin, a pheomelanin indicator. Values exceeding 100 mg/mg are underlined.
[c]Values above 1.0 or below 0.1 are underlined, provided that either or both of PTCA and AHP are above 100 ng/mg.
[d]E, eumelanic: PTCA > 100 ng/kg; PTCA/AHP ratio > 1.0. P, mainly pheomelanic AHP > 100 ng/mg; PTCA/AHP ratio < 0.1. M, mixed type: either or both of PTCA and AHP > 100 ng/mg; PTCA/AHP ratio 0.1 to 1.0.

siderably. The llama samples are intensely pigmented, either eumelanin (black) or pheomelanin (red brown). The intensity of the pheomelanin indicates that little if any selection has occurred for pale pheomelanin types in llamas.

The most commonly used allele to generate white sheeps wool is A^{Wh}, which is the top dominant allele at the agouti locus (Adalsteinsson, 1983; Vsevolodov et al., 1987). Comparative coat color genetics indicates that this should be wholely red (pheomelanic) (Searle, 1968), but selection for whiteness has evidently reduced the pheomelanin present in wool-producing breeds of sheep (Vsevolodov et al., 1987). Indeed, the only pheomelanic fibers available in sheep are from hair sheep (Barbados Blackbelly) and pelt sheep (Karakul). Karakul lambs of the A^{Wh} phenotype are born a reddish brown but quickly fade to a light beige or off-white color. It is extremely rare to find a pheomelanic sheep that has retained much pigment in the wool. This phenomenon of fading pheomelanic phenotypes is probably the end result of centuries of selection against the formation of pheomelanin, resulting in white wool (Adalsteinsson, 1983).

The exact genetic mechanism of pigment reduction in sheep has not been characterized. Several loci in rodents do dilute color by a variety of mechanisms, but these may or may not be acting in the sheep. The C-locus of mice is a multiple allelic series that tends to dilute pheomelanin more than eumelanin, especially with the alleles of intermediate dominance. The most recessive allele, albino, is unlikely to be acting in most white sheep since the ocular effects of this allele are lacking in sheep. Adalsteinsson (1977) has reported al-

TABLE 2. Eumelanin and Pheomelanin Contents in Goat Hair

Color	Breed	PTCA[a] (ng/mg hair)	AHP[b] (ng/mg hair)	PTCA/AHP ratio[c]	Melanogenesis type[d]
White					
26	Angora	1.5	18	0.083	
Fading red					
37	Angora kid	77	91	0.85	
38	Angora kid	44	23	1.9	
39	Angora kid	7.3	480	0.015 P	
40	Angora kid	9.0	461	0.020 P	
25	Angora	5.3	16	0.33	
27	Angora	3.4	82	0.041	
28	Angora	5.3	75	0.071	
33	Angora	1.7	34	0.050	
34	Angora	16	68	0.24	
35	Angora	3.8	18	0.21	
Gray					
23	Angora	193	6.8	28	E?
Black					
22	Angora	601	22	27	E (most)
24	Angora	354	18	20	E
Brown					
1	Toggenburg	20	22	0.91	
36	Angora	17	33	0.52	
41	Angora	27	22	1.2	
42	Angora	36	21	1.7	
43	Angora	13	62	0.21	

[a]Pyrrole-2,3,5 Tricarboxyic acid, an eumelanin indicator. Values exceeding 100 ng/mg are underlined.
[b]Aminohydroxyphenylalanin, a pheomelanin indicator. Values exceeding 100 mg/mg are underlined.
[c]Values above 1.0 or below 0.1 are underlined, provided that either or both of PTCA and AHP are above 100 ng/mg.
[d]E, eumelanic: PTCA > 100 ng/kg; PTCA/AHP ratio > 1.0. P, mainly pheomelanic AHP > 100 ng/mg; PTCA/AHP ratio < 0.1. M, mixed type: either or both of PTCA and AHP > 100 ng/mg; PTCA/AHP ratio 0.1 to 1.0.

TABLE 3. Eumelanin and Pheomelanin Contents in Llama Hair

Color	PTCA[a] (ng/mg hair)	AHP[b] (ng/mg hair)	PTCA/AHP ratio[c]	Melanogenesis type[d]
Red				
84	13	1120	0.012	P
85	<1.0	134	<0.01	P?
86	18	441	0.040	P
87	18	314	0.057	P
88	18	1540	0.012	P
90	31	2170	0.014	P (most)
Black				
89	220	1900	0.12	P~M
91	990	31	32	E

[a]Pyrrole-2,3,5 Tricarboxyic acid, an eumelanin indicator. Values exceeding 100 ng/mg are underlined.
[b]Aminohydroxyphenylalanin, a pheomelanin indicator. Values exceeding 100 mg/mg are underlined.
[c]Values above 1.0 or below 0.1 are underlined, provided that either or both of PTCA and AHP are above 100 ng/mg.
[d]E, eumelanic: PTCA > 100 ng/kg; PTCA/AHP ratio > 1.0. P, mainly pheomelanic AHP > 100 ng/mg; PTCA/AHP ratio < 0.1. M, mixed type: either or both of PTCA and AHP > 100 ng/mg; PTCA/AHP ratio 0.1 to 1.0.

binism in sheep. The chinchilla-type alleles could be responsible, as these remove essentially all visual expression of pheomelanin but leave eumelanin relatively unaffected (Searle, 1968). If this is the locus responsible for reduction of expression, then the hair sheep (Barbados Blackbelly) crosses should demonstrate segregation of a single allele. Although extensive data are not available, it is observed (Sponenberg) that the crosses are usually born intense red but fade quickly to be white-fleeced. Haired portions of the sheep (face, legs) usually retain the reddish pheomelanic hairs for all of their life. No segregation seems to occur (although untested directly) in the lambs resulting from the backcross of Barbados Blackbelly and wool sheep ewes to wool sheep rams. The lambs tend to be red or red spotted at birth. The expectation of the C-locus hypothesis would be half of the lambs red and half white, which is not observed. Searle (1968) also finds no evidence of the C-locus being active in sheep.

The P-locus of rodents acts to reduce eumelanin while leaving pheomelanin relatively unaffected (Searle, 1968). This is the opposite of the situation observed in sheep, and so is unlikely to be the mechanism resulting in white fleeces. The D-locus alleles reduce pigment of hair, but act on both eumelanin and pheomelanin. The fact that most eumelanic phenotypes of sheep are fully intense makes the D-locus an unlikely candidate for the reduction of pheomelanin in white sheep. So whereas the exact mechanism of pigment reduction in white wooled sheep is undetermined, there are sound reasons for believing that the alleles responsible are not at the C-, P-, or D-locus, although the situation at the C-locus does need more investigation.

The genetics of color in Angora goats (the mohair-producing goat) has not been documented, but several agouti locus patterns similar to those in sheep have been noted. In these patterns the areas expected to be pheomelanic are white, which is similar to the same patterns in sheep where selection against expression of pheomelanin results in very pale or white pheomelanic areas in agouti locus patterns. In goats, then, as in sheep, white phenotypes seem to be accomplished by a selection for reduction of pheomelanin to yield white fiber while selecting for a wholely pheomelanic phenotype (A^{Wh} in sheep) to eliminate eumelanic fibers as well. Since the most usual nonwhite Angora goat is a fading red, it is tempting to suppose that the mechanisms at work for white fiber production in sheep are also at work in goats. Both the close relationship of the animals and the common geographic area of domestication and postdomestication development favor this interpretation.

Llamas, in contrast, are camelids, not bovids, and were domesticated in an entirely different geographic area than sheep and goats. Still, white fiber is much preferred in llamas and alpacas when they are raised for fiber production. However, intensely pheomelanic fibers do occur commonly in the llama. These vary from a dark brown to a paler reddish brown, but in any event they are intensely pigmented. The presence of intense pheomelanin as a common color in llamas indicates that the genetic mechanisms used in selection for white fleeces are probably different than those used in sheep and goats or that selection for white has been less intense. These are probably white-spotting alleles, but this remains to be proven. That such could be used to generate pure white fiber is demonstrated in selection experiments in which spotted sheep were selected for extensive spotting until white-fleeced animals resulted (Cook, 1950; Panfilova, 1939).

Chocolate brown animals were present in sheep and goats but not llamas. All chocolate brown goats had low melanin content of fiber, and it is not possible to say definitely if the pigment is mainly eumelanin, pheomelanin, or some combination. Such low pigment concentrations raise the possibility that these colors in goats are due to a dilution of color that is independent of and more severe than the brown (*B*) locus action of other mammals. The genetic mechanism for the brown of goats has not yet been proven, and these results do not help in formulating a hypothesis. Brown sheep, in contrast, are usually conditioned by a *bb* genotype at the brown locus (Adalsteinsson, 1983). The results on most of the brown sheep indicate concentrations of eumelanin consistent with this hypothesis. The decreased concentration of eumelanin in *bb* sheep as compared to *B-* sheep is similar to results found in mice (Ito et. al. 1984; Russell, 1946).

CONCLUSION

These results indicate that black and gray in fiber-producing species are due to eumelanin. Red is generally due to pheomelanin, but is much reduced in sheep and goats to give white fiber (wool or mohair in this study). Intensely pheomelanic types were not found in fiber-producing breeds studied, except in the Karakul sheep, which is selected for very intensely pigmented birth coat with little or no selection for color of adult wool. Llamas, in contrast, have intensely pheomelanic phenotypes, probably as a result of less intense selection for white-fleeced animals. Those llamas that are white-fleeced probably result from genetic mechanisms that are different than those typical of sheep and goats. Brown fiber in sheep is commonly eumelanic, in keeping with the *bb* genotype proposed for this. In brown mohair the melanogenesis is weak and of indefinite type, and is therefore probably not of the *bb* genotype. Brown llama fiber was not available for study.

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