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Study of Hair Melanins in Various Hair Color Alpaca (*Lama Pacos*)*

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ABSTRACT : The aim of this study was to measure the hair melanins of various colors and to find the relationship between the quantity of melanins and hair color phenotypes in alpacas. According to the Munsell color system, 3 healthy alpacas were selected for each of the 22 different hair color phenotypes (66 alpacas altogether). Alpaca hair was taken from the lateral thoracic region and then dissolved with different solutions to obtain melanins. The values of alkali-soluble melanins (ASM), eumelanin (EM) and pheomelanin (PM) were measured by spectrophotometric assay, and labeled as Sp.ASM, Sp.EM and Sp.PM, respectively. Data were analyzed using SPSS11.5 software. Results showed that average Sp.ASM and Sp.PM were increased as the color deepened from white to black, ranging from 0.500 to 4.543 for Sp.ASM and from 0.268 to 1.457 for Sp.EM. However, average Sp.PM had no such apparent relationship with color. Based on the value of Sp.ASM and EM, 7 hues were produced and gray was a single hue. Most of the data were in a normal distribution ($p>0.10$). ANOVA analysis showed that mean values of Sp.ASM, Sp.EM and Sp.PM were significantly different ($p<0.05$). The results also showed that Sp.ASM was positively correlated with Sp.EM but the correlation between Sp.ASM and Sp.PM was not significantly different from 0. It is concluded that EM is the major constituent of alpaca hair melanin; there is a significant correlation among ASM, EM and alpaca hair colors, and EM is the most reliable parameter for distinguishing these groups. (**Key Words** : Alkali Soluble Melanin, Eumelanin, Pheomelanin, Alpaca Hair Color, Correlation)

INTRODUCTION

Alpacas (*L. pacos L.*) are classified in the Tilopods suborder together with llamas (*Lama glama L.*), guanacos (*L. guanicoe L.*) and vicuñas (*Vicugna vicugna M.*), and they belong to South American Camelids. Domesticated by the pre-conquest Andean cultures, alpaca is currently used by people all over the world for its fiber. Craftsmen and the international textile industry especially appreciate the natural, fine, colorful fiber produced by alpacas. Because alpaca fibers have 22 colors (Hoffman, 2006), they can be used as commercial material, meanwhile its direct use without chemical dye can protect the body from irritation and the natural environment from being polluted. Therefore, color of the alpaca fiber is an important trait for its commercial use, and thus is important to know the color formation of alpacas.

Mammalian melanogenesis, i.e. the production of the colour pigments eumelanin (EM) and pheomelanin (PM) in melanocytes, or the lack of it, is the main material basis of the color patterns observed in mammals (StepHane et al., 2004). These two types of melanin pigment are chemically distinct (Ito et al., 2000). The eumelanosome is large (~0.9×0.3 μm) and ellipsoidal with a highly ordered glycoprotein matrix that is integral to the production of the black or brown coloured eumelanin pigments, whereas the red or yellow pheomelanins are produced within smaller and spherical (~0.7 μm diameter) pheomelanosomes that are composed of a loosely aggregated and disordered glycoprotein matrix (Sturm et al., 2001). Eumelanin is a highly heterogeneous polymer consisting of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) units in reduced or oxidized state, as well as the pyrrole units derived from their peroxidative cleavage, while pheomelanin consists mainly of sulfur-containing benzothiazine derivatives. Both eumelanin and pheomelanin derive from the common precursor dopaquinone that is produced from tyrosine by the action of tyrosinase (Lamoreux et al., 2001). The color of hair, skin and eyes in animals mainly depends on the quantity, quality and distribution of the pigment melanin (Ito et al., 2003). Eumelanin and pheomelain of several species such as

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humans, mice, fish, and birds were quantitatively analyzed to find the relationship between the quantity of melanin pigments and the hair or skin color (Ito et al., 2000; Lamoreux et al., 2001). Burchill et al. (1986) first reported the application of analytical methods to study the regulation of melanogenesis in mice. Subsequently, a spectrophotometric method for assaying eumelanin in pigmented tissues was developed (Ito et al., 1993; Ozeki et al., 1995; Ozeki et al., 1996). Though high performance liquid chromatography (HPLC) was established to quantify eumelanins and pheomelanins in tissue samples (Ito, 1993), the spectrophotometric method was considered the most suitable for several reasons: i) specific eumelanin solubilization does not discriminate between DHI and DHICA derived units as the HPLC method does; ii) specific pheomelanin solubilization is possible; iii) pheomelanins and some eumelanins can be differentially solubilized (Cecchi et al., 2004). Thus, the spectrophotometric method was used to characterize melanin pigments transferred to hair shafts.

Based on the above biological evidence and methods, an experiment was conducted to improve our understanding of the relationship between pigment contents and hair color phenotypes to supply the evidence of mechanism of hair color formation in alpacas.

Figure 1. Twenty-two different colors of alpaca hair used in the study. 1-4 were white, 5-14 were yellow to brown, 15-19 were grey, 20-22 were black.

MATERIALS AND METHODS

Chemicals

Sepia melanin was purchased from Sigma and used as a standard for spectrophotometric assay of total melanin, eumelanin and pheomelanin. 1 M PBS, hydroiodic acid, hypophosphoric acid and NaOH were purchased from Takaya.

Alpacas and hair

According to the Munsell Color System, which is the standard color measurement of fiber, 3 healthy alpacas were selected for each of the 22 different hair color phenotypes (Figure 1)(66 alpacas altogether). The Munsell color system is a color space that specifies colors based on three color dimensions: hue, value (lightness), and chroma (color purity or colorfulness): hue, measured by degrees around horizontal circles; chroma, measured radially outward from the neutral (gray) vertical axis; and value, measured vertically from 0 (black) to 10 (white). Munsell determined the spacing of colors along these dimensions by taking measurements of human visual responses. Each horizontal circle was divided by Munsell into five principal hues: Red, Yellow, Green, Blue, and Purple, along with 5 intermediate hues halfway between adjacent principal hues. Each of these 10 steps is then broken into 10 sub-steps, so that 100 hues are given integer values. Two colors of equal value and chroma, but on opposite sides of a hue circle, are complementary colors, and mix additively to the neutral gray of the same value. Value, or lightness, varies vertically along the color solid, from black (value 0) at the bottom, to white (value 10) at the top. Neutral grays lie along the vertical axis between black and white. *Chroma*, measured radially from the center of each slice, represents the "purity" of a color, with lower chroma being less pure. Based on the colors determined by the Munsell Color System, the 7 hues were formed. For example, if the result was 2B 5/10, then 2B means the color in the middle of the yellow hue band, 5 means medium lightness, and a chroma of 10 means high purity (from http://en.wikipedia.org). Those phenotypes were from black, dark brown, light brown, yellow to white (Figure 1). All alpacas were female with similar body weight (65-70 kg) and age (2-3). Fibres were washed in mild abulent. Alpacas were not subjected to any unnecessary suffering, following the Shanxi Agricultural University animal care procedures.

Spectrophotometric assay of eumelanin

Sepia melanin suspension (1 mg/ml, prepared by sonication for 5 min) was used as a standard for the spectrophotometric assay of eumelanins. Hair sample suspensions (10 mg/ml) were homogenized with a Tenbroeck tissue grinder (Wheaton, Milville, NJ, USA). For

the spectrophotometric assay of eumelanin, hair samples were hydrolyzed in hot 30% hypophosphoric acid and hydroiodic acid. After cooling, 50% ethanol was added and samples were centrifuged at 2,234 g with a model 4,225 (ALC) centrifuge for 10 min. Insoluble eumelanic pigments were selectively solubilized in hot sodium hydroxide and hydrogen peroxide; they were cleared by centrifugation at 10 700 g for 1 min with a Sorvall Ultracentrifuge (DuPont Instruments, Wilmington, DE, USA). Supernatants were analyzed for absorbance at 350 nm. A350/mg = spectrophorophotometric eumelanin (Sp.EM).

Spectrophotometric assay of pheomelanin

For the spectrophotometric assay of pheomelanins, hair sample suspensions were solubilized in a phosphate buffer (pH 10.5) and cleared by centrifugation at 10 700 g for 10 min. Chloroform was added to supernatants to remove fatty impurities. Pale yellow aqueous layers containing pheomelanins were cleared by centrifugation at 10 700 g for 10 min and analyzed for absorbance at 400 nm. A400/mg = spectrophotometric pheomelanin (Sp.PM).

Spectrophotometric assay of alkali-soluble melanin

For the spectrophotometric assay of alkali-soluble melanin (pheomelanins and some eumelanins) the procedure was the same as for SpPM, but hair sample suspensions were solubilized in 8 M urea/1 M sodium hydroxide. A400/mg = spectrophotometric alkali soluble melanin (Sp.ASM).

Statistical analysis

Data were analyzed using the statistical software SPSS11.5. Kolmogorov-Smirnov and ANOVA analysis were used to test for normal data distribution and mean difference among Sp.ASM, Sp.EM and Sp.PM, respectively. The correlation coefficients among the 3 parameters were also calculated with default setting.

RESULTS

After melanins were extracted, the mixed melanins in different hair colors appeared and looked different (Figure 2). Though the phenotypes of eumelanin and pheomelanin of alpaca were not identified, the phenotype of the alkali-soluble melanin (including pheomelanins and eumelanins) can represent grossly the color of hair (Figure 2). Eumelanins, pheomelanins and alkali-soluble melanins (including pheomelanins and some eumelanins) were measured by spectrophotometric methods (the value of Sp.EM, Sp.PM and Sp.ASM) (Table 1). Average Sp.ASM and Sp.PM were increased as the color deepened from white to black, ranging from 0.500 to 4.543 for Sp.ASM and from 0.268 to 1.457 for Sp.EM. Perhaps because of the small quantity of Sp.PM in the samples, Sp.PM values were

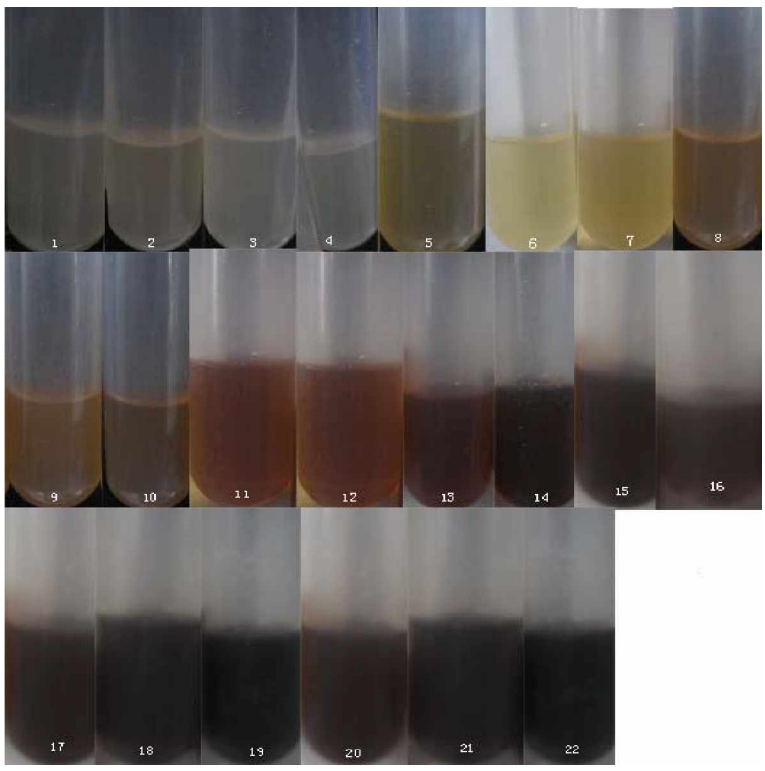

Figure 2. Different phenotypes of alkali-soluble melanins from 22 different hair colors.

Table 1. Melanin contents in different color hair from alpaca

C / Pr	White1	White 2	White 3	White 4	LightYellow1	LightYellow2	LightYellow3
Sp.ASM	0.500±0.004	0.503±0.003	0.523±0.007	0.532±0.003	0.514±0.006	0.548±0.004	0.544±0.005
Sp.EM	0.268±0.006	0.273±0.003	0.272±0.006	0.273±0.003	0.306±0.006	0.328±0.011	0.394±0.005
Sp.PM	0.007±0.001	0.004±0.001	0.011±0.0006	0.002±0.001	0.003±0.0006	0.014±0.001	0.015±0.003
	DeepYellow1	DeepYellow2	DeepYellow3	LightBrown1	LightBrown2	LightBrown3	DeepBrown1
Sp.ASM	0.721±0.004	0.828±0.008	0.950±0.006	1.077±0.005	1.293±0.009	1.448±0.009	1.976±0.006
Sp.EM	0.411±0.010	0.452±0.006	0.464±0.005	0.469±0.004	0.473±0.002	0.470±0.002	0.666±0.011
Sp.PM	0.008±0.0011	0.009±0.003	0.010±0.003	0.009±0.003	0.009±0.003	0.012±0.002	0.016±0.003
	DeepBrown 2	DeepBrown 3	Gray1	Gray2	Gray3	Black1	Black2
Sp.ASM	1.854±0.009	2.027±0.013	1.168±0.009	1.222±0.015	1.931±0.098	3.003±0.111	3.818±0.029
Sp.EM	0.761±0.009	0.912±0.017	0.367±0.006	0.401±0.011	0.571±0.007	0.717±0.019	1.414±0.020
Sp.PM	0.012±0.003	0.016±0.003	0.007±0.001	0.008±0.003	0.018±0.0017	0.002±0.002	0.002±0.002
	Black3						
Sp.ASM	4.543±0.077						
Sp.EM	1.457±0.013						
Sp.PM	0.01205±0.002						

Hair homogenates were examined for the contents of alkali-soluble melanin (ASM), eumelanin (EM) and pheomelanin (PM). Sp.EM = spectrophotometric absorbances of eumelanin at 350 nm; Sp.PM = spectrophotometric absorbances of pheomelanin at 400 nm; Sp.ASM = spectrophotometric absorbances of alkali soluble melanin at 400 nm. The data in the above table are means±SD. The abbreviations were as follows C(color); Pr(parameter).

not directly related to the colors of mixed melanins. Based on the value of Sp.ASM and Sp.EM, 7 hues were produced (Table 2). Both average Sp.ASM and Sp.EM in gray (white+black) were between that in white and black, and gray was a single hue. ANOVA analysis showed that there was no significant difference from white to dark yellow for Sp.ASM and Sp.EM. Differences between means of Sp.ASM, Sp.EM and Sp.PM were significant at the 0.05 level. For Sp.ASM, there was a significant difference from white to black, except from white to dark yellow and between light brown and gray. For Sp.EM, there was a significant difference from white to black, except from white to dark yellow and gray. For Sp.PM, there was no significant difference between light yellow, light brown and dark brown, and between white, dark yellow and black. Generally, there was difference in the 3 parameters among 7 hues (Table 2). Sp.ASM was positively correlated with Sp.EM ($p<0.01$). However, correlations between Sp.ASM and Sp.PM, Sp.EM and Sp.PM were not significant. (Table 3).

DISCUSSION

The hair color of alpacas varies from white, grey, brown, yellow to black with subtle variations in each color (Hoffman, 2006). The most important factors in

Table 2. Estimates for difference in ASM, EM and PM in different hue hair from alpaca

Parameter / Hue	Sp.ASM	Sp.EM	Sp.PM
White	0.5144±0.00415[a]	0.2715±0.00127[a]	0.0059±0.00101[b]
Light Yellow	0.5353±0.00555[a]	0.3427±0.01340[a]	0.0107±0.00207[a]
Dark Yellow	0.8331±0.03308[a]	0.4423±0.00829[a]	0.0089±0.00075[b]
Light Brown	1.2727±0.05384[b]	0.4707±0.00097[b]	0.0101±0.00086[a]
Dark Brown	1.9522±0.02586[c]	0.7797±0.03606[c]	0.0147±0.00101[a]
Gray	1.4403±0.12403[b]	0.4466±0.03167[a]	0.0110±0.00188[a]
Black	3.7881±0.22364[d]	1.1961±0.12005[d]	0.0062±0.00159[b]

22 different hair colors were divided into 7 hues by Munsell color system. Sp.EM = spectrophotometric absorbances of eumelanin at 350 nm; Sp.PM = spectrophotometric absorbances of pheomelanin at 400 nm; Sp.ASM = spectrophotometric absorbances of alkali soluble melanin at 400 nm. The data in the above table was means±SD. In each column, means with different letters a, b, c, and d are significantly different.

Table 3. Correlations among Sp.ASM, Sp.EM and Sp.PM in hair from alpaca

		Sp.EM	Sp.PM
Sp.ASM	Pearson correlation	0.950**	-0.031
	Sig. (2-tailed)	0.000	0.808
	N	66	66
Sp.EM	Pearson Correlation		-0.064
	Sig. (2-tailed)		0.612
	N		66

** Correlation is significant at the 0.01 level (2-tailed). Sp.EM = spectrophotometric absorbances of eumelanin at 350 nm; Sp.PM = spectrophotometric absorbances of pheomelanin at 400 nm; Sp.ASM = spectrophotometric absorbances of alkali soluble melanin at 400 nm. Sp.ASM was positively correlated with Sp.EM (p = 0.000). Sp.ASM was not correlated with Sp.PM (p = 0.808). Sp.EM was not correlated with Sp.PM (p = 0.612).

determining hair color are the quantity and quality of the melanins, and the amounts that are transferred to the hair shafts (Ozeki et al., 1995). The switch between eumelanogenesis and pheomelanogenesis is also the main factor whose mechanism has drawn considerable attention and is clearly complex (Barsh et al., 2000). In a previous study on contents of eumelanin and pheomelanin in some species such as humans and mice, the methods were based on measuring the pyrrole-2,3,5-tricarboxylic acid (PTCA, the chemical degradation of eumelanin) and aminohydroxyphenylalanine isomers (AHP, the chemical degradation of pheomelanin) by HPLC (Ito et al., 2003) . Subsequently, spectrophotometric methods were proposed by Ito (1993) and Ozeki et al. (1995). For example, the melanins of llama (lama pacos) were quantified by this method (Cecchi et al., 2007). In this report, we characterized the contents and their relations among three different types of melanin in 22 alpaca hair colors.

The contents of the pheomelanins in different hair colors were very low, and had no relationship to the alpaca hair color. This result agreed with Cecchi et al. (2007). They analyzed melanins and melanosomes in hair and skin from adult pigmented Argentine llamas, and their results showed that pheomelanins were present in small quantities in each sample. However, we found that the amounts of alkali-soluble melanins and eumelanins had a close relationship to the alpaca hair color and both of them increased as the color deepened from white to black. Similar results were obtained by Cecchi who reported that the amount of alkali-soluble melanins and eumelanins both decreased as hair changed from black to reddish brown color (Cecchi et al., 2004; Cecchi et al., 2007). Studies have been conducted in several species using the methods suggested by Ozeki et al. (1996). They believed that eumelanin results in the black phenotype, while the mixture of eumelanin and pheomelanin in varying ratios and levels results in the tan and brown to red phenotypes. Sponenberg et al. (1988) analyzed hair samples of various colors of horses for eumelanin and pheomelanin contents, and the results indicated that eumelanin results in the black phenotype and pheomelanin results in red to yellow phenotypes. Results showed that pheomelanin contents were highest in human "fire red" hair, and eumelanin was highest in black, gray and golden human hair; while in yellow mouse hair, pheomelanin contents were higher (Gregory, 1996); in yellow-brown, brown and red sheep, eu- and pheo- melanins were higher (Aliev et al., 1990); in red and yellow horses, eu- and pheo- melanins were higher (Sponenberg et al., 1988). From the results of this study, eumelanin was the main component in the alpaca hair shaft, which was different from other species, such as the horse and sheep.

The results also showed that eumelanins and pheomelanins existed in small quantities in white alpaca hair. Commo et al. (2004) found that no melanocytes can also cause the whitening of human hair due to the lack of melanins in the hair shaft. From these results, it was concluded that the mechanism of formation of white alpaca hair may be different from other animals such as pigs and humans.

REFERENCES

Aliev, G., M. Rachkovsky, S. Ito, K. Wakamatsu and A. Ivanov. 1990. Pigment types in selected color genotypes of Asiatic sheep. Pigment Cell Res. 3:177-180.

Barsh, G. S., T. Gunn, L. He, S. Schlossman and J. Duke-Cohan. 2000. Biochemical and genetic studies of pigment-type switching. Pigment Cell. Res. (Suppl) 8:48-53.

Burchill, S. A., A. J. Thody and S. Ito. 1986. Melanocyte-stimulating hormone, throsinase activity and the regulation of eumelanogenesis and pheomelanogenesis in the hair follicular melanocytes of the mouse. J. Endocrinol. 109:15-21.

Cecchi, T., A. Valbonesi, P. Passamonti, E. Frank and C. Renieri. 2007. Quantitative variation of melanins in llama (*Lama glama L.*). Small. Rumin. Res. 71:52-58.

Cecchi, T., C. Cozzali, P. Passamonti, P. Ceccarelli, F. Pucciarelli and A. M. Gargiulo. 2004. Melanins and melanosomes from llama (*Lama glama L.*). Pigment Cell Res. 17:307-311.

Commo. S, O. Gaillard and B. A. Bernard. 2004. Human hair greying is linked to a specific depletion of hair follicle melanocytes affecting both the bulb and the outer root sheath. Br. J. Dermatol. 150:435-443.

Gregory, S. B. 1996. The genetics of pigmentation: from fancy genes to complex traits. Tig. August. 12:299-305.

Hoffman, E. 2006. The complete alpaca book. 2nd Edition. Bonny Doon Press, California.

Ito, S. 1993. High-performance liquid chromatography (HPLC) analysis of eu- and pheomelanin in melanogenesis control. J. Invest. Dermatol. 100:166S-171S.

Ito, S. and K. Wakamatsu. 2003. Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. Pigment Cell Res. 16:523-531.

Ito, S., K. Wakamatsu and H. Ozeki. 1993. Spectrophotometric

assay of eumelanin in tissue samples. Anal. Biochem. 215:273-277.

Ito, S., K. Wakamatsu and H. Ozeki. 2000. Chemical analysis of melanins and its application to the study of the regulation of melanogenis. Pigment Cell Res. 13(Suppl. 8):103-109.

Lamoreux, M. L., K. Wakamatsu and S. Ito. 2001. Interaction of major coat color gene functions in mice as studied by chemical analysis of eumelanin and pheomelanin. Pigment Cell Res. 14:23-31.

Ozeki, H., S. Ito, K. Wakamatsu and A. J. Thody. 1996. Spectrophotometric characterization of eumelanin and pheomelanin in hair. Pigment Cell Res. 9:265-270.

Ozeki, H. S. Ito, K. Wakamatsu and T. Hirobe. 1995. Chemical characterization of hair melanins in various coat-color mutants of mice. J. Invest. Dermatol. 105:361-366.

Sponenberg, D. P., S. Ito, L. A. Eng and K. Schwink. 1988. Pigment types of various color genotypes of horses. Pigment Cell Res. 1:410-413.

StépHane, C., G. Olivier, T. Sonia and A. Bruno. 2004. Absence of TRP-2 in melanogenic melanocytes of human hair. Pigment Cell Res. 17:488-497.

Sturm, R. A., R. D. Teasdele and N. F. Box. 2001. Human pigmentation genes: Identification, Structure and Consequences of PolymorpHic variation. Gene 277:49-62.