PAPER

Quantitative variation of melanins in alpaca (*Lama pacos* L.)

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Abstract

The amount of melanin pigments was investigated in 95 Peruvian alpaca, representative of six different fleece colours, by means of spectrophotometric assays: SpEM (Spectrophotometric Eumelanin), SpPM (Spectrophotometric Pheomelanin), SpASM (Spectrophotometric Alkali Soluble Melanin), and SpTM (Spectrophotometric Total Melanin). It was found that these melanin pigments were suitable for identifying three homogeneous groups, each consisting of two closely related colours. A low, an intermediate, and a high amount of SpASM, SpTM, and SpPM characterize pinkish grey and light reddish brown, brown and reddish brown, dark reddish brown and black fleeces, respectively. SpEM and SpTM provide a further split within this latter group; higher concentrations of these pigments distinguish black fleece from dark reddish brown.

Introduction

Alpaca (*Lama pacos* L.) is a domestic mammal specialized in fibre production. Domesticated during the prehistoric times in the Andean region from the wild vicuna (*Vicugna vicugna*), the alpaca is reared in Peru and Bolivia, and outside South America (Australia, New Zealand, U.S.A., many European countries) (Wheeler, 1995; Bonavia, 1996). Among the characteristics taken in consideration for fibre quality in textile production, fibre colour is important in two different ways, white fibre and naturally coloured fibre (Mc Quarry, 1995; Vinella, 1994; Renieri *et al*., 2004). For some decades a high intensity selection has been made in alpaca populations for full white, and about 85% of the species is at present estimated to show this phenotype (Fernandez-Baca, 1994). In order to preserve biodiversity and produce ecological fibre for textile industries, a new interest exists now for coloured fibre, especially for uniform black, brown, red, grey and diluted (light fawn) phenotypes (Renieri *et al*., 2004).

Coat variation in coloured alpaca populations is generally very large and several classifications have been proposed in different countries and by international industries (Hoffman, 2006; Lauvergne, 1994; Lauvergne *et al*., 2001; Renieri, 1995). Generally based on the colour appearance, the relation by colour classes and both melanin type and amount in South American domestic camelids has been poorly explored (Cecchi *et al*., 2007; Fan *et al*., 2010).

Chemical proprieties of melanins in alpaca have been described by Renieri *et al*. (1991) and Cecchi *et al*. (2001). The morphology of melanosomes has been illustrated by Cozzali *et al*. (1998, 2001).

With the present work we propose to investigate the relation between the most frequent fleece colours in alpaca and the quantitative variation of melanin pigments carried out by means of spectrophotometric assays: SpEM (Spectrophotometric Eumelanin), SpPM (Spectrophotometric Pheomelanin), SpASM (Spectrophotometric Alkali Soluble Melanin, i.e., pheomelanins and brown melanins), and SpTM (Spectrophotometric Total Melanin). The aim was to identify (and hence distinguish) homogeneous groups of fleece colours according to the distribution of these pigments.

Materials and methods

Animals and management

Ninety-five Peruvian young female alpaca, reared in the Toccra Experimental Station (DESCO, Peru), were chosen as representative of six fleece colours: pinkish grey (27 animals), light reddish brown (6 animals), brown (6 animals), reddish brown (10 animals), dark reddish brown (37 animals), and black (9 animals). The different colours are defined according the Munsell Colour System (Macbeth, 1975), generally used in the classification of colours in fine-fibre-producing animals (Renieri, 1995).

Fibre samples were cut from the mid-side of each specimen and shipped to Italy.

Laboratory analyses

The melanins chemical proprieties analysis was carried out using the spectrophotometric methods proposed by Ito *et al*. (1993) and Ozeki *et al*. (1995, 1996a,b, 1997). These are considered the most eligible since: i) they easily allow the quantification of combined eu- and pheomelanins by dissolving them in Soluene-350 and examining the absorbance at 500 nm (A500); ii) specific eumelanin solubilization does not discriminate between DHI- and DHICA-derived units as the HPLC method does; iii) specific pheomelanin solubilization is possible; iv) pheomelanins and brown type eumelanins can be differentially solubilized under the conditions in which black type eumelanins remain insoluble.

A Hewlett Packard 8452A diode array spectrophotometer equipped with a 500 µL cuvette (10 mm optical path) and with external computer control (HP 89531A MS-DOS - UV/VIS operating software) was used. Sepia melanin suspension (1 mg/mL, prepared by sonicating for 5 min) was used as a standard for spectrophotometric assay of eumelanins. Hair sample suspensions (10 mg/mL) were homogenized with a Tenbroeck tissue grinder (Wheaton Industries Inc., Millville, NJ, USA). For the spectrophotometric assay of eumelanins (SpEM) hair samples were hydrolyzed in hot 30% hypophosphorous acid and

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hydroiodic acid. After cooling, 50% ethanol was added and samples were centrifuged at 3000 rpm (g = 2234) with a 4225 (ALC Industries Srl, Cologno Monzese, Italy) model centrifuge for 10 min. Insoluble eumelanic pigments were selectively solubilized in hot sodium hydroxide and hydrogen peroxide; they were cleaned by centrifugation for 1 min at 10000 rpm (g=10734) with a mod. S 323 K apparatus (HERMLE, Wehingen, Germany). Supernatants were analyzed for absorbance at 350 nm.

For the spectrophotometric assay of pheomelanins (SpPM), hair sample suspensions were solubilized in a phosphate buffer, pH 10.5, and cleared by centrifugation at 10000 rpm for 10 min. Chloroform was added to supernatants to remove fatty impurities. Pale yellow aqueous layers containing pheomelanins were cleared by centrifugation at 10000 rpm for 10 mins and analysed for absorbance at 400 nm. For the spectrophotometric assay of alkali-soluble melanins (SpASM, i.e., pheomelanins and brown eumelanins) the procedure was the same as for SpPM, but hair sample suspensions were solubilized 8 M urea /1 M sodium hydroxide. For the spectrophotometric assay of total melanins (SpTM), eumelanins and pheomelanins from hair sample suspensions were completely solubilized in hot toluene-350 and cleared by centrifugation at 10000 rpm for 10 min. Supernatants were analysed for absorbance at 500 nm.

The reproducibility of Ito's method agrees with that found by the present authors; the coefficient of variation (n=10 and triplicate measurements) is: 4.3% for SpEM, 3.5 % for SpPM, 4.6 % for SpASM, and 4.0 % for SpTM.

Statistical analyses

Statistical analyses were performed using the SPSS 12.0 statistical software package (SPSS, 2003). Since the original data related to the above mentioned melanin pigments differed significantly from a normal symmetric distribution, they were successfully normalized by using the arcsine square root transformation. A model I, one-way ANOVA was utilized for testing difference among fleece group colours. Pairwise comparisons (or Post Hoc test) were based on the GT2-method because of the unequal group size. Correlations were estimated by the Pearson correlation coefficient (*r*).

Results

All four melanin pigments investigated (viz.: SpEM, SpPM , SpASM, and SpTM) were strongly affected by fleece colours ($P<0.001$). The confidence of this result was supported by R-squared values ranging from 0.78 (registered in the SpPM ANOVA) to 0.91 (in SpTM). Concerning SpPM and SpASM, the pairwise comparisons, carried out among the six fleece colours, showed three homogeneous groups, which significantly differed from each other (Table 1). The first group comprised the colours exhibiting the lowest values (i.e.: pinkish grey, and light reddish brown); the second one the colours with intermediate values (brown, and reddish brown); the third one the colours showing the highest values (dark reddish brown, and black). Differences in SpTM and in SPEM allowed a further split within this latter group, as the black fleece is characterised by a higher content of both these two melanin pigments.

A positive, highly significant (at the 0.01 level) correlation was observed among all the four melanin pigments in all the pairwise combinations (Table 2).

Discussion

In full accordance with previous works on the fibre melanin pigment in llama (Cecchi *et al*., 2007) and alpaca (Fan *et al*., 2010), the content of pheomelanin was very low in all the coloured fleeces investigated. In fact, the spectrophotometric pheomelanin values ranged from 0.005 (pinkish gray fibre) to 0.091 (black fibre).

The content of the melanic pigments here investigated increased as the colour deepened from pinkish gray to black. Hence the black fleece is characterized by the largest content of all the melanin pigments here investigated. In particular, SpEM exhibited values from about thirteen-fold up to sixty-four-fold larger than those observed in dark reddish brown and in reddish brown, respectively. Dark reddish brown results in having a large amount of both SpASM and SpTM, yet joined with a low amount of SpEM. Pinkish gray, light reddish

Table 1. Absorbance values of Spectrophotometric pheomelanin), SpASM (Spectrophotometric alkali soluble melanin), SpTM (Spectrophotometric total melanin), and SpEM (Spectrophotometric eumelanin), monitored in 95 Peruvian alpaca representative of six fleece colours.

			Melanin pigments			
Fleece colours	n		SpPM	SpASM	SpTM	SpEM
Pinkish grey	27	Mean	0.005[a]	0.028[a]	0.012[a]	0.015[a]
		SD	0.003	0.009	0.006	0.010
Light reddish brown	6	Mean	0.006[a]	0.043[a]	0.024[ab]	0.011[a]
		SD	0.002	0.019	0.003	0.011
Brown	6	Mean	0.016[b]	0.115[b]	0.052[bc]	0.010[a]
		SD	0.006	0.024	0.006	0.011
Reddish brown	10	Mean	0.031[b]	0.193[b]	0.088[c]	0.007[a]
		SD	0.017	0.087	0.038	0.005
Dark reddish brown	37	Mean	0.086[c]	0.409[c]	0.193[d]	0.034[a]
		SD	0.045	0.111	0.066	0.045
Black	9	Mean	0.091[c]	0.542[c]	0.5430[e]	0.451[b]
		SD	0.036	0.149	0.116	0.195

[a-e]Different letters indicate significantly different mean values at $P<0.05$; significance was tested by using the GT2-method performed on absorbance data transformed into arcsine square root.

Table 2. Pearson correlation coefficients computed on the absorbance values° of SpPM (Spectrophotometric pheomelanin), SpASM (Spectrophotometric alkali soluble melanin), SpTM (Spectrophotometric total melanin), and SpEM (Spectrophotometric eumelanin).

	SpASM	SpTM	SpEM
SpPM	0.941**	0.869**	0.414**
SpASM	-	0.946**	0.512**
SpTM		-	0.713**

°Absorbance original data were transformed into arcsine square root; **correlation is significant at the 0.01 level (2-tailed).

brown, brown, and reddish brown have the same low amount of SpEM, whereas they are assigned to two different colour classes by the amount of SpPM and SpASM.

Conclusions

As already highlighted in the introduction, the textile industry is largely interested in natural, fine coloured fibre that allows to avoid chemical dying and pollution of natural environment. Hence, in a selection program addressed to improve alpaca fibre quality, colour is an important commercial trait. As the colour of the hair, as well as skin and eyes, in animal mainly depends on the quantity, quality and distribution of the melanin pigments, the quantification of eu- and pheomelanins in fibre samples by means of spectrophotometric method (Ito, 1993) may be a suitable and reliable criterion for fleece colour identification. Yet, a strict correspondence between quality and quantity of melanin pigments can not be established for all the six fleece colours, here investigated. This result suggests that different closely related fleece colours may share the same genetic background.

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