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# Colorimetry analysis of coat color and its relationship with fiber traits in alpacas



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#### ABSTRACT

Addressing the improvement of the textile characteristics is currently required in natural color production of alpaca fiber. This study analyses the possibility of implementing a genetic improvement program aiming to reduce the fiber diameter and the percentage of medullation in natural colors under the incomplete definition of the natural colors of alpaca fiber. The study considers color determination analysis in three separate steps. The first step aimed at finding the values of lightness ( $L^*$ ), red/green axis ( $a^*$ ), yellow/blue axis  $(b^*)$  of three-dimensional space of color and chroma  $(C^*_{ab})$ , tone  $(h^*_{ab})$  and color difference  $(\Delta E)$  with mathematical models for the description of the coat color. The second analysis is aimed at estimating genetic parameters of color traits and their correlation with fiber traits (fiber diameter, standard deviations and percentage of medullation - PM). The third step was to determine the potential selection criteria of breeding animals based on the parameters provided by a three-dimensional space values regarding the coat color assignment in alpacas. The colorimetric data were taken using a Chroma meter device analyzing 3 008 records from Huacaya type alpacas, collected between 2018 and 2019. In the first objective of the study, the color traits were subjected to a principal component analysis. The analysis of variance components and the estimation of genetic parameters were carried out using a restricted maximum likelihood procedure. The discriminant analysis was used for the correct assignment of the coat color. The principal component analysis results showed that the  $L^*$ ,  $a^*$ ,  $b^*$ ,  $h^*$  and  $\Delta E$  values can be grouped into two Principal Components (PC) to describe the color, where the  $L^*$  value is mainly distributed in PC2,  $b^*$  is distributed in PC1, while  $a^*$  is distributed in both components. The heritabilities found were 0.144, 0.128, 0.151, 0.104 and 0.152 for L\*, a\*, b\*, PC1 and PC2. The relevant genetic correlations were between  $L^*$ -PM (-0.557) and  $b^*$ -PM (-0.622). The discriminant analysis showed a high percentage of correct assignment in white (99.15%) and black (99.19%) coat colors for Huacaya type alpacas, while for the intermediate colors, the accuracy was lower. The three analyses showed that there is no pure natural color, but a range of color variation. It is better to use the values of the threedimensional space and within them, the values of  $L^*$  and  $b^*$  are potential selection criteria to be included in a genetic improvement program.

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# **Implications**

Our results indicated that is possible to predict the white and black colors in alpacas using lightness, red/green axis, yellow/ blue axis of three-dimensional space color. This method could be

helpful for assigning to an alpaca fiber fleece, could be helpful for assigning to an alpaca fiber fleece, the right natural color for genetic improvement and textile manufacture.

#### Introduction

The big textile industry is often blamed for being an important contaminating agent in the world. The large amounts of chemicals

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employed for its production and the excessive use of energy and water, plus the generation of toxic waste are at the core of this accusation. There are, however, particular commercial niches in the textile industry such as the one known as "Noble Fibers" that differ from the mass production operations. These fibers are of animal origin and are usually considered as luxury items. Among the Noble Fibers, the alpaca is the one that comes in more shades of natural colors than any other fiber producing livestock. There are up to 22 different natural Alpaca colors ranging from white to black and including different shades of browns and grays (Morante et al., 2009). Although both the demand and the production of natural alpaca color textiles are limited, there is an important niche for this type of textiles that fit admirably well with the modern concept of sustainability. Natural Alpaca colors do not need to be dyed; thus, there is no need for the use of chemicals nor energy or excessive water for its production, and there is no waste involved as well. The Twenty-First century consumer is aware of the importance of reducing contamination on our planet. The production of natural alpaca colors should therefore be promoted in order to fulfill this increasing demand.

Although the perception of the human eye can group 22 different colors in alpaca fiber, it is recommended to group animals in only nine basic colors: White, Light Fawn intensity X, Y or Z (LFX, LFY, LFZ), Light Brown, Dark Brown, Dark Brown Black, Black and Gray (Cruz, 2017).

Since the color perception has physical and psychological components as established by the Commission Internationale de l'Eclairage or International Commission on Illumination, it is necessary to consider these factors that influence the observer when assigning a coat color in alpacas.

Hetrick et al. (2013) explain that a form of subjective visual qualification of color is used for the assignment of color and this perception is correlated with instrumental measurement, which uses the three-dimensional space of color by pouring values of lightness ( $L^*$ ), red/green axis ( $a^*$ ), yellow/blue axis ( $b^*$ ). From these values ( $L^*$ ,  $a^*$  and  $b^*$ ), the perception of the appearance of color responds to mathematical models to understand better the magnitudes, denoted as a "color appearance model" (Fairchild, 2005). Among the common models used to describe color, we have the tone, chroma value, and colorimeter difference (Fairchild, 2005; Hetrick et al., 2013).

The first advances regarding the influence of color on fiber traits were reported by (Cruz et al., 2017), and this work would complement the research carried out regarding the production of fiber in natural colors with textile characteristics since the objective continues producing fine fiber (Gutiérrez et al., 2009; Gutierrez et al., 2011; Cruz et al., 2020) and reduce the percentage medullation as a possible cause of itching (Pinares et al., 2018 and 2019; Cruz et al., 2019). Therefore, this study would address the possibility of implementing a genetic improvement program aiming to reduce the fiber diameter and the percentage of medullation under the incomplete definition of the natural colors of alpaca fiber.

The purpose of this work was to analyze the quantification of the color of the alpaca fiber and therefore three objectives were proposed. The first objective was to propose the utility of the use of the total of color traits or the reduction in linear combinations through a principal component analysis in the description of the coat color. The second objective was to estimate the variance components of the color traits and how this correlate with the main fiber traits and analyze the possibility of using them as possible selection criteria. The third objective was to analyze the possibility of predicting and correcting the assignment of coat color in alpacas by a linear discriminant analysis as the potential selection criteria to choose.

#### Material and methods

The data were obtained from PacoPro v.5.10, of Pacomarca Genetic Center. The data of colorimetry were collected annually between 2018 and 2019. A total of 3 008 records of colorimetry were analyzed by Huacaya type. In addition, 452 records of Suri type alpaca were used to test the assignment of the coat color and 674 records for cross-validation of Huacaya type alpacas. The records for fiber traits were collected between 2001 to 2019, a total of 25 147 records for fiber diameter (FD), Standard Deviation (SD), and Percentage of Medullation (PM). Table 1 shows a more detailed description of the dataset regarding distribution across sex, range of ages and contemporary groups. The pedigree consisted of 11 438 animals, with 314 sires and 2 404 dams, 172 grandsires and 793 granddams, leading to families of 2.43 fullsibs and 9.70 halfsibs on average. The pedigree had six maximum generations and three complete generations on average, with a global mean inbreeding of 0.22%. The mean inbreeding values considered by maximum number of generations known were 0%, 3.14%, 7.28%, 3.65%, 5.30% and 33.33% from the first to the sixth. Pedigree information was computed using Endog v4.8 (Gutierrez and Goyache, 2005).

The assignment of coat color was made at birth using a color chart with nine basic colors ranging from white to black, including an intermediate color such as gray. However, the coat color can be corrected during routinary animal management activities, especially in extreme colors such as white and black, where it is sought to assign these colors to animals without spots or moles, and when any of these defects are found, they are reassigned as the Light Fawn intensity X color if it is adjacent to white or dark brown black color if it is adjacent to black. Fiber samples of approximately 100 g were taken before shearing from the mid-side to quantify the color and fiber traits. Impurities were removed by washing the samples with a neutral detergent and drying them at room temperature. The chroma meter v3.0 equipment was used to quantify color. The three-dimensional space values such as  $L^*$ ,  $a^*$ ,  $b^*$ , and  $C^*_{ab}$ ,  $h_{ab}^*$ ,  $\Delta E$  were computed as described by Martínez (2001), where, under the three-dimensional color space, L\* describes lightness,  $a^*$  describes color saturation from red to green and  $b^*$  describes

**Table 1**Description of the number of records of fiber and coat color traits by sex, contemporary groups and range of age (days) in Huacaya type alpacas.

	Fiber t	traits	Coat color traits		Range of age (days)
Contemporary group	Male	Female	Male	Female	
2001	5	68			752-2 232
2002	12	840			289-3 446
2003	39	984			499-3 993
2004	98	152			157-4 323
2005	220	942			84-4 938
2006	413	927			71-4 994
2007	410	854			69-5 164
2008	426	981			79-5 766
2009	427	1 074			81-6 150
2010	529	1 181			96-6 494
2011	513	1 138			62-6 870
2012	563	1 158			82-7 212
2013	518	982			61-6 424
2014	326	908			113-6 639
2015	383	910			85-6 861
2016	467	1 015			102-7 065
2017	517	1 013			82-5 932
2018	463	1 533	243	682	102-5 977
2019	518	1 640	498	1 585	61-6 281

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color saturation from yellow to blue. Concepts to describe the color appearance from the three-dimensional space values where: Chroma  $(C^*_{ab})$  is the color of an area judged in proportion to the brightness of a similarly lit area that appears white or highly transmissive; the equation is  $C_{ab}^* = \sqrt{(a*)^2 + (b*)^2}$ . Tone  $(h^*_{ab})$  is the attribute of a visual sensation according to which an area resembles one of the perceived colors denoted as red, yellow, green, and blue, or a combination of them; the equation is  $h_{ab}^* = arctg(\frac{bs}{as})$ . Color difference ( $\Delta E$ ) is expressed as the relative distance between two reference points, and the equation is  $\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$  (Martínez, 2001). The mean and standard deviations of colorimetric traits are shown in Table 2. The fiber traits were FD, SD, described by Gutiérrez et al. (2009) and PM described by Cruz et al. (2019), analyzed in the Optical Fibre Diameter Analyser - OFDA 100® device (Lupton and Pfeiffer, 1998).

#### Principal component analysis

The principal component analysis for  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*_{ab}$ ,  $h^*_{ab}$  and  $\Delta E$  was performed in order to represent the best dimensionality of the dataset regarding the measurement, prediction and assignment of the coat color. Variables were transformed into linear combinations defined by the decomposition of the covariance matrix into eigenvectors after the data were centered and scaled, so that the principal components represent the variance captured in a descendent order in each of them. The functions *scale* and *prcomp* of R language version 3.5.3 (R Core Team, 2019) were used. A component is retained if its eigenvalue is greater than the 95th percentile of the distribution of eigenvalues generated from the random data (Horn, 1965), and Kaiser criterion where the variance of components is >1 (Kaiser, 1960), the function *paran* of R language version 3.5.3 was used (R Core Team, 2019).

# Variance component estimation

The variance components were estimated using a restricted maximum likelihood procedure under a multitrait animal model, in which three values were taken from the three-dimensional space and the linear coefficients of the two principal components retained. These have been correlated with the FD and SD described by Gutiérrez et al. (2009) and the percentage of medullation as described by Cruz et al. (2019) and Pinares et al. (2018).

And the model fitted for  $L^*$ ,  $a^*$ ,  $b^*$ , PC1, PC2, FD, SD and PM was as follows:

$$y = Xb + Zu + Wp + e$$

In matricial notation 
$$\begin{pmatrix} u \\ p \\ e \end{pmatrix} \tilde{\mathbf{N}} \left( \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} A \otimes G_0 & 0 & 0 \\ 0 & I_p \otimes P_0 & 0 \\ 0 & 0 & I_e \otimes R_0 \end{bmatrix} \right)$$

where  $\mathbf{y}$  is the vector of observations,  $\mathbf{b}$  is the vector of fixed effects,  $\mathbf{u}$  is the vector representing the additive genetic effects,  $\mathbf{p}$  corresponds to the vector of permanent environments, and  $\mathbf{e}$  is the vector of residuals;  $\mathbf{X}$ ,  $\mathbf{Z}$  and  $\mathbf{W}$  are the incidence matrices for respectively fixed, genetic and permanent effects,  $\mathbf{A}$  the numerator relationship matrix,  $\mathbf{G}_0$  the covariance matrix for additive genetic effects,  $\mathbf{I}_p$  the identity matrix of equal order to the number of permanent environmental subclasses,  $\mathbf{P}_0$  the covariance matrix for permanent environmental effects,  $\mathbf{I}_e$  the identity matrix of equal order to the number of records,  $\mathbf{R}_0$  the residual covariance matrix among measurements on the same animal and  $\otimes$  the Kronecker product.

The fixed effects included in the model for colorimetry traits were as follows: sex (2 levels) coat color (9 levels), year of recording as contemporary group (2 levels) and age as linear and quadratic covariate. The fixed effects for fiber traits were coat color (9 levels), year of recording as contemporary group (19 levels), sex (2 levels) and age as linear and quadratic covariate. Coat color was fitted as fixed in order to accommodate the market demand that requires alpaca fiber in pure natural colors, aiming to reduce variability and homogenize the coat color within each group (level of fixed effect), thus assuming the assignment error to the corresponding level. Genetic parameters were estimated using the VCE 6.0 program (Neumaier and Groeneveld, 1998).

#### Discriminant analysis

The linear discriminant analysis used three data files, the first with 3 008 records of Huacaya type alpacas that allows creating the linear discriminant coefficients, and another analysis to create the linear coefficients with groups of coat colors that exceed 100 records. In the testing, the obtained linear coefficients were used to test them on a second group of colorimetric data of the Suri type alpacas with 452 records. Finally, the cross-validation was carried out using a third group of 674 colorimetry records belonging to Huacaya type alpacas. The MASS package with the *lda* function of R language version 3.5.3 was used (R Core Team, 2019).

#### Results

Principal component analysis

Table 3 shows the Pearson's correlations between color traits. There was one very high correlation between  $b^*$  and  $C^*_{ab}$  (0.991).

**Table 2**Descriptive statistic (mean and SD) of fiber samples of Huacaya type alpaca grouped by coat color.

Coat color <sup>1</sup>	Animals	Animals with repeated records	Total Records	$L^{*2}$	a*2	b*2	$C^*_{ab}^3$	$h^*_{ab}^{4}$	$\Delta E^5$
W	809	21	830	87.10 (3.97)	-0.34 (0.55)	8.27 (1.40)	8.29 (1.40)	-0.79 (1.28)	3.45 (2.47)
LFX	268	58	326	77.50 (10.40)	2.46 (2.57)	12.20 (3.64)	12.60 (4.04)	0.68 (1.22)	9.79 (5.73)
LFY	111	53	164	58.20 (8.25)	6.79 (1.59)	17.60 (2.01)	18.90 (2.35)	1.21 (0.05)	7.17 (4.79)
LFZ	66	37	103	40.40 (6.25)	9.18 (1.05)	18.10 (2.01)	20.3 (2.06)	1.10 (0.05)	5.51 (3.68)
LB	100	59	159	31.40 (4.97)	9.15 (0.86)	14.60 (2.48)	17.20 (2.38)	1.00 (0.06)	4.55 (3.28)
DB	122	165	187	26.40 (4.44)	7.44 (2.05)	10.30 (3.83)	12.8 (4.13)	0.93 (0.11)	5.09 (3.54)
DBB	61	38	99	18.10 (1.41)	1.44 (0.81)	0.79 (1.05)	1.74 (1.19)	0.30 (0.41)	1.50 (1.22)
В	665	445	1 110	17.40 (1.09)	0.85 (0.45)	-0.02(0.61)	0.99 (0.580)	-0.18(0.49)	1.03 (0.93)
G	22	8	30	44.60 (13.7)	0.55 (0.58)	1.42 (1.22)	1.72 (1.07)	0.50 (1.06)	12.2 (5.93)

<sup>1</sup> W = white, LFX, LFY and LFZ = light fawn (intensity X, Y or Z), LB = light brown, DB = dark brown, DBB = dark brown black, B = black, G = gray.

 $<sup>^2</sup>$   $L^*$ ,  $a^*$ ,  $b^*$  = represent the three-dimensional color space.

 $<sup>^{3}</sup>$   $C^{*}_{ab}$  = chroma.

 $<sup>^{4}</sup> h^{*}_{ab} = \text{tone.}$ 

<sup>&</sup>lt;sup>5</sup>  $\Delta E$  = subjective color appearance.

**Table 3**Pearson's correlations between color traits in Huacaya type alpacas.

	$a^{*1}$	$b^{*1}$	$C^*_{ab}^2$	$h^*_{ab}^3$	$\Delta E^4$
L*1	-0.254	0.526	0.426	-0.221	0.355
a*		0.655	0.744	0.631	0.315
$b^*$			0.991	0.389	0.534
$C^*_{ab}$				0.429	0.519
$h^*_{ab}$					0.281

<sup>&</sup>lt;sup>1</sup>  $L^*$ ,  $a^*$ ,  $b^*$  = represent the three-dimensional color space.

**Table 4**Principal components (PCs) analysis of color traits in Huacaya type alpacas.

	PC1	PC2	PC3	PC4	PC5
L*1	-0.157	0.743	0.229	-0.339	0.507
$a^{*1}$	-0.514	-0.376	0.273	0.447	0.566
$b^{*1}$	-0.568	0.237	0.432	0.104	-0.650
$h^*_{ab}^2$	-0.437	-0.416	-0.139	-0.785	0.013
$\Delta E^3$	-0.443	0.279	-0.817	0.243	-0.015
SD	1.567	1.247	0.748	0.638	0.158
Variance of components	2.455	1.555	0.560	0.407	0.025
Proportion of variance	0.491	0.311	0.1120	0.081	0.005
Cumulative proportion (%)	49.10	80.20	91.40	99.50	100.00

<sup>&</sup>lt;sup>1</sup>  $L^*$ ,  $a^*$ ,  $b^*$  = represent the three-dimensional color space.

High correlations were found between  $L^*-b^*$  (0.526),  $a^*-C^*_{ab}$  (0.744),  $a^*-h^*_{ab}$  (0.631),  $-b^*-\Delta E$  (0.534) and  $C^*_{ab}-\Delta E$  (0.519). The high correlation between two supposedly independent parameters  $a^*-b^*$  (0.655) shows that, in alpacas, the coat color moves on both scales simultaneously. The rest of correlations were moderate. The extreme high genetic correlation found between  $b^*$  and  $C^*_{ab}$  (0.991) suggests removing the  $C^*_{ab}$  variable within the principal component analysis that was done on the other five.

The results of principal component analysis are shown in Table 4. PC1 and PC2 explained 49.10% and 31.10% respectively of the total variance. These first two principal components explained 80.20% of the total variance. The retention of the principal components in which the color can be grouped was based on the eigenvalues and variance components being >1 (Supplementary Figure S1), explaining the global variance of the traits of color

in the first two components, PC1 (2.455) and PC2 (1.555) showed in the Table 4. According to the decomposition, the value  $b^*$  was explained in the first component (-0.568). The value  $L^*$  was explained in the second component (0.743), while the values  $a^*$ ,  $h^*_{ab}$  and  $\Delta E$  were explained in both components.

The distributions of eigenvectors of  $L^*$  of each animal with PC1 and PC2 are shown in Fig. 1, grouped by coat color, showing overlapping between adjacent colors, and an elliptic distribution between white and black, where the intermediate colors move away from both extreme colors, except the gray color.

#### Variance component estimation

The principal component analysis results suggest using the first two components as a linear combination to describe the coat color in alpacas. These two linear coefficients of PC1 and PC2 together with the  $L^*$ ,  $a^*$  and  $b^*$  values were declared as traits for the variance component estimation. The solution of the fixed effects of age (covariate), coat color, sex and contemporary group is shown in Supplementary Table S1; it is necessary to randomly zero some levels of fixed effects to solve the system of equations.

The genetic parameters are shown in Table 5. The heritabilities for  $L^*$ ,  $a^*$ ,  $b^*$ , PC1, and PC2 were moderate to low (0.104–0.152). Similarly, the heritabilities for fiber traits were moderate (0.251–0.374). The relevant genetic correlations within color traits were high between  $L^*$ - $a^*$  and  $b^*$ - $a^*$  (–0.676 and 0.764, respectively). The genetic correlations between PC1 and PC2 with  $L^*$ ,  $a^*$  and  $b^*$  were high (0.625 to 0.944 in absolute value, since PC1 and PC2 are linear combinations of the  $L^*$ ,  $a^*$  and  $b^*$ .

The genetic correlations of color traits with fiber traits showed two important correlations, between  $L^*$ -PM (-0.557) and  $b^*$ -PM (-0.622), and the remaining genetic correlations were low to very low. The permanent environment variances were high for  $L^*$ ,  $a^*$ ,  $b^*$ , PC1 and PC2 (0.556-0.658) and moderate for fiber traits. The repeatabilities were high for  $L^*$ ,  $a^*$ ,  $b^*$ , PC1 and PC2 (0.707-0.782) and moderate to high for fiber traits.

# Discriminant analysis

The estimation of the variance components provided relevant correlations between the values of  $L^*$ ,  $a^*$  and  $b^*$ , and only two important correlations with the fiber traits that involve the values of  $L^*$  and  $b^*$ . Therefore, for the discriminant analysis, only the three-dimensional space  $(L^*, a^*, b)$  values were used as potential

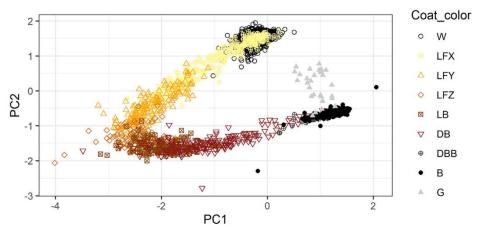


Fig. 1. Distribution of the coat color white (W), light fawn intensity X, Y or Z (LFX, LFY, LFZ), light brown (LB), dark brown (DB), dark brown black (DBB), black (B) and gray (G) in the principal components (PCs) 1 and 2 in Huacaya type alpacas.

 $<sup>^{2}</sup>$   $C^{*}_{ab}$  = chroma.

 $<sup>^3</sup> h^*_{ab} = \text{tone.}$ 

<sup>&</sup>lt;sup>4</sup>  $\Delta E$  = subjective color appearance.

 $<sup>^2</sup> h^*_{ab} = \text{tone.}$ 

<sup>&</sup>lt;sup>3</sup>  $\Delta E$  = subjective color appearance.

**Table 5**Heritabilities (in diagonal), genetic correlations (above diagonal), permanent environmental variances ratio (row c<sup>2</sup>), repeatabilities (last row, R) and their corresponding SE (in brackets) for color traits and fiber traits in Huacaya alpacas type. All heritabilities and genetic correlations higher than 0.30 in absolute values are in bold.

	L*1	a*1	b*1	FD <sup>2</sup>	SD	$PM^3$	PC1 <sup>4</sup>	PC2 <sup>4</sup>
L*	0.144 (0.033)	- <b>0.676</b> (0.086)	-0.165 (0.107)	-0.037 (0.080)	-0.124 (0.046)	- <b>0.557</b> (0.062)	<b>0.671</b> (0.233)	<b>0.820</b> (0.055)
$a^*$		0.128 (0.034)	0.764 (0.055)	-0.144(0.084)	-0.098(0.091)	-0.166(0.072)	- <b>0.944</b> (0.032)	- <b>0.913</b> (0.115)
$b^*$		SE 172	0.151 (0.042)	-0.069(0.035)	-0.107(0.049)	- <b>0.622</b> (0.073)	- <b>0.628</b> (0.077)	- <b>0.625</b> (0.077)
FD				0.337 (0.008)	0.687 (0.025)	0.300 (0.035)	0.271 (0.104)	0.282 (0.134)
SD				,	0.374 (0.013)	0.324 (0.025)	0.094 (0.080)	0.102 (0.083)
PM						0.251 (0.044)	0.033 (0.139)	-0.031 (0.085)
PC1							0.104 (0.038)	0.882 (0.099)
PC2							And the second s	0.152 (0.054)
$c^2$	0.626 (0.035)	0.654 (0.035)	0.556 (0.038)	0.140 (0.007)	0.151 (0.009)	0.153 (0.034)	0.658 (0.047)	<b>0.559</b> (0.038)
R	<b>0.770</b> (0.048)	<b>0.782</b> (0.049)	<b>0.707</b> (0.057)	<b>0.477</b> (0.011)	<b>0.525</b> (0.016)	<b>0.404</b> (0.056)	0.762 (0.060)	0.711 (0.066)

<sup>&</sup>lt;sup>1</sup>  $L^*$ ,  $a^*$ ,  $b^*$  = represent the three-dimensional color space.

**Table 6**Percentage of coat colors correctly assigned (CCA) by linear discriminant analysis in Huacaya and Suri type alpacas.

	CCA (%)	CCA (%)								
	White	LFX <sup>1</sup>	LFY <sup>1</sup>	LFZ <sup>1</sup>	LB <sup>2</sup>	DB <sup>3</sup>	DBB <sup>4</sup>	Black	Gray	
Huacaya type	99.28	32.52	79.88	62.14	62.26	62.57	1.01	99.91	73.33	
Huacaya type (testing data) <sup>5</sup>	99.28	32.82	79.88	64.08	61.64	62.57	_	99.91	-	
Suri Type (testing data)	99.26	32.73	58.49	78.68	30.45	55.79	35.81	97.45	0	
Huacaya type (cross-validation)	99.15	25.00	92.31	77.78	37.50	40.00	0	99.19	-	

<sup>1</sup> Light Fawn intensity X, Y or Z.

criteria to describe the coat color in alpacas. The coefficient of discriminant analysis for coat color assigned in alpacas is shown in the Supplementary Table S2. The first coefficient of linear discriminant represents 88%, the second represents 11% and the third represents only 1%.

The results of the performance of the assignment are shown in Table 6. The testing study to assign the coat color in the Huacaya type alpacas was done from the calculated coefficients with its own Huacaya type data. However, assignment of coat color was also performed in Suri type alpacas using the calculated coefficients assuming similarity between both genetic types of the quantitative characteristics of the coat color. A final cross-validation study was performed to assign the coat color in a new dataset of Huacaya type alpacas. The correct assignment of coat colors reached a value of 82.21% and 81.20% for Huacaya and Suri type alpacas respectively in the test, and 78.64% for Huacaya type alpacas in the cross-validation.

The percentage of colors correctly assigned by discriminant analysis within the color group in both types were high in the extreme colors (White and black). Showing a correct assignment in the tests with 99.28% and 99.26% for white coat color and 99.91% and 97.45% for black coat color for Huacaya and Suri type alpacas respectively. Similarly, the cross-validation show a correct assignment of 99.15% for white coat color and 99.19% for black coat color in Huacaya type alpacas, but a lower percentage for the assignment of the other colors, in the tests and the cross-validation.

#### Discussion

This is the first study that addresses the colorimetry of coat color in alpacas, facing a growing market demand for textiles in natural colors. This growing unique market niche requires fewer chemicals having lower environmental impact and, above all, being sustainable (Malviya et al., 2020). However, the same market demands homogeneous colors.

Unlike the improvement programs that have so far focused on fiber traits, the production of natural colors has its first limitation in the incomplete definition of the color classes, with a no uniform distribution of the population with respect to the coat color (Tables 1 and 2). Therefore, the selection pressure will be very variable in each group, with inbreeding concerns, as the population presents a high degree of kinship. Exceptionally, a great population of black alpacas is available as a consequence of a special recovery and conservation program for this coat color. However, the natural structure of the population with respect to coat color shows low percentages of brown and black alpacas, even with the gray alpacas on the verge of extinction. A detailed study of the particularities of the different color classes and their relationship with fiber quality is of concern in this scenario.

The distribution of colorimetry data (except to gray) for  $L^*$  shows a decrease in the values of white to black (Table 2). Nevertheless, for  $a^*$ ,  $b^*$ ,  $C^*$ <sub>ab</sub>, and  $h^*$ <sub>ab</sub>, the distributions shown a value higher in the intermediate colors as light fawn intensity Y and light brown (Table 2). Our results are in accordance with findings reported in horses with bay and black coat color by Druml et al. (2018), and with lower ranges for a much smaller population of alpacas, grouping the coat color only into four categories (Guridi et al., 2011). Table 2 and Fig. 1 show a great overlapping in the adjacent coat colors, this is evident between dark brown black and black, where the range of  $L^*$  between both colors is small. The difference between these adjacent colors is the  $b^*$  value, dark brown black having a positive value and black having a negative one. The gray color, unlike the rest of the colors, had a particular special distribution.

<sup>&</sup>lt;sup>2</sup> FD = fiber diameter.

<sup>&</sup>lt;sup>3</sup> PM = percentage of medullation.

<sup>&</sup>lt;sup>4</sup> PC = coefficients of Principal Components 1 and 2.

<sup>&</sup>lt;sup>2</sup> LB = light brown.

<sup>&</sup>lt;sup>3</sup> DB = dark brown.

<sup>&</sup>lt;sup>4</sup> DBB = dark brown black.

<sup>&</sup>lt;sup>5</sup> Creation of linear coefficients and test with groups greater than 100 records.

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The assignment of the coat color category in alpacas is done at birth, making it difficult to control environmental conditions to standardize the psychophysical and psychological color perception of the observer. However, the phenotypic correlations between the three-dimensional space values and the mathematical models to describe color show that they can be grouped (Table 3). The principal component analysis shows that of the five color traits analyzed, only the first two components can be retained, where  $L^*$ and  $b^*$  are represented in separate components and the other traits are distributed in both components. These same results were found for the analysis for visible and near infrared reflectance spectroscopy for four group samples in alpaca fiber reported by Canaza-Cayo et al. (2012). The overlap in the components distribution occurs in all adjacent colors, except for the gray color. The distribution of this color was not influenced by the first eigenvector nor the PC1 ( $a^*$  or  $b^*$ ), but only by the PC2 that corresponds to the  $L^*$ , showing an intermediate distribution between the white and black coat colors (Fig. 1), where it resembles a brown color but the brown-yellow tint has been removed. One possible explanation is that there is a different genetic control mechanism for the color gray as reported by Jones et al. (2019) in alpacas and by Curik et al. (2013) for horses.

Two types of genes regulate genetic control of coat color in camelids, a few that control the main color and many polymorphisms in the modifier genes (Daverio et al., 2016). By including the coat color as a fixed effect in the model, the main colors effect is mapped to the effect of the coat color, and variability is sought in modifier genes within each group, showing moderate to low heritability (0.104 - 0.152). When this effect was not included in the model, the heritability was much higher, as reported by Bartolomé et al. (2009) for Spanish alpacas. Nevertheless, the relevant genetic correlation for colorimetric traits between  $L^*$ - $a^*$ (-0.676) and  $a^*-b^*$  (0.764) were similar to that reported by Bartolomé et al. (2009). Likewise, the very high genetic correlation between the two principal components is very striking (0.882), although they are theoretically independent by construction. It is a consequence from how the color parameters were defined in the space, from red to green  $(a^*)$  and from yellow to blue  $(b^*)$ , being the natural coat color moving in a reduced area of the possible space simultaneously to an increase in the yellow and red axis while increasing the light. These make that although PC1 and PC2 were independent by construction, unavoidably they trend to move the three together in a common undefined axis in the space. A clear exception to this pattern is the gray coat color.

The heritabilities, repeatabilities and genetic correlations of fiber traits were similar than those reported by Cruz et al. (2019) and Cruz et al. (2020). The genetic correlations of the color traits with fiber traits showed two relevant correlations between  $L^*$ -PM (-0.557) and  $b^*$ -PM (-0.622); the rest of the correlations were low. However, these correlations should be handled with caution since the selection focused on the white coat color, having a very low selection pressure for diameter and percentage of medullation in the dark color, so the results may show spurious correlations.

Then, of traits analyzed in the principal component analysis, the Pearson correlations identified that the values  $b^*$  and  $C^*_{ab}$  addressed basically the same concept. Also, it revealed that the mathematical models do not provide information to describe the color, being the values of the three-dimensional space that can describe the coat color in alpacas. However, the correlations with the PM trait showed that only  $L^*$  and  $b^*$  have the potential to be used as selection criteria. A similar correlation between coat color and percentage of medullation was also reported for llama fiber samples (Frank et al., 2006). These correlations suggest that the percentage of medullations was lower in alpacas with a lighter coat color. The white alpaca is perfect for the objective of garment manufacturing because it is finer and with less prickle factor. However,

suppose the objective was to produce fiber in dark colors, in that case, the correlations are unfavorable to produce fiber with desirable textile characteristics, and especially in black fiber, it would be necessary to decide whether to produce black alpacas at the cost of increasing the percentage of medullation or using a genetic index (Gutierrez et al., 2014).

The success of producing pure natural colors will largely depend on the correct assignment of the coat color. However, a reclassification of the color can be made, given the results of the test and cross-validation of the discriminant analysis in both types of alpaca (Table 6), showed that there was a high accuracy of assignment in the black and white colors, but a lower accuracy in the intermediate colors. Canaza-Cayo et al. (2012) reported this same trend in cross-validation for four groups of coat colors, with high accuracy of assignment in extreme colors (light and dark) but lower accuracy in intermediate colors.

The probability of coat color assignment from the linear discriminant was similar for two groups of test, 82.21% for Huacaya types and 81.20% for Suri types and 78.64% for cross-validation in Huacaya type alpacas. The same proportions in correct classification of coat color, higher in the extreme colors and lower in the intermedium colors, was reported by Canaza-Cayo et al. (2012). The coat color classification was mainly influenced by the  $L^*$  (value 0 – 100) and this trait shows the greatest overlap of adjacent colors (Table 2 and Fig. 1). This influences the low classification rates in the intermedium colors, but mainly near the extreme colors as the light fawn intensity X and dark brown black in both types and exceptionally in light brown for Huacaya type (Table 6).

The present work demonstrated that to produce color fiber to satisfy an increasing demand focused on the sustainability of natural colors, there are limitations that must be overcome since the perception of subjective color has deficiencies in the assignment in intermediate colors. Color segregation would respond to polygenic control for its quantitative expression. Therefore, there is no pure natural color, but rather a color range, and the consumption of natural color garments would have to accept this range of color variation. It has also been shown that the color in alpacas is mainly influenced by  $L^*$  and  $b^*$ , and lesser influence of  $a^*$ , since there are no green alpacas involving the yellow-green scale of tridimensional space. Instead, the gray color has a different quantification from the rest of the colors, and it is not possible to use a discriminate analysis to evaluate its correct assignment, which would obey its genetic control (Jones et al., 2019).

Of the five traits analyzed, these analyses suggest retaining two components involving three traits:  $L^*$ ,  $a^*$ , and  $b^*$  values of three-dimensional space. Heritability and genetic correlations showed that the values of  $L^*$  and  $b^*$  were the important traits that can be used as selection criteria, alone or combined in a genetic index within a breeding program. There was a high percentage of correct color assignment in black and white coat color alpacas, but the percentage was lower in intermediate colors. There are no separated categories of pure natural colors, but a range of color variation.

#### Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100219.

### **Ethics approval**

Not applicable.

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# Data and model availability statement

None of the data were deposited in an official repository. Available upon request.

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#### Declaration of interest

None.

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