

Comparison of projection microscope with OFDA100 in alpaca fibers medullation

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SUMMARY

The aim of this research was to compare Projection Microscope (PMic) and Computerized Projection Microscope (CPMic) with Optical Fiber Diameter Analyzer (OFDA100), for the determination of the medullation in alpaca fibers expressed in percentage (MED). Fiber samples were taken from Pacamarca Scientific Station. Three experiments were carried out. At experiment 1, the MED was determined in 36 Huacaya alpaca males using PMic and OFDA100. At experiment 2, the MED was determined in 200 alpacas using CPMic and OFDA100 in Arequipa; and at experiment 3 carried out at Huancavelica, the MED was measured in 105 fiber samples with CPMic and OFDA100. OFDA medullation mean was very low in the three experiments (between 9.81 ± 0.61 and $18.30 \pm 1.68\%$), compared with the total medullation obtained by the PMic ($67.43 \pm 1.74\%$) and CPMic (between 28.71 ± 1.37 and $41.14 \pm 2.00\%$). The continuous medullation + strongly medullated fibers reported by CPMic was even greater than OFDA medullation. These results indicate that the OFDA100 procedure provides very low and different reports of MED than PMic and CPMic. Although the OFDA100 was a system for rapid evaluation of wool MED, it would not be recommended in alpaca fibers.

Comparación de la medulación de fibras de alpaca utilizando microscopio de proyección y OFDA100

RESUMEN

El objetivo del presente trabajo fue comparar el microscopio de proyección (MicProy) y el microscopio de proyección computarizado (MicProyCom) con el analizador óptico de diámetro de fibra, denominado OFDA100, en torno a la determinación de la medulación de fibras de alpacas, expresado en porcentaje (MED). Las muestras de fibras fueron tomadas de la Estación Científica de Pacamarca. Tres experimentos fueron llevados a cabo. En el experimento 1, la MED fue determinada en 36 alpacas Huacaya machos utilizando el MicProy y el OFDA100. En el experimento 2, la Med fue determinada en 200 alpacas utilizando el MicProyCom en Arequipa; y el experimento 3, fue realizado en Huancavelica, donde la MED fue determinada en 105 muestras de fibras de alpaca con el MicProyCom y el OFDA100. En los tres experimentos llevado a cabo, la MED obtenida OFDA100 es muy bajo (entre 9.81 ± 0.61 and $18.30 \pm 1.68\%$), comparado con la MED total obtenida mediante el MicProy ($67.43 \pm 1.74\%$) y el MicProyCom (28.71 ± 1.37 and $41.14 \pm 2.00\%$). Asimismo, la MED de fibras con medulación continuas más las fibras fuertemente meduladas obtenidas con el MicProyCom fue más alta comparada con la MED obtenida con el OFDA100. Por lo tanto, aunque el OFDA100 es un sistema para la evaluación rápida de la medulación de lanas, éste no podría ser recomendado para la evaluación de la medulación de fibras de alpaca.

INTRODUCTION

The animal fiber trade represents an important resource for many low-income families in South American countries, being an essential raw material for the textile industry. Although fineness is the characteristic that has a higher impact on the quality of camelid and

goat fibers, there are also complementary characteristics (Wang *et al.*, 2005), such as the medullation incidence, which directly affect their textile manufacturing and consequently the characteristics of the resulting fabric, such as its comfort, softness, lightness, thermoregulation capacity, homogeneity and economic/commercial value, among others (Balasingam, 2005).

Medullated fibers are those that have a central channel containing residual cells and air pockets continuously or interrupted along the bark (Wang *et al.*, 2005; Botha & Hunter, 2010) while objectionable fibers (also known as “kemps”, in the wools), have a very wide medullar canal surrounded by a thin layer of cortex (Botha & Hunter, 2010). In llamas and alpacas, it is preferred to call them objectionable (Frank *et al.*, 2014) or strongly medullated fibers (Pinares, *et al.*, 2018).

There is evidence that strongly medullated fibers and fibers with continuous medullation, due to their high thickness rate, would be responsible for an uncomfortable and unpleasant feeling in the skin, called “prickling” (Frank *et al.*, 2014), therefore, their elimination and/or decrease should be pursued; in addition, the fineness, softness, handle and homogeneity of the textile fabric would improve, along with its economic value. The llamas and alpacas selection for fiber that includes the percentage of medullation fiber as a new selection criteria would help to produce fibers with low medullations and of better quality (Pinares, *et al.*, 2018; Cruz, *et al.*, 2019; Torres, 2020).

Medullation is undesirable because of its rigid nature and low degree of buckling (Balasingam, 2005), and it affects the yielding of the textile processing (scouring, carding, combing, and others), particularly if they are used for the production of dresses. Because of the relatively stiff nature of medullated fibres, they tend to break easily, obtaining small fragments that end up being lost in the fabric production process. Also, they do not absorb dye to the same extent as non-medullated fibers and tend to produce yarn with a harsh handle and rough hairy appearance (Wood, 2003). The strongly medullated fibers are undesirable because the large medulla makes the dyed fibers have a paler shade than in the case of non-medullated fibers. This effect is enhanced by the reflection of light on the medulla that makes these fibers appear chalky white (Balasingam, 2005).

Alpacas and llamas have fleeces with medullated fibers, whose incidence varies from 10 to 90%, (Villa-rruel, 1963; Wang *et al.*, 2005; McGregor, 2006). The percentage of fiber medullation show a high and direct relationship with fiber thickness (Martinez *et al.*, 1997; McGregor, 2006; Cruz, *et al.*, 2019), but medullas are present even in very fine fibers, such as 13 μm thick (Pinares, *et al.*, 2018).

The measurement of the medullation can be performed with the Projection Microscope (PMic), the most reliable method, but with the disadvantage of being expensive, laborious (Boguslavsky *et al.*, 1992; Balasingam, 2005; Shakyawar *et al.*, 2013), and may even harm the visual health of the operator (Perez *et al.*, 2008), although there are also computer-aided systems that decreases by 80% the analysis time required (Shakyawar, *et al.*, 2013; Quispe & Quispe, 2018). In this sense, several attempts to attain new objective and indirect MED test, with varying degrees of success, have been developed. Devices like the WRONZ Medullameter (Lappage & Bedfor, 1983), the NIR analysis (Boguslavsky *et al.*, 1992; Hammersley & Tonsend,

1995), and the OFDA system (Brims & Peterson, 1994; Balasingam, 2005) are some examples.

The OFDA100 is a computerized system based on opacity, considering as medullated fibers those ones that have opacity $\geq 80\%$, and kemp fibers those having opacities greater than 94% (Turpie & Steenkamp, 1995; Balasingam, 2005; IWTO, 2015a). This device has undergone validation tests on wools and mohair fibers, with good results in wool (IWTO, 2015a), and somewhat debatable results in mohair (Lee *et al.*, 1996; Lupton & Pfeiffer, 1998). The OFDA is the most commonly used equipment in fieldwork and research. Although OFDA validation tests are still lacking for South American camelid fibers and other types of fibers, there are research reports and recommendations for the use of OFDA100 in alpacas and llama fibers (McGregor, 1995; Lupton, McColl, & Stobart, 2006; Wuliji, 2017; Cruz, *et al.*, 2019). This is against some evidence showing strong differences in MED calculated by the Projection Microscope and OFDA100 (Lee *et al.*, 1996; Pinares, *et al.*, 2018; Torres, 2020).

There are evidences showing that removing or decreasing the objectionable or strongly medullated fibers from alpaca fleece would solve the problem of prickling (McGregor, 1997; Frank *et al.*, 2014; Pinares, *et al.*, 2018). It would be necessary to have the appropriate fieldwork equipment to determine the types of fiber present, and thus incorporate effective selection criteria. The OFDA100 could be an important tool for alpaca genetic improvement programs, however, validation should be implemented first. The present work has been carried out to assess OFDA100 ability to determine MED in alpaca fibers, and compare its results with the ones provided by the Projection Microscope and the Computerized Projection Microscope.

MATERIAL AND METHODS

Three trials or experiments were carried out using three types of instruments: OFDA100 (one of them belonging to the Inca Group located at Arequipa, Perú, and another of Universidad Nacional de Huancavelica, located at Huancavelica, Perú), Projection Microscope (one of them belonging to the Universidad Nacional Agraria La Molina, and another of Maxcorp Technologies, located at Lima, Perú.) and Computerized Projection Microscope belonging to Maxcorp Technologies, located at Lima, Perú. The total medullation of fibers was expressed in percentage (MED).

Experiment 1:

The MED of 36 white male alpaca fiber samples (taken from the Pacamarca Scientific Station, belonging to the Inca Group) have been assessed using the Projection Microscope (PMic) and OFDA100. Each sample was divided into two subsamples, one for each device. The OFDA opacity calibration was greater than 80% for the first subsample following the IWTO-57 (IWTO 2015a) standard. The second subsample was evaluated in a PMic according to the IWTO-8 standard (IWTO 2015b) at the Wool and Fiber Laboratory of the National Agricultural University La Molina (Lima, Peru).

As a whole, 600 fibers per subsample were randomly recorded and classified according to the category of their medullation: non-medullated (NM), with fragmented medullation (Fr), discontinuous medullated (Disc), continuous medullated (Con) and strongly medullated (SM). Each medullation type was expressed in percentage: NMed, MedFr, MedDisc, MedCon, MedSM, respectively. Total MED was MedFr+MedDisc+MedCon+MedSM. Additionally, the individual fiber diameters were measured, and the average fiber diameter (AFD) determined for each subsample.

Experiment 2:

Fiber samples from Experimental farm of Pacamarca, and belonged to males and females between 4 to 104 months old. The analyses were carried out in the Quality Control Laboratory of the Company Inca Tops S.A. (Arequipa), in a controlled environment (environmental relative humidity: $65\% \pm 3\%$; ambient temperature $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$).

Each of the samples was divided into two subsamples. One of which was evaluated by OFDA 100, following the procedure indicated in IWTO-57 (IWTO 2015a), while the other subsample was evaluated using a Computerized Projection Microscope (CPMic) known as "Medullometer", which is actually a Projection Microscope to which a software was implemented that allows to determine the diameter and distinguish the types of medullation objective and manually using a computer mouse, and whose results are automatically saved (Quispe y Quispe 2018). The procedure for the use of the CPMic followed the procedure of IWTO-8 (IWTO 2015b), obtaining fiber fragments using a Hardy's microtome, which were then placed on a slide, dispersed with circular movements using a drumstick inside a droplet of immersion oil and then covered with an object cover, which were then placed on the CPMic plate for semi-automatic evaluation. Only MedCon+MedSM of 200 samples of Huacaya and Suri white alpaca fibers were measured. In addition, the diameter of each fiber was also measured.

Experiment 3:

Of the 200 samples from experiment 2, 105 samples were taken from animals between 12 to 60 months. Each sample was divided into two samples and they were assessed with OFDA100 (at Fibers and Wool Laboratory of Universidad Nacional de Huancavelica, located in Huancavelica, Perú) and CPMic (at Fibers Laboratory of Natural Fiber's Tech SAC, located in Lima, Perú). The evaluation procedure was similar to that carried out in the Experiment 2, but 1000 fibers images per subsample were randomly recorded and classified according to the category of their medullation.

MED values obtained from the devices (OFDA, CPMic and PMic) were evaluated through comparative and relationship tests. The paired t-means test, regression and correlation analysis were used to compare PMic/OFDA100 and CPMic/OFDA100 MED results. Also, for the assessment of biases, determined by the evaluation of averages and differences, the MED obtained between the PMic and CPMic with OFDA100,

according the procedure indicated by IWTO-0 (IWTO 2015c), was used to evaluate the relationship between devices results; then the Pearson correlation analysis and simple linear regression were used. All analyses were carried out using the free statistical package R.

RESULTS AND DISCUSSION

The descriptive statistics of alpaca of medullation percentage obtained from PMic, CPMic and OFDA100 are shown in **Table I**. Significant differences of Med average were appreciated between OFDA100 and the other two devices, obtaining lower medullation averages with OFDA100 compared with PMic or CPMic, with a difference greater than 49% and 27% of Med, for Experiment 1 and 3, respectively. Furthermore, the maximum Med obtained with the OFDA100 (34.00 and 53.20%) is lower or close to the Med average obtained with the PMic or CPMic (34.00% vs 67.43%) according experiment 1 and 3, while the minimum Med obtained with OFDA100 is the smallest (about 1.00% and 0.80%, Experiment 1 and 3, respectively).

In addition, the MedSM+MedCon evaluated with the CPMic are higher than total MED obtained with OFDA100 (Experiment 2), confirming that OFDA100 provides lower MED average. Also, variations in MED values (expressed as standard error and standard deviation) obtained with the OFDA100 are lower than those of the PMic and CPMic.

According **Table II**, significative MED differences between evaluations with OFDA100 carried out at Arequipa and Huancavelica (10.84 vs 13.79%) were found, but this difference was lower compared with OFDA100 with CPMic procedure (27.40% and 30.13, respectively). These results indicate that OFDA100 provides lower Med of CPMic.

On other hand, the analysis of the fibers according medullation type with the PMic and CPMic, it was found that about 20% of fibers were MedSM + MedCon fibers (between 16.42 to 24.14%), while between 24.71 to 43.30% of fibers showed MedFr or MedDisc, although it is observed that MedSM fibers only would have a low incidence (0.24% and 1.34%). Also, comparing the MedFr, MedDis, MedCon or MedSM fibers obtained with PMic and CPMic with the total MED obtained with OFDA100 significative differences were found ($p < 0.001$). Only at Experiment 1, MedDisc did not differ between the two devices (See **Table II**).

Bias evaluation is show at **Table III** and **Figure 1**. Thus, OFDA100 with PMic or OFDA100 with CPMic estimates of MED were biased, because the differences for MED increased linearly and geometrically as averages incremented. At analysis statistical, the slopes and correlation estimates were highly significant. The lineal slope varied between 0.46 to 0.82, and the correlation coefficients varied between 0.41 to 0.86, for experiment 1 and 2.

The plotter graphics, Pearson correlation coefficients and regression equations for MED with MedFr, MedDisc, MedCon and MedSM can be seen in **Figure 2**. The strongest relationships were found between MedCon and MED, obtained by the PMic and CPMic with

OFDA100. Pearson correlation coefficients between 0.85 and 0.56 respectively were found, and weak relationships for MedFr of alpaca fibers (-0.14) was found.

At relationship analysis of MedFr, MedDisc, and MedSM (obtained with PMic) with MED (obtained with OFDA100), the Pearson correlation coefficients no different from zero were found, but for MedCon the Pearson correlation coefficient was high and significant in the experiment 1. But, at Experiment 3, all relationships were significant. Pearson correlation coefficients were higher for MedDisc and MedCon (between 0.67 and 0.89), moderate for MedSM (0.43) and low for MedFr (0.24). According these results, we inferred that there would be relationship between these Med when alpaca fiber is evaluated for OFDA100, PMic and CPMic.

Finally, a high relationship between the AFD and MED (obtained with OFDA100, PMic and CPMic) was found. Pearson correlations were lower using OFDA100 (0.47 and 0.57) than PMic and PMicCom (0.67), and so did regression coefficients: OFDA100 1.7 and 1.8, and PMic and PMicCom (3.7 and 4.5, respectively). More information in **Figure 3**.

Based on the mean test, correlation and regression analyses, it is shown that the MED of alpaca fibers obtained with OFDA100 is different and low from those of PMic and CPMic. In addition, the results show that the MedFr or MedCon or MedSM of alpaca fibers samples are very different at total MED obtained by OFDA100, concluding that OFDA100 would not provide accurate results around total MED or MedFr or MedCon or MedSM. This observation was similar to that of Lee *et al.* (1996), Lupton & Pfeiffer (1998), who reported that OFDA underestimated medullated mohair fibers by a factor about 8.20%. Pinares *et al.* (2018) indicated that the OFDA100 would only be used to register the percentage of alpaca fibers with continuous medullation, but our results do not support this assertion.

Although, difference between MedDisc (obtained with PMic) and total MED (obtained with the OFDA100) is no significant, it is not indicator that OFDA100 measures MedDisc with accuracy, because between the correlation of these medullation types is not significant (at experiment 1), and because, at experiment 2, significant differences found between MedDisc of CPMic and OFDA100. Differences of MED

Table I Medullation percentage statistics (%) of alpaca fibers, obtained with the PMic and OFDA100 (Experiment 1); with CPMic and OFDA100 (Experiment 2); and CPMic and OFDA100 (Experiment 3). The percentage of fibers according their medullation types with the PMic and CPMic are also appreciated (Estadísticas porcentuales de medulación (%) de fibras de alpaca, obtenidas con PMic y OFDA100 (Experimento 1); con CPMic y OFDA100 (Experimento 2); y CPMic y OFDA100 (Experimento 3). También se aprecia el porcentaje de fibras según sus tipos de medulación con el PMic y CPMic).

Experiments	Medullation (%)					
	N	Average	S.E.	S.D.	Minimum	Maximum
Experiment 1						
MED_PMic	36	67.43	1.74	18.30	12.3	91.7
MED OFDA100 ¹	35	18.30	1.68	10.06	1.0	34.0
Percentage per fiber type by PMic						
Non-medullated	36	32.57	1.74	18.30	8.30	87.70
Fragmented medullation	36	29.29	1.84	11.67	10.00	51.50
Discontinuous medullation	36	14.01	1.69	10.13	1.20	44.50
Continuous medullation	36	23.90	0.92	13.01	1.20	65.50
Strongly medullated	36	0.24	0.07	0.44	0.00	1.80
Experiment 2						
MED_CPMic	200	28.71	1.37	19.42	0.67	78.83
MED OFDA100 ¹	200	9.81	0.61	8.61	0.80	45.25
Experiment 3						
MED_CPMic	105	41.14	2.00	20.46	8.51	90.91
MED OFDA100 ¹	105	10.84	0.88	8.97	0.80	45.25
MED OFDA100 ²	105	13.79	1.06	10.87	0.90	53.20
Percentage per fiber type by CPMic						
Non-medullated	105	58.86	2.00	20.46	9.09	91.50
Fragmented medullation	105	17.80	0.75	7.67	3.85	37.38
Discontinuous medullation	105	6.91	0.52	5.29	0.60	26.15
Continuous medullation	105	15.08	1.12	11.49	1.25	55.24
Strongly medullated	105	1.34	0.19	1.95	0.00	10.97

MED_PMic: Percentage of total medullation obtained with the projection microscope; MED OFDA100¹: Percentage of total medullation obtained with OFDA100 of Arequipa; Med OFDA100²: Percentage of total medullation obtained with OFDA100 of Huancavelica; MED_CPMic: Percentage of total medullation obtained with the computerized projection microscope; S.E.: Standard Error; S.D.: Standard deviation.

Table II. Paired t-test and correlation analysis by experiment 1, 2 and 3, about total medullation (MED) and categories of medullation obtained with OFDA100, PMic and CPMic in white Huacaya alpaca fibers (Prueba t pareada y análisis de correlación por experimento 1, 2 y 3, sobre medulación total (MED) y categorías de medulación obtenidas con OFDA100, PMic y CPMic en fibras blancas de alpaca Huacaya).

Experiments	Mean of differences (%)	p-value	Sign.	Pearson's r	Sign.
Experiment 1					
MED_PMic – MED_OFDA100 ¹	49.20	<0.001	***	0.56	***
MedFr_PMic – MED_OFDA100 ¹	10.40	<0.01	**	-0.14	n.s.
MedDisc_PMic–MED_OFDA100 ¹	-4.09	0.119	n.s.	0.15	n.s.
MedCon_PMic – MED_OFDA100 ¹	5.95	<0.001	***	0.79	***
MedSM_PMic–MED_OFDA100 ¹	-18.10	<0.001	***	0.19	n.s.
Experiment 2					
MED_CPMic – MED_OFDA100 ¹	18.90	<0.001	***	0.85	***
Experiment 3					
MED_CPMic – MED_OFDA100 ²	27.40	<0.001	***	0.81	***
MED_CPMic – MED_OFDA100 ¹	30.13	<0.001	***	0.81	***
MED_OFDA100 ³ –MED_OFDA100 ¹	2.93	<0.001	***	0.96	***
MedFr_PMic – MED_OFDA100 ²	6.92	<0.001	***	0.24	*
MedDisc_PMic – MED_OFDA100 ²	-3.95	<0.001	***	0.67	***
MedCon_PMic – MED_OFDA100 ²	4.13	<0.001	***	0.89	***
MedSM_PMic – MED_OFDA100 ²	-9.48	<0.001	***	0.43	***

MED_PMic: Percentage of medullation obtained with the projection microscope; MED_OFDA100¹: Percentage of total medullation obtained with OFDA100 of Arequipa; MED_OFDA100²: Percentage of total medullation obtained with OFDA100 of Huancavelica; MedFr_PMic: Percentage of fibers with fragmented medullation obtained with MicrProy; MedDisc_PMic: Percentage of fibers with discontinuous medullation obtained with MicrProy; MedCon_PMic: Percentage of fibers with continuous medullation, obtained with the PMic; MedSM_PMic: Percentage of strongly medullated fibers obtained with PMic; Med_CPMic: Percentage of strongly medullated + continuous medullation fibers, obtained with the computerized projection microscope.

Table III. Summary of statistical data of the Functional Regression of Geometric Mean (FRGM) and Simple Linear Regression (SLR) of the difference against the average MED of white Huacaya alpacas, obtained with Projection Microscope and OFDA100 (Experiment 1) and Computed Projection Microscope and OFDA100 (Experiment 2) (Resumen de datos estadísticos de la Regresión Funcional de Media Geométrica (FRGM) y Regresión Lineal Simple (SLR) de la diferencia frente al MED promedio de alpacas Huacaya blancas, obtenidos con Microscopio de Proyección y OFDA100 (Experimento 1) y Microscopio de Proyección Computarizada y OFDA100 (Experimento 2)).

Statistical	Type of regression	
	Geometric Functional Mean	Linear differences against average
Experiment 1		
Intercept estimator	-79,06	29,31
Estimated slope	1,44	0,46
Standard slope error	0,21	0,18
Significance of the slope	t-value	-2,16
	Significance	*
Correlation significance	r-value	0.56
	t-value	3,84
	Significance	***
Experiment 2		
Intercept estimate	-2,91	3,01
Standard intercept error	0,57	0,81
Significance of the intercept	***	***
Estimated slope	0,44	0,82
Standard slope error	0,02	0,03
Significance of the slope	t-value	23,44
	Significance	***
Correlation significance	r-value	0,85
	t-value	22,50
	Significance	***

MED_PMic: Percentage of total medullation obtained with the projection microscope; MED OFDA100¹: Percentage of total medullation obtained with OFDA100 of Arequipa; Med OFDA100²: Percentage of total medullation obtained with OFDA100 of Huancavelica; MED_CPMic: Percentage of total medullation obtained with the computerized projection microscope; S.E.: Standard Error; S.D.: Standard deviation.

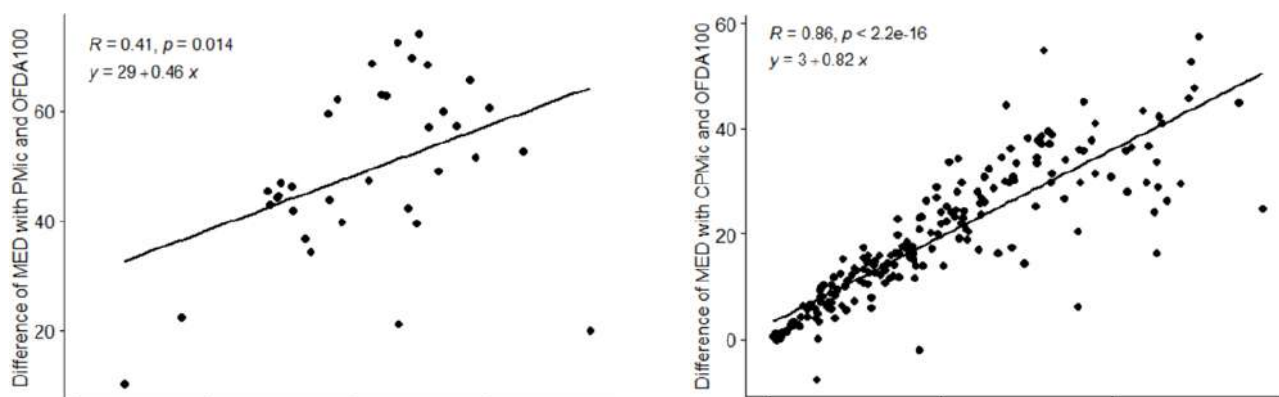


Figure 1. Plotting between the average and the difference of percentage of medullation of fibres (MED) obtained with the Projection Microscope (Pmic) and OFDA100 (above) and the Computer Projection Microscope (CPMic) and OFDA100 (below). The correlation coefficient, equation, and regression adjustment line are displayed. Note that higher values are more dispersed (Trazar entre la media y la diferencia de porcentaje de medulación de fibras (MED) obtenida con el Microscopio de Proyección (Pmic) y OFDA100 (arriba) y el Microscopio de Proyección por Computador (CPMic) y OFDA100 (abajo). Se muestran el coeficiente de correlación, la ecuación y la línea de ajuste de regresión. Tenga en cuenta que los valores más altos están más dispersos).

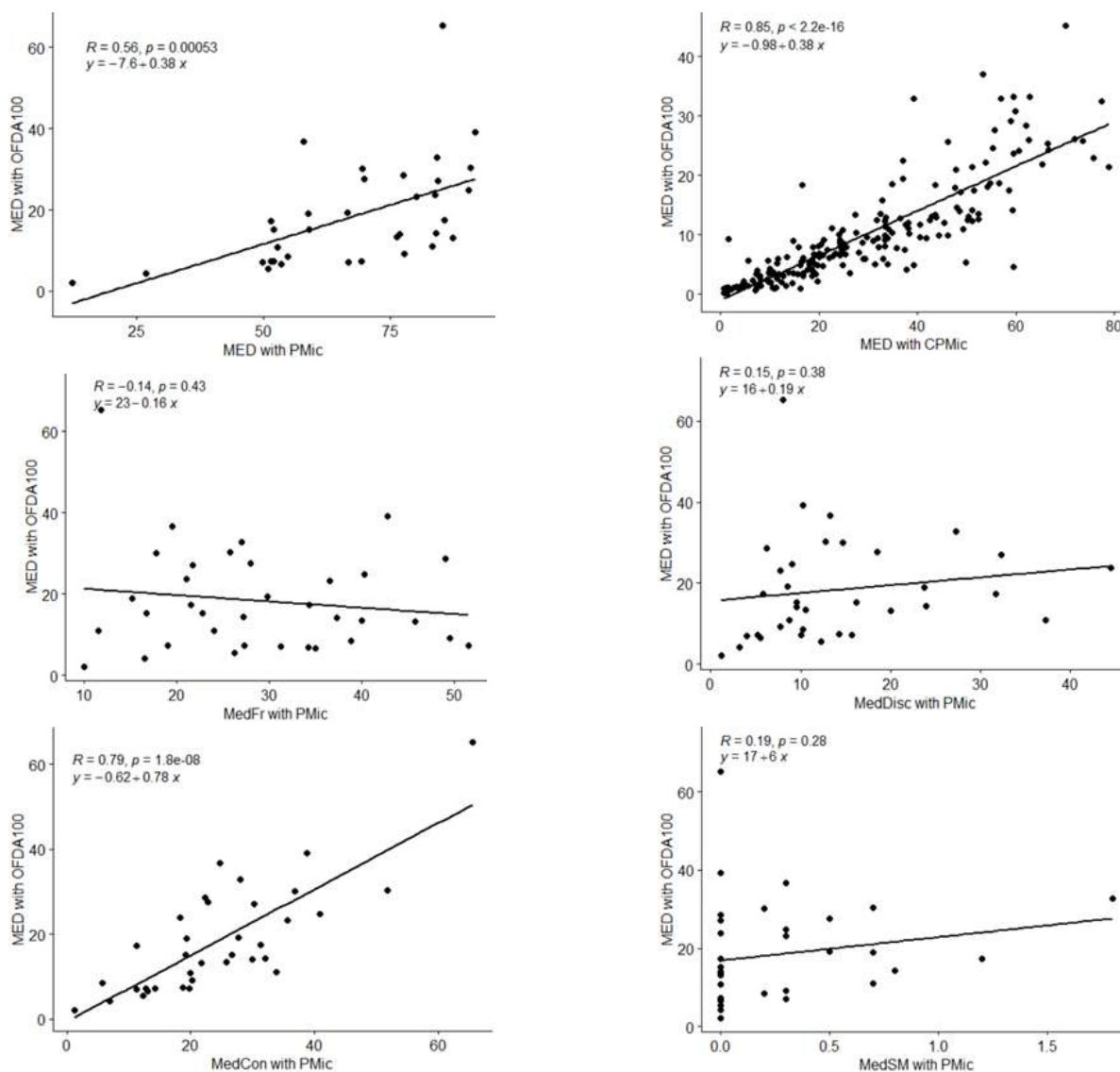


Figure 2. Plotting between the total percentage of total medullation (MED) Figure 2a and 2b, fragmented (MedFr) Figure 2c, discontinuous (MedDisc) Figure 2d, continuous (MedCon) Figure 2e and strongly medullated (MedSM) Figure 2f obtained with the Projection Microscope (Pmic) or Computer Projection Microscope (CPMic) and OFDA100. The correlation coefficient, equation, and regression adjustment line are displayed (Trazando entre el porcentaje total de medulación total (MED) Figure 2a y 2b, fragmentado (MedFr) Figura 2c, discontinuo (MedDisc) Figura 2d, continuo (MedCon) Figura 2e y fuertemente medulado (MedSM) Figura 2f obtenido con el Microscopio de Proyección (Pmic) o Microscopio de Proyección por Computador (CPMic) y OFDA100. Se muestran el coeficiente de correlación, la ecuación y la línea de ajuste de regresión).

in experiment 1 and 3, could be due to: the number of fiber images evaluated (Lupton & Pfeiffer, 1998) because at experiment 1 and 3 were evaluated 600 and 1000 fiber images, respectively; because there is high variability between samples (Hansford, 1992; McGregor, 2006; Pinares, *et al.*, 2018) and sub-sampling.

On the other hand, from the results shown in **Table II**, it can be indicated that OFDA100 would not be measuring MedCon+MedSM either, because the difference found, between 2.63% to 18.90%, is highly significant. However, it has been showed that there is a high relationship around MedCon+MedSM when alpaca fibers are assessed with OFDA100 and CPMic. Lupton & Pfeiffer (1998) found correlations between 0.56 and 0.98 for total MED.

The methodologies followed by the PMic and CPMic are direct and objective, so it is the main reference method for determining the percentage of medullation, and the AFD (Cottle & Baxter, 2015). The OFDA100 measure AFD and medullation content indirectly (Brims & Peterson, 1994; Balasingam, 2005; Botha & Hunter, 2010; Cottle & Baxter, 2015). It was tested to assessed wool and mohair fibers (IWTO, 2015a; Lupton & Pfeiffer, 1998), but not on alpaca fibers. Thus, according our results it would not be adequate to measure the MED of alpaca fibers, and its results should be taken with great caution and prudence. In addition, when evaluating the differences and average values of MED between the OFDA100 with PMic and CPMic, it was found that there is bias about results obtained with these devices (**Table III** and **Figure 2**). This indicates

that, there is a greater difference as the medullation percentages increase.

Previously many researchers evaluated MED with PMic. They found MED greater than 40% (Villarroel, 1963; Hack *et al.*, 1999; Wang *et al.*, 2005; Córdova, 2015; Pinares *et al.*, 2018; Radzik-Rant *et al.*, 2018; Radzik-Rant & Wiercinska, 2021) which do not match the much lower results obtained with OFDA100. Therefore, the opacity threshold should be redefined as an indirect measure of the percentage of medullation, as before, Turpie & Steenkamp (1995), would have warned that in some cases even for mohair fibres no significant correlation was found between the number of counted medullated fibers (including flattened fibres) with the opacity threshold > 80%, which OFDA100 establishes to determine the percentage of medullation in wool or mohair fiber.

Based on the results obtained, it is recommended that, before carrying out measurements with any equipment, a validation and appropriate use for a particular material to be used should be considered (Rubio *et al.*, 2020). Several publications in alpacas fiber about medullation incidence assessed with OFDA100 have been made (Aylan-Parker & McGregor, 2002; Lupton *et al.*, 2006; McGregor, 2006; Cruz *et al.*, 2019) without doing a previous validation; but that would be inappropriate, because alpaca and llama fiber have their peculiarity around the presence, variability, incidence and type of medullation, which is reflected in characteristics such as their density, which would be leading

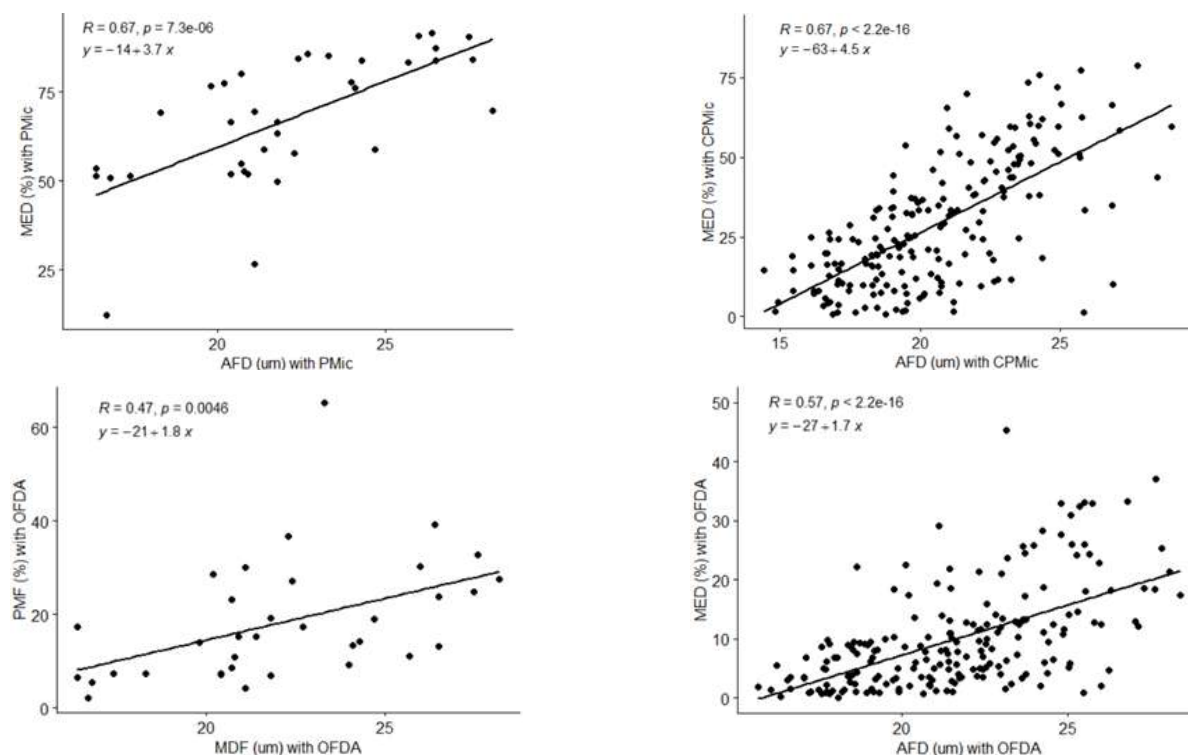


Figure 3. Left: Plotting between the average fiber diameter (AFD) and percentage fiber medullation (MED), obtained with PMic and OFDA100 up and down respectively, respect of Experiment 1 (36 samples). Right: Plotting between the AFD and MED, obtained with CPMic and OFDA100, up and down respectively, corresponding to Experiment 2 (200 samples) (Izquierda: Trazando entre el diámetro medio de la fibra (AFD) y el porcentaje de medulación de la fibra (MED), obtenidos con PMic y OFDA100 hacia arriba y hacia abajo respectivamente, respecto del Experimento 1 (36 muestras). Derecha: Trazado entre AFD y MED, obtenido con CPMic y OFDA100, arriba y abajo respectivamente, correspondiente al Experimento 2 (200 muestras).

to the defined opacity threshold, would not be suitable for this case.

On the other hand, it has been possible to supplement information that effectively the MED, as well as MedCon and MedSM, have high direct relationship with the diameter of fiber (obtained with both PMic, CPMic and OFDA100), as indicated by other researchers (Frank, *et al.*, 2014), which has importance in the selection and improvement of alpacas fiber, because selecting for finesse, the medullation would also be indirectly reduced, however, to decrease MED, and specifically the percentage of strongly medullated and continuously medullated fibres (which are the ones that would be responsible for the unpleasant feeling of itching on the skin) requires that this characteristic be measured accurately and accurately (which unfortunately is not provided by OFDA100) in order to be considered as a selection criteria. Unfortunately, the diameter of fiber has not proven to be a criterion that can improve comfort and avoid the unpleasant pricking (Pinares *et al.*, 2018; Cruz, *et al.*, 2019). Therefore, it is urgently necessary to have an equipment that allows to evaluate the medullation of alpaca fibers in a practical, economical, and fast way, characteristics that are denied to the PMic, so its use is not practical for productive purposes. With use of CPMic is possible to reduce time, but it is not enough to be practical.

CONCLUSIONS

MED obtained with OFDA100 is underestimated in alpaca fibers, and MedFr, MedDisc, MedCon, and MedSM are very different at MED of OFDA100. However, there is linear relationship with those MED obtained with PMic and CPMic between medium to high. Also, there is medium linear relationship between MedCon obtained with PMic and MED obtained with OFDA100. In addition, the two instruments, PMic and CPMic determines MED directly.

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