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Analysis of the echogenicity and echotexture of the walls of the palmar and plantar digital arteries of horses and mules

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Abstract: The grayscale histogram (GH) is a tool available in some software that allows evaluating the quantity and distribution of the grayscale frequency of certain region studied in an image. In ultrasonography, GH has been applied to assess the echogenicity and echotexture of different organs, revealing clinical applicability. This study makes a comparative analysis of the echogenicity and echotexture of the palmar and plantar digital arteries in healthy horses and mules using the grayscale histogram (GH). It also set out to compare the possible variability between the superficial and deep tunica intima and media (IM) of the vessels evaluated. B-Mode ultrasonography was performed in the longitudinal plane of the lateral and medial palmar and plantar digital arteries in 10 healthy horses and 10 mules. Subsequently, the images were analyzed using the GH tool to acquire the variables Mean (echogenicity) and Standard deviation (StdDev) (ecotexture). It was observed that mules showed higher values of brightness intensity (Mean) than horses. Differences were observed between superficial and deep IM, and the deep wall showed higher echogenicity and heterogeneity in both the equine species and in the mule hybrid. The GH proved to be a viable tool to quantify the echogenicity and echotexture of the walls (superficial and deep) of the palmar and plantar digital arteries of horses and mules. In addition, it allowed to highlight the differences found between animals (horses and mules) and IM (superficial and deep).
Keywords: grayscale histogram, IM, ultrasound.

1. Introduction

Ultrasound is a diagnostic imaging method that provides real-time information on organ architecture. The tissue interfaces reflect sound, where the reflection is processed by the equipment and produces an image on a monitor (Peixoto et al., 2010). Ultrasound examination may present divergence in the interpretation of its findings, making it necessary to use techniques that allow the evaluation and quantification of echogenicity and echotexture of the region of interest (Lee et al., 2006).

Echogenicity refers to the ability of different structures to reflect ultrasound waves (echoes). Organs and tissues are visualized in various shades of gray (brightness), which are relatively constant from animal to animal. Some disease states can alter the normal echogenicity of tissues (Farrow, 1992).

The term “anechoic” is used when there is no echo, when ultrasound waves pass through liquids without generating an echo, being represented with a black pattern of the studied structure (absence of brightness intensity). “hypoechoic” represents images with few echoes (hypoechogenic = low brightness intensity). The term “hyperechoic” refers to the high reflection of ultrasound waves and appears as a bright image (hyperechogenic = high brightness intensity) (Barr, 1990; Farrow, 1992; Peixoto et al., 2010). Echotexture, on the other hand, can be defined as homogeneous (where there is no or little variation in gray tones) or heterogeneous (where there is a lot of variation in gray tones), however, the echotexture of the structure depends on the diversity of the tissues that compose it (Chen et al. al., 2004; Callas et al., 2007).

Some techniques make it possible to quantify the echogenicity and echotexture of the evaluated region, allowing the reduction of possible errors in image interpretation (Maeda et al., 1998). The grayscale histogram (GH) is a tool available in some software and allows the evaluation of the amount and distribution of the gray frequency (brightness intensity) of certain regions studied. This procedure is efficient and allows for obtaining real values (Lee et al., 2006; Silva et al., 2015).

To perform the GH, it is necessary to delimit an area of interest (ROI) and subsequently, a graphic representation will be obtained with the number of pixels present in the ROI (Queiroz and Gomes, 2006). The graphical representation has a horizontal axis (illustrates the scale with 256 shades of gray) and a vertical axis (percentages of the number of pixels in each grayscale (degree of brightness) (Santos-Filho et al., 2010).

Some studies use the Image J software as a HEC analysis method and to evaluate the area of interest, a graphical representation is performed with information on some variables. The Mean variable represents the mean brightness intensity of the pixels referring to the ROI, where zero (0) represents a completely black pixel (hypoechoic/hypoechogenic), and 255, a completely white pixel (hyperechoic/hyperechogenic). The variable StdDev corresponds to the degree of variation (standard deviation) of the Mean, the lower the value, the more homogeneous the echotexture (less variation in brightness intensity), the higher the value, the more heterogeneous the echotexture (greater variation in brightness intensity) (Ferreira and Rasband, 2011; Fogaça et al., 2019).

GH has been applied to evaluate echogenicity and echotexture in ultrasound images of different organs, revealing clinical applicability (Lee et al., 2006). GH is available in some software and has been the subject of research for the standardization of values, for application in clinical routines in humans and animals, and for the elaboration of possible experimental protocols (Maeda et al., 1998).

GH has been applied in ultrasound imaging for the evaluation of organs and tissues in humans (Kim et al., 2011; Yang et al., 2012; Tsai et al., 2013; Kim et al., 2015; Harris-Love et al., 2013; al., 2016; Shin et al., 2016; Gollie et al., 2018) and in the assessment of vessel walls that have atherosclerotic plaques (Picano et al., 1986; Marks et al., 2008; Andersson et al., 2009, Sarmento et al., 2014).

Atherosclerotic plaques are not frequent in animals when compared to humans (Ribeiro and Shintaku, 2004; Aguiar et al., 2014). Although there are already reports in dogs (Hess et al., 2003) and in horses (Aguiar et al., 2014). According to Colles and Hickman (1977), they observed that the disease of the navicular is accompanied by vascular changes and suggested that this disease is caused by arteriosclerosis and thrombosis of main arteries that are responsible for supplying the blood flow to the distal sesamoid bone.

Mules are hybrid animals resulting from the cross between horses and donkeys, and as a result, they present anatomical and physiological differences compared to horses (Anderson, 1939; Camac, 1997; Salles et al., 2013). In recent years, these animals have aroused interest in the development of studies highlighting the normal differences found (Salles et al., 2013; Mendoza et al., 2018).

In view of the growing demand for scientific research, this work aimed to compare the echogenicity and echotexture of the palmar and lateral and medial digital arteries, of horses and mules, using GH. Furthermore, to verify the differences (echogenicity and echotexture) between the superficial tunica and deep tunica and tunica media and tunica intima (EIM) of the evaluated arteries.

2. Material e Methods

This research was approved by the Committee on Ethics in the Use of Animals (CEUA) of the Faculty of Veterinary Medicine and Zootechnics of Botucatu (FMVZ/UNESP) (Protocol No. 0257/2018) and was developed at the Edgárdia Farm belonging to the Botucatu Campus of the FMVZ /UNESP.

Ten healthy mules (5 males and 5 females) with a body weight of 347 to 439 kilograms (kg) and ages between 4 and 15 years were selected. Ten healthy horses (5 males and 5 females) with a body weight of 336 to 390 kg and ages ranging from 2 to 18 years were also used. Animals with normal blood count, serum biochemistry (gamma-glutamyl transferase, aspartate aminotransferase, alkaline phosphatase, urea and creatinine) and physical examination (resting heart rate, respiratory rate, intestinal motility and rectal temperature) were included in the study.

To perform the B-Mode ultrasound exams, trichotomy was performed in the region of the metacarpophalangeal joint, and later, isopropyl alcohol at a concentration of 30% water and 70% alcohol was applied to the proximal region of the fetlock. To obtain images in the longitudinal plane, a mobile ultrasound device, model Logiq V2 from Healthcare, with a high-frequency linear transducer (8 to 12 MHz) was used. The examinations were performed with the animals in a standing position without much movement of the locomotor limbs, with the body weight equally distributed between the limbs.

The technical parameters (gain of brightness and depth) were adjusted and modified according to the need for each animal evaluated, always to obtain better quality images of the vessels. The images of the lateral and medial palmar and plantar digital arteries in the longitudinal plane of all animals were evaluated using the GH tool available in the ImageJ software – National Institutes of Health. For GH measurements in superficial and deep IM, an ROI with a sample size (Count) between 300 and

500 pixels were standardized (Figure 1). Subsequently, the values of the variables: Mean and StdDev were used for statistical analysis.

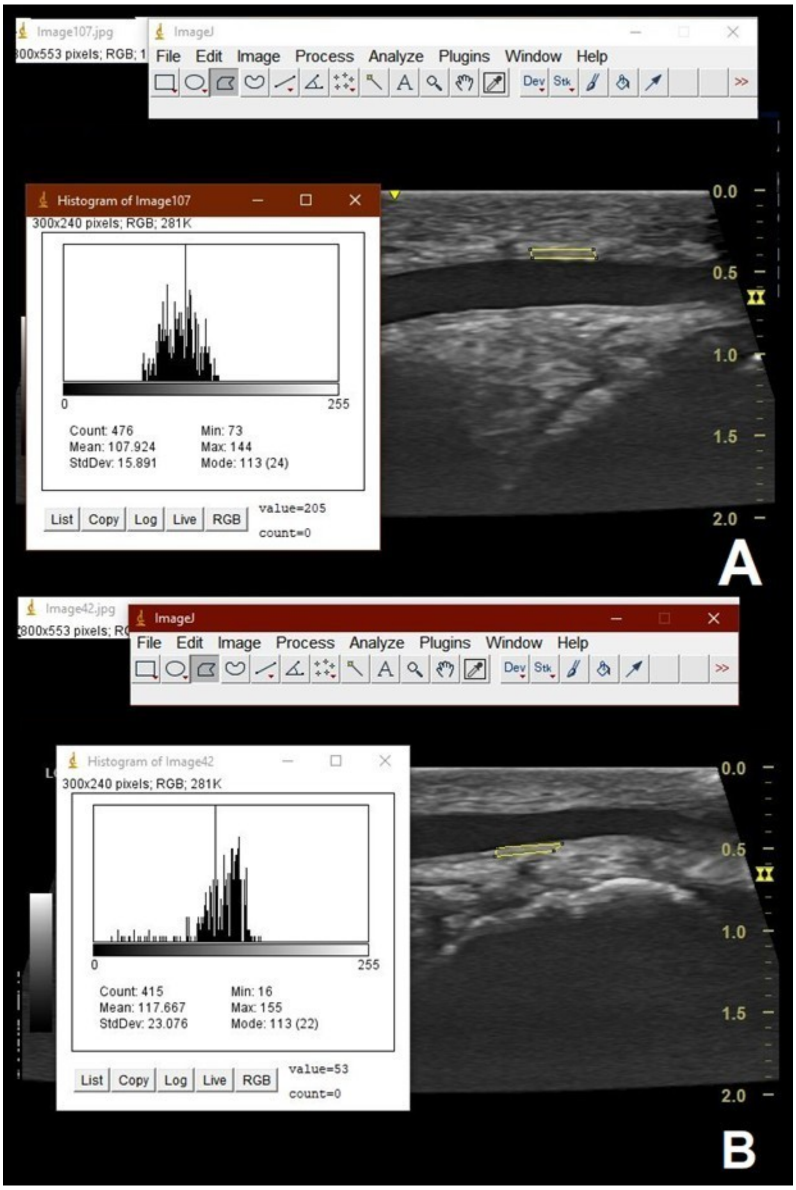

Figure 1 – GH of the superficial wall of the digital artery (A) and the deep wall (B).

The Mann-Whitney test was used for data analysis, in addition to exploratory data analysis, such as mean, median, and standard deviation. The software used in the analysis was the Statistical Package for Social Science (SPSS) V20, developed by IBM. All hypothesis tests developed in this study considered a significance of 5%, i.e., the null hypothesis was rejected when the p-value was less than or equal to 0.05.

3. Results

There was a significant difference between horses and mules (Table 1) in the variable Mean (p=0.031) in the right thoracic limb lateral palmar digital artery (RTL) in the superficial wall, with mules having higher values. However, the medial palmar digital artery of the same limb did not show statistical significance in the comparison between horses and mules. In the left thoracic limb (LTM), the lateral palmar digital artery showed a significant difference between horses and mules in the variable Mean (p<0.001), and the mules showed higher values. As for the medial palmar digital artery of the same limb, there was statistical significance in the variable Mean (p=0.03) of the superficial wall, and the mules showed higher values.

When comparing lateral and medial plantar digital arteries between horses and mules, no statistical significance was observed that there was (Table not illustrated).

Toracic Limb	Face	Wall	GH	Animals	Media	Median	Standard deviation	Q1	Q3	P-value
Right	Lateral	Superficial	*Mean*	Horses	83.4	78.0	20.0	67.0	96.0	**0.031***
				Mules	101.4	105.0	21.7	92.0	111.0	
			StdDev	Horses	17.1	17.0	5.1	14.0	20.0	0.857
				Mules	16.7	16.0	4.7	13.0	20.0	
		Deep	*Mean*	Horses	102.8	100.0	23.8	81.0	120.0	0.35
				Mules	92.6	96.0	23.6	78.0	105.0	
			StdDev	Horses	19.6	18.0	3.8	18.0	20.0	0.35
				Mules	22.8	24.0	7.1	17.0	30.0	
	Medial	Superficial	*Mean*	Horses	88.7	82.0	20.9	76.0	109.0	0.243
				Mules	102.3	98.0	33.0	77.0	119.0	
			StdDev	Horses	17.5	18.0	3.0	16.0	19.0	0.436
				Mules	19.4	21.0	6.8	13.0	26.0	
		Deep	*Mean*	Horses	104.4	102.0	26.3	78.0	123.0	0.309
				Mules	114.9	119.0	36.7	84.0	147.0	
			StdDev	Horses	21.8	20.0	4.5	19.0	23.0	0.799
				Mules	22.3	20.0	8.7	14.0	27.0	
Left	Lateral	Superficial	*Mean*	Horses	84.2	84.0	10.3	75.0	90.0	**<0.001***
				Mules	112.0	115.0	23.8	101.0	128.0	
			StdDev	Horses	18.5	19.0	4.2	17.0	19.0	0.927
				Mules	18.3	16.0	5.1	14.0	23.0	
		Deep	*Mean*	Horses	102.9	100.0	21.5	83.0	123.0	0.403
				Mules	110.7	110.0	28.3	93.0	134.0	
			StdDev	Horses	19.4	18.0	6.2	15.0	22.0	0.826
				Mules	20.4	24.0	6.9	13.0	25.0	
	Medial	Superficial	*Mean*	Horses	93.7	93.0	18.2	85.0	109.0	**0.03***
				Mules	117.3	127.0	28.6	90.0	137.0	
			StdDev	Horses	19.9	20.0	6.8	16.0	23.0	0.509
				Mules	20.6	21.0	5.8	15.0	25.0	
		Deep	*Mean*	Horses	116.2	119.0	16.1	111.0	123.0	0.402
				Mules	122.9	129.0	32.8	90.0	140.0	
			StdDev	Horses	23.7	23.0	8.8	19.0	25.0	0.308
				Mules	20.7	21.0	6.0	16.0	23.0	

Table 1 – Mean, median, standard deviation, Q1 and Q3 of the HEC variables (Mean and StdDev) on the walls (superficial and deep IM) of the palmar digital arteries, followed by the comparison between horses and mules.

Comparing superficial and deep IMT (Table 2) in horses, there was a significant difference in the lateral palmar digital artery of the right thoracic limb (RTL) in the variable Mean (p=0.05), with the deep wall presenting higher values. As for the medial palmar digital artery of the same limb (RTL), there was a significant difference in the variable StdDev (p=0.009), with the deep wall presenting higher values.

In horses, the left forelimb lateral palmar digital artery (LTL) did not present statistical significance in the comparison of IM (superficial and deep). However, the medial palmar digital artery of the same limb (LTL) showed a significant difference in the variable Mean (p=0.012), where the deep wall showed higher values.

Concerning the mules, the lateral palmar digital artery of the right forelimb (MTD) showed a significant difference in the StdDev variable (p=0.005) where the deep wall showed higher values. Regarding variables of the other member, there was no statistical significance significance.

Toracic Limb	Face	GH	Animals	Wall	Mean	Median	Standard Deviation	Q1	Q3	P-value
Right	Lateral	*Mean*	Horses	Deep	102.8	100.0	23.8	81.0	120.0	**0.05***
				Superficial	83.4	78.0	20.0	67.0	96.0	
			Mules	Deep	92.6	96.0	23.6	78.0	105.0	0.19
				Superficial	101.4	105.0	21.7	92.0	111.0	
		StdDev	Horses	Deep	19.6	18.0	3.8	18.0	20.0	0.299
				Superficial	17.1	17.0	5.1	14.0	20.0	
			Mules	Deep	22.8	24.0	7.1	17.0	30.0	**0.005***
				Superficial	16.7	16.0	4.7	13.0	20.0	
	Medial	*Mean*	Horses	Deep	104.4	102.0	26.3	78.0	123.0	0.204
				Superficial	88.7	82.0	20.9	76.0	109.0	
			Mules	Deep	114.9	119.0	36.7	84.0	147.0	0.23
				Superficial	102.3	98.0	33.0	77.0	119.0	
		StdDev	Horses	Deep	21.8	20.0	4.5	19.0	23.0	**0.009***
				Superficial	17.5	18.0	3.0	16.0	19.0	
			Mules	Deep	22.3	20.0	8.7	14.0	27.0	0.242
				Superficial	19.4	21.0	6.8	13.0	26.0	
Left	Lateral	*Mean*	Horses	Deep	102.9	100.0	21.5	83.0	123.0	0.059
				Superficial	84.2	84.0	10.3	75.0	90.0	
			Mules	Deep	110.7	110.0	28.3	93.0	134.0	0.955
				Superficial	112.0	115.0	23.8	101.0	128.0	
		StdDev	Horses	Deep	19.4	18.0	6.2	15.0	22.0	0.94
				Superficial	18.5	19.0	4.2	17.0	19.0	
			Mules	Deep	20.4	24.0	6.9	13.0	25.0	0.518
				Superficial	18.3	16.0	5.1	14.0	23.0	
	Medial	*Mean*	Horses	Deep	116.2	119.0	16.1	111.0	123.0	**0.012***
				Superficial	93.7	93.0	18.2	85.0	109.0	
			Mules	Deep	122.9	129.0	32.8	90.0	140.0	0.481
				Superficial	117.3	127.0	28.6	90.0	137.0	
		StdDev	Horses	Deep	23.7	23.0	8.8	19.0	25.0	0.233
				Superficial	19.9	20.0	6.8	16.0	23.0	
			Mules	Deep	20.7	21.0	6.0	16.0	23.0	0.938
				Superficial	20.6	21.0	5.8	15.0	25.0	

Table 2 – Mean, median, standard deviation, Q1 and Q3 of the HEC variables (Mean and StdDev) on the walls (superficial and deep IM) of the palmar digital arteries, followed by the comparison between the deep wall with the superficial wall of the forelimbs of horses and mules.

According to Table 3, it was possible to observe that there were few significant differences between the IM (deep and superficial) in the pelvic limbs of horses and mules. The lateral plantar digital artery of the right pelvic limb (RPL) showed a difference between the IM (superficial and deep) in horses in the variable StdDev (p=0.01), being greater in the deep wall. While the medial plantar digital artery of the same limb (RPL) did not show statistical significance.

The lateral plantar digital artery of the left pelvic limb (LPL) in mules showed a difference between the walls (superficial and deep) in the variable StdDev (p=0.011), where the deep wall showed the highest value.

Pelvic Limb	Face	GH	Animals	Wall	Mean	Median	Standard Deviation	Q1	Q3	P-value
Right	Lateral	*Mean*	Horses	Deep	102.9	104	22.4	83	122	0.279
				Superficial	93.7	101	17.1	82	104	
			Mules	Deep	103.7	101	20.8	93	119	0.189
				Superficial	110.2	106	27.2	97	131	
		StdDev	Horses	Deep	24.0	24	5.6	23	28	**0.01***
				Superficial	18.1	17	4.0	16	20	
			Mules	Deep	19.7	19	5.7	16	21	0.067
				Superficial	17.0	16	3.9	15	17	
	Medial	*Mean*	Horses	Deep	92.3	82	27.2	72	123	0.791
				Superficial	87.1	86	14.6	81	90	
			Mules	Deep	110.9	99	40.4	79	150	0.639
				Superficial	102.5	97	27.9	91	109	
		StdDev	Horses	Deep	22.6	18	9.1	15	30	0.825
				Superficial	21.4	21	3.9	19	23	
			Mules	Deep	21.6	19	10.2	13	29	0.152
				Superficial	17.6	18	7.2	10	23	
Left	Lateral	*Mean*	Horses	Deep	93.4	98	20.9	76	108	0.65
				Superficial	95.7	87	33.7	76	105	
			Mules	Deep	106.9	104	26.6	93	131	0.613
				Superficial	101.6	110	27.6	77	120	
		StdDev	Horses	Deep	21.2	20	6.6	17	24	0.199
				Superficial	17.3	17	3.6	15	18	
			Mules	Deep	22.1	23	5.7	20	27	**0.011***
				Superficial	17.1	16	6.0	14	20	
	Medial	*Mean*	Horses	Deep	103.0	98	25.1	82	121	0.29
				Superficial	93.4	80	28.8	76	114	
			Mules	Deep	103.0	94	38.6	76	135	0.754
				Superficial	103.9	95	33.9	77	140	
		StdDev	Horses	Deep	20.9	20	5.3	19	24	0.406
				Superficial	19.7	18	4.4	17	23	
			Mules	Deep	20.3	21	7.5	13	26	0.696
				Superficial	19.8	19	6.6	15	24	

Table 3 – Mean, median, standard deviation, Q1 and Q3 of the HEC variables (Mean and StdDev), followed by the comparison between the deep wall and the superficial wall of the pelvic limbs of horses and mules.

4. Discussion

For the B-mode ultrasound examinations in the present study, the parameters of the techniques (gain in brightness and depth) were modified for each animal. The objective was to obtain the best quality images since changing the techniques does not interfere significantly with the GH results, according to Sarmento et al. (2014).

In this study, the longitudinal plane was used to perform the GH measurements, since in the longitudinal plane the arteries present tubular aspects (Mattoon et al., 2020) facilitating the delimitation of the area of interest (ROI), and in the transverse plane the arteries present appear as rounded anechoic structures (Yanik, 2002; Mattoon et al., 2020). The evaluation of the arteries in the longitudinal plane is the most indicated way, since in the measurement of the intima-media thickness (IMT) in the transverse plane, values above or below the true may occur (Evans et al., 1989; Liguori et al., 2002).

Studies with GH involving healthy animals are pertinent, as they collaborate with the elaboration of experimental protocols (Maeda et al., 1998). Therefore, the healthy animals in the present study were carefully selected through physical and laboratory examinations. Furthermore, it is essential to perform studies comparing horses and mules, as mules are hybrids (Anderson, 1939; Camac, 1997; Salles et al., 2013), presenting anatomical and physiological differences from horses (Burnhan, 2002; Alsafy et al., 2008; Smith, 2009; Miranda and Palhares, 2017).

In this study, the walls of the lateral and medial palmar digital arteries of the forelimbs of horses and mules were compared by GH. It was observed that mules showed higher brightness intensity values (Mean) than horses in the superficial wall of the lateral surface of the LTL and RTL and also in the superficial wall of the medial face of the LTL, while in the pelvic limbs, there was no statistical significance in the comparison between horses and mules. In a study comparing the superficial wall of the carotid arteries between horses and mules by GH, it was observed that mules also showed a higher brightness intensity (Mean) than horses (Fogaça et al., 2019). Although the study by Fogaça et al. (2019) was in another vessel, when comparing horses and mules, the brightness intensity behavior (Mean) was similar to what we found in the present study with palmar digital arteries, where mules showed higher values than horses in the thoracic limb.

Regarding the comparison of the IM (superior x deep) of horses and mules by GH, it was verified that the deep wall showed a greater increase in brightness intensity and heterogeneity (Mean and StdDev). It is attributed that this increase in brightness intensity and heterogeneity (Mean and StdDev) may be related to the artifact of acoustic reinforcement (Vargas et al., 2008). According to Vargas et al. (2008), acoustic reinforcement is an increase in the echo amplitude that occurs subsequently to a low attenuation structure, this occurs in structures that have liquids in their interior.

The deep walls showed higher heterogeneity, meaning they have more brightness intensity variations. Also, the brightness intensity (echogenicity) is higher in the deep wall, which may be related to the acoustic reinforcement artifact. According to Vargas et al. (2008) and Lima et al. (2013), acoustic reinforcement is an increase in the amplitude of the echo that occurs subsequently to a low attenuation structure, this occurs in structures that have liquids inside.

In ultrasonographic evaluation, enhancement appears as an area of more intense brightness. However, in most GH studies in human vessels beings (Wohlin et al., 2009; Noto et al., 2012; Sarmento et al., 2014), measurements are performed in the deep wall, due to the possibility of the presence of atherosclerotic plaques. However, it is not possible to state whether or not the reinforcement artifact interferes with the GH results.

We believe that the GH performed in the transverse plane can minimize changes in measurements influenced by the artifact of acoustic reinforcement, as the arteries in this plane appear as anechoic rounded structures (Yanik, 2002; Mattoon et al., 2020) that may result in limiting ROI. While in the longitudinal plane, where the vessel presents a tubular appearance, facilitating the obtaining of a larger sample (ROI) (Yanik, 2002; Mattoon et al., 2020).

We believe that studies with GH should be performed in animals with limbs compromised by vascular alterations, allowing us to verify whether or not this tool collaborates with the diagnosis and therapeutic follow-up. As well as the possibility of applying this tool in other regions of the locomotor limbs, such as muscles, tendons and ligaments, because in the present study, we limited the evaluation of the GH in healthy animals.

5. Conclusion

Mules have higher values of palmar digital arteries brightness intensity (Mean) than horses in the deep wall on the lateral surface of the RTL and LTL and in the medial face of the LTL. The differences occur in the walls (superficial and deep) of the palmar and lateral and medial digital arteries of horses and mules, with greater echogenicity and heterogeneity in the deep wall. GH proved to be a viable tool to quantify the echogenicity and echotexture of the walls (superficial and deep) of the palmar and plantar digital arteries of horses and mules and to help to highlight differences in echogenicity and echotexture.

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