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Radiographic Texture of the Trabecular Bone in the Proximal Phalanx of Horses

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Abstract

Trabecular bone is highly dynamic in response to external and internal stimuli, and changes in its structure can be quantified through fractal analysis. However, fractal analysis is still an incipient technique in equine research. This study aimed to evaluate the complexity, heterogeneity, and density of the trabecular bone of the proximal phalanx (P1) of healthy adult horses of different breeds and sexes by measuring the values of fractal dimension (FD), lacunarity, and bone area fraction (BA/TA) in 65 radiographic examinations of the metacarpophalangeal joint and evaluate the agreement between the *BoneJ* and *FracLac* plugins for measuring FD. Regions of interest of 50 × 50 pixels were manually selected on the trabecular bone in the proximal epiphysis of the P1. No differences were observed for FD, lacunarity, and BA/TA between horses of different breeds and sexes ($p > 0.1$). The *BoneJ* and *FracLac* plugins showed no agreement when measuring FD ($p < 0.01$). Therefore, the radiographic texture of the trabecular bone of the P1 in horses had no influence depending on the analyzed breed or sex. The *FracLac* plugin measured higher FD values, and hence standardization using the *BoneJ* plugin is recommended. Further studies are required to evaluate other breeds, age groups, and training levels.

Keywords

Fractal analysis; fractal dimension; bone area fraction; lacunarity; trabecular bone; horses

1. Introduction

Trabecular bone has a complex structure that is difficult to measure accurately [1]. Its fractal nature has been described for decades and has been the target of several studies carried out in humans, mainly to evaluate bone changes resulting from diseases such as osteopenia and osteoporosis [2,3]. Trabecular bone has a higher metabolism and remodeling rate than cortical bone, which makes it more dynamic in response to variations in the magnitude and direction of mechanical loads [4]. This plasticity of internal bone morphology makes trabecular bone a good indicator of changes in bone structure [3,5].

Fractal analysis is a method used to describe complex structures and can be used to study biological systems, such as bone trabecular tissue, considered a natural fractal [2,6]. Fractal analysis is expressed as a fractal dimension (FD) and reflects the degree of complexity of an object, being correlated with the mechanical properties of the bone [7,8]. Lacunarity reflects the heterogeneity of an object, indicating the distribution of gaps in space [8]. The bone area fraction, called BA/TA (or BV/TV for three-dimensional images), is correlated with bone density and indicates the fraction of bone tissue in a two-dimensional image, consisting of a potential determinant of stiffness, elasticity, and trabecular strength [9–11]. Therefore, FD, lacunarity, and BA/TA are used for morphological descriptions of trabecular architecture and are potential indicators of bone quality [1,8].

The quantitative assessment of bone microarchitecture can be performed using two-dimensional digital images, such as radiographic examinations, or three-dimensional images, such as images obtained by computed tomography [12]. The *ImageJ* software is the most used in the medical field for the scientific evaluation of bone tissue images, as it is free, modern, simple to use, and easy to download [13].

Fractal analysis is useful in the clinical and scientific evaluation of horse bone tissue, but it is little studied and used in the species. Similarly, it is not yet known exactly how the trabecular bone architecture may vary in humans according to factors such as sex, age, anatomical region, and body mass, in addition to other (epi)genetic factors. Moreover, there is no methodological standardization for calculating the fractal dimension, which results in high variability of technique and results in studies that use fractal analysis.

This study aimed to measure the values of FD, lacunarity, and BA/TA of the trabecular bone of the proximal phalanx of male and female Pure Blood Lusitano and Brazilian Sport Horse and evaluate the agreement between two plugins for measuring the fractal dimension values. We hypothesize that the radiographic texture of the trabecular bone of the proximal phalanx of horses is different between breeds and sexes and that there is no agreement between the different plugins for measuring the fractal dimension.

2. Materials and Methods

2.1. Data Collection

The study evaluated 15 radiographic examinations of the left metacarpophalangeal joint of 15 female Pure Blood Lusitano (PSL) horses aged between 3 and 6 years old and weighing between 450 and 530 kg, and 50 radiographic examinations of the metacarpophalangeal joint of both forelimbs of 25 male (12/25) and female (13/25) Brazilian Sport Horses (BH) aged between 3 and 4 years old and weighing between 450 and 700 kg. The radiographic examinations were obtained from two studies previously carried out and approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science of the University of São Paulo, under protocols numbers 4119210917 and 8840030417 [14,15]. The horses were evaluated through physical examination of the locomotor system and radiographic examination (lateromedial, dorsopalmar, dorsolateral-palmaromedial oblique, dorsomedial-palmarolateral oblique, and flexed lateromedial projections), showing to be clinically healthy and not presenting lameness or radiographic or ultrasound changes of the metacarpophalangeal joint.

2.2. Definition of Regions of Interest

Radiographic images in JPEG format were processed and analyzed using the *Fiji* image processing package of the *ImageJ* software (National Institutes of Health, USA). Regions of interest (ROIs) of 50 × 50 pixels from the dorsopalmar projection of the metacarpophalangeal joint were defined in the proximal epiphysis of the proximal phalanx, manually selected on the trabecular bone, distal to the subchondral region, medial and lateral to the sagittal groove. ROIs were positioned 30 pixels away from the joint line, centered on a line that ran from the sagittal groove to the lateral/medial end, to standardize their selection. ROIs were cropped, converted to 8 bits, duplicated, and binarized **(Figure 1)**.

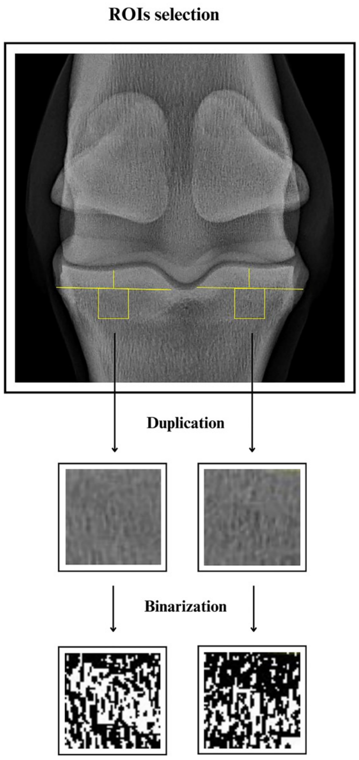

Figure 1: Demonstration of the definition and pre-processing of regions of interest (ROIs).

2.3. Fractal Dimension, Lacunarity, and Bone Area Fraction

Fractal dimension, using the box-counting method, and bone area fraction (BA/TA) of the binary images were measured using the *BoneJ* plugin, using the "Fractal Dimension" and "Area/Volume Fraction" tools, respectively. Fractal dimension and lacunarity were measured by the *FracLac* plugin using grayscale images ("Gray 1: Differential" image type), two scan positions and the differential box-counting method, which is the most suitable method for assessing the texture of objects [8,16–18]. All measurements were carried out by a single previously trained observer.

2.4. Statistical Analysis

Statistical analyses were performed using the Jamovi program (version 2.3.21). The analyzed variables were presented as a single population, being described with the mean, median, standard deviation, minimum, maximum, and 95% confidence interval. Box plots were used to present data separated by sex and breed. The normality of residuals and homogeneity of residuals of variances were analyzed using the Shapiro-Wilk and Levene tests, respectively. Q-Q plots were used to evaluate the data distribution. The Mann-Whitney U test was used to compare within the sex and breed categories. The Bland-Altman test [19] from the *blandr* package [20] was used to evaluate the agreement between the two methods for measuring FD. The one-sample T-test was used to test the hypothesis of similarity to zero of the differences relative to the means of the method data. Values of $p < 0.05$ were considered significant and $p < 0.1$ were considered a trend.

3. Results

3.1. Differences in Trabecular Bone Structure between Breeds

No significant difference was observed between the mean values of FD (calculated using the *BoneJ* plugin), lacunarity, and BA/TA of the medial and lateral ROIs for Brazilian Sport Horse and Pure Blood Lusitano horses ($p > 0.1$) **(Figure 2)**. The descriptive analysis of the data is available in **Supplementary Table 1**.

3.2. Differences in Trabecular Bone Structure between Sexes

A trend for a difference relative to lower mean fractal dimension (*BoneJ*) values was observed in the lateral ROI for male horses ($p = 0.06$). The other variables showed no significant differences between sexes ($p > 0.1$) **(Figure 3)**. The descriptive analysis of the data is available in **Supplementary Table 2**.

3.3. Comparison of Plugins for Measuring Fractal Dimension Values

No agreement was observed between the two plugins for measuring fractal dimension values ($p < 0.01$). The *FracLac* plugin tended to calculate higher values when compared to *BoneJ* (**Figure 4** and **Figure 5**). The mean FD values obtained using *FracLac* were 1.69 ± 0.07 and 1.66 ± 0.08 for the medial and lateral ROIs, respectively. The mean values obtained using *BoneJ* were 1.55 ± 0.04 for the medial ROI and 1.54 ± 0.05 for the lateral ROI **(Table 1)**.

4. Discussion

This study evaluated the fractal dimension, lacunarity, and bone area fraction of horses of different breeds and sexes, as well as the agreement between two plugins for measuring the fractal dimension. The results indicated no difference in the complexity, heterogeneity, and density of trabecular bone between Brazilian Sport Horse and Pure Blood Lusitano horses. Different breeds are subjected to different breeding systems, functions, and training programs. Consequently, horses of different breeds are subjected to different frequencies, magnitudes, and directions of mechanical stress, which cause tension in the bone and remodeling of the external and internal bone structure [3].

The close relationship between bone architecture and mechanical load causes exercise to alter bone density and the orientation of the trabeculae, and studies have suggested that this is a protective mechanism and a normal physiological condition for horses in training [21–28]. Therefore, no difference in the bone trabecular microstructure of horses of different breeds observed in our study can be explained by the similarity in the intensity and frequency of exercises to which these animals were subjected, as Pure Blood Lusitano mares were kept in stables and Brazilian Sport Horses were at the beginning of the training program for show jumping. There is still a lot that we do not understand about the influence of genetics and development on trabecular architecture, but perhaps changes in bone microstructure may be more closely related to the sport modality and training routine of horses than to the breed.

Our results demonstrated no difference in bone microstructure related to sex, except for the fractal dimension of the lateral ROI, which had a trend to differ between sexes ($p = 0.06$), with lower mean values observed in male horses. Bone growth is mediated by hormones at local and systemic levels, and the opposing action of sex steroids in humans is known to be responsible for sexual dimorphism in the skeleton, but the mechanisms underlying sexual dimorphism in cortical and trabecular bone are not well understood [3,4,29]. The main target of testosterone is cortical bone, while estrogens act essentially on trabecular bone and are powerful regulators of bone maturation and structure [30,31]. Circulating estrogen concentrations fluctuate significantly throughout the estrous cycle, as do concentrations of bone formation and resorption markers, which may be associated with altered bone remodeling in mares [29]. Prado Filho and Sterman [32] evaluated bone mineral density in male and female racing foals at the beginning of their athletic activity and found no significant difference between the sexes, which corroborates the findings of this study. On the other hand, Jackson *et al.* (2003) observed sex-associated differences in bone remodeling in racing horses, with males showing higher serum concentrations of biochemical markers of bone formation and resorption than females [33]. Further studies are needed to establish more precisely the influence of sex and sex steroids on horse bone microstructure and its clinical relevance.

Table 1: Descriptive analysis of fractal dimension (FD), lacunarity, and bone area fraction (BA/TA) values.

	Mean	Lower limit	Upper limit	Median	Standard deviation	Minimum	Maximum
Medial BA/TA	0.53	0.5	0.55	0.56	0.11	0.29	0.71
Medial FD (*BoneJ*)	1.55	1.54	1.56	1.55	0.03	1.43	1.61
Medial FD (*FracLac*)	1.69	1.67	1.71	1.7	0.07	1.35	1.76
Medial lacunarity	0.02	0.01	0.02	0.01	0.02	1.00e-4	0.07
Lateral BA/TA	0.49	0.46	0.52	0.47	0.12	0.3	0.74
Lateral FD (*BoneJ*)	1.54	1.52	1.55	1.54	0.05	1.34	1.61
Lateral FD (*FracLac*)	1.65	1.64	1.67	1.66	0.08	1.33	1.76
Lateral lacunarity	0.02	0.02	0.03	0.01	0.02	9.00e-4	0.12

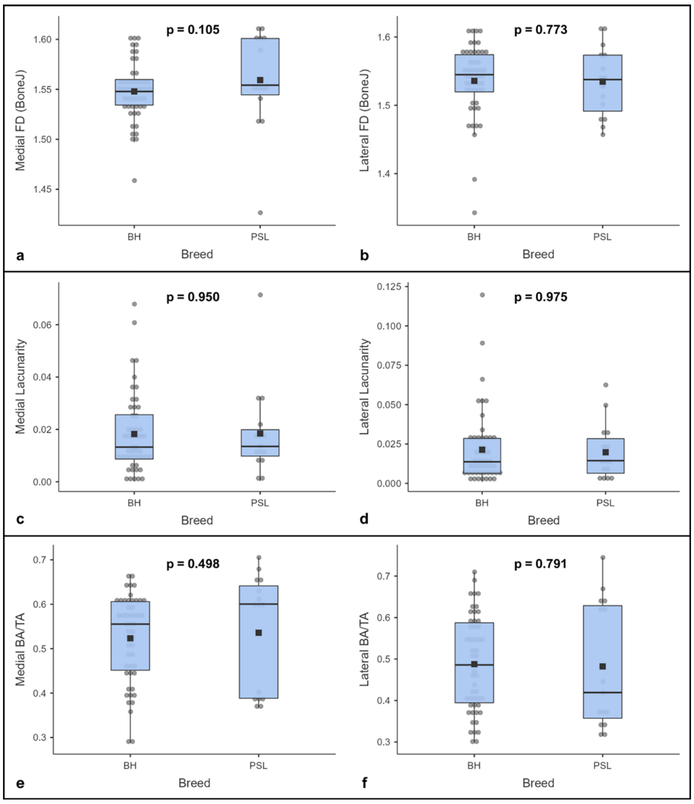

Figure 2: Box plots showing data on fractal dimension (a and b), lacunarity (c and b), and bone area fraction (e and f) of the medial and lateral ROIs for horses of BH and PSL breeds. FD = fractal dimension; BA/TA = bone area fraction; BH = Brazilian Sport Horse; PSL = Pure Blood Lusitano.

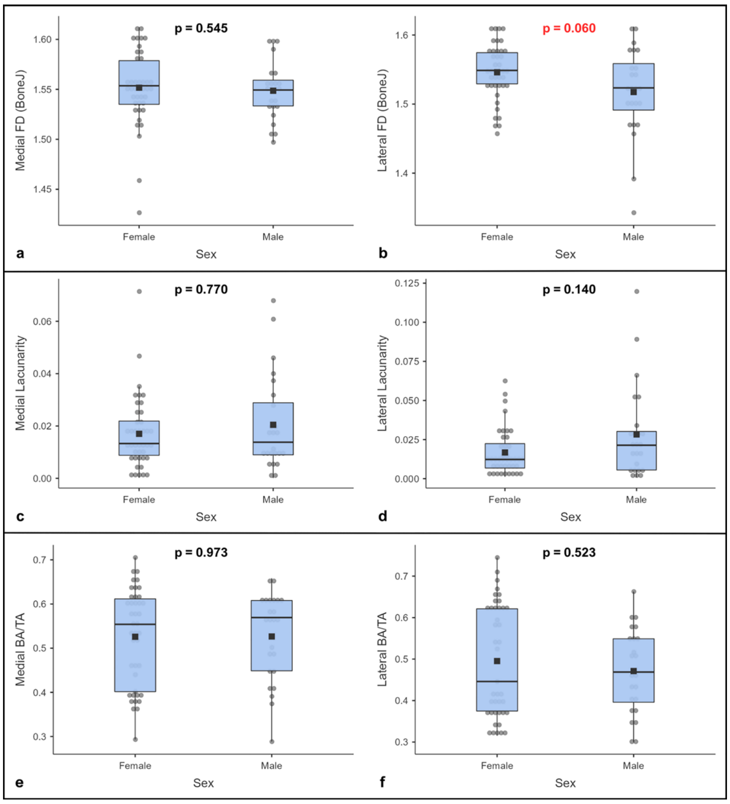

Figure 3: Box plots showing data on fractal dimension (a and b), lacunarity (c and b), and bone area fraction (e and f) of the medial and lateral ROIs for male and female horses. FD = fractal dimension; BA/TA = bone area fraction.

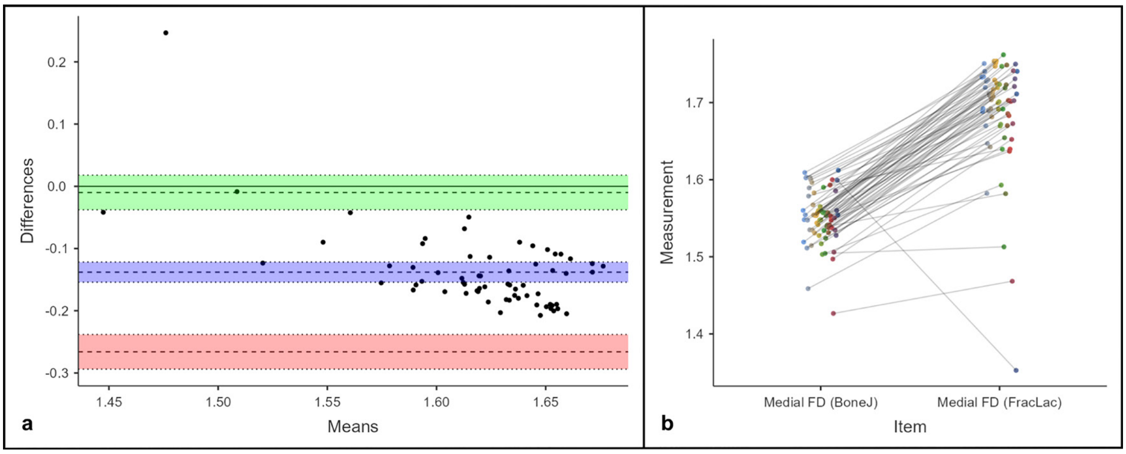

Figure 4: Bland Altman plot (a) and data reliability plot (b) representing the differences between the mean fractal dimension (FD) values of the medial ROI measured by two plugins.

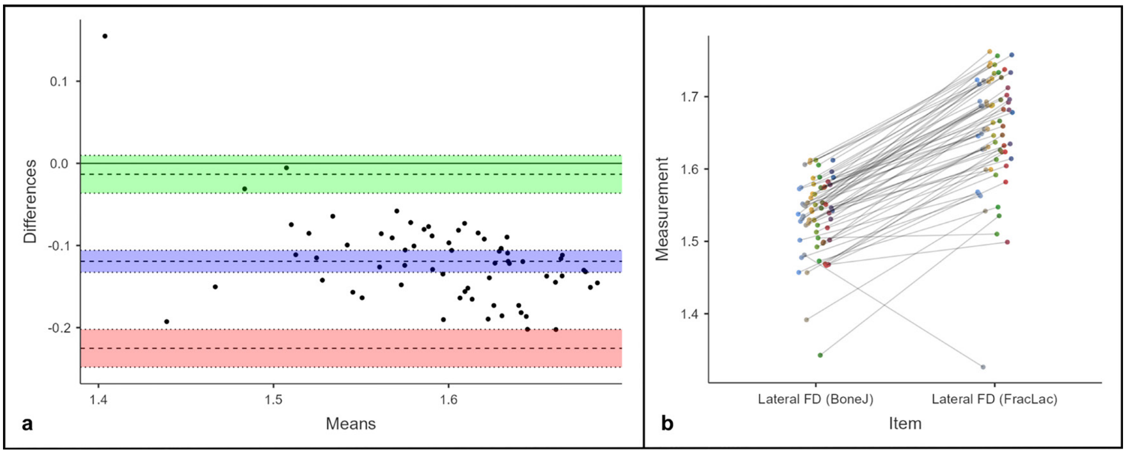

Figure 5: Bland Altman plot (a) and data reliability plot (b) representing the differences between the mean fractal dimension (FD) values of the lateral ROI measured by two plugins.

The values found in our study suggest that the trabecular bone of the proximal phalanx of healthy adult horses presents high complexity, density, and homogeneity, represented by high values of FD and BA/TA and low values of lacunarity. The body of the femur in a study evaluating human trabecular bone presented mean values of 1.2 ± 0.06 for FD and 11.05 ± 4.38 for BA/TA [34]. The subchondral trabecular bone of the femoral head in individuals with severe hip osteoarthritis presented lower FD and higher BV/TV values (1.05 ± 0.02 and 36.97 ± 10.39, respectively) than control individuals (1.10 ± 0.05 and 28.09 ± 0.06) [2]. Women with osteoporotic fractures have lower FD of the cortical bone of the femoral neck (2.11 ± 0.01) than women with hip osteoarthritis (2.57 ± 0.01) [8]. A systematic review that evaluated the use of fractal analysis in dental images found FD values that ranged from 0.78 to 2.79 for panoramic radiographs, 0.78 to 1.84 for periapical radiographs, and 0.91 to 2.4 for cone-beam computed tomography [12]. Yaşar and Akgünlü [35] evaluated dental radiographs of edentulous and dentate regions and observed that the edentulous regions had higher FD values (1.65 ± 0.08) and lower lacunarity values (0.34 ± 0.05) than dentate regions (1.36 ± 0.12 and 0.41 ± 0.06, respectively), possibly due to regional anatomical differences and occlusal loads. Foals presented BA/TA of 26.7 ± 4.6 for the trabecular bone of the parasagittal groove and 31.0 ± 4.4 for the condyle of the third metacarpal bone, while adult horses (>6 years) presented values of 56.6 ± 1.7 and 62.9 ± 5.4, respectively, demonstrating an increase in bone fraction with age and training [21]. These studies showed that the values of FD, lacunarity, and BA/TA (analogous to BV/TV) varied according to the methodology, such as the imaging modality (2D or 3D), image processing, ROI positioning (cortical, subchondral, or trabecular bone), and anatomical region, and the characteristics of the evaluated individuals, such as age, level of sporting activity, and clinical changes.

In order to perform fractal analysis, image pre-processing is necessary to standardize images and reduce artifacts by removing large-scale variations in image brightness, which can be caused, for example, by overlapping soft tissues. Many authors follow the methodology detailed by White and Rudolph [36], where a sequence of steps is carried out to assess trabecular bone, using *ImageJ* software [12,36]. The *BoneJ* and *FracLac* plugins automate image pre-processing, reducing the number of steps required for standardization, making it easier and faster. The several methods for calculating FD often result in obtaining different dimensions for the same object [37]. Ensuring standardization in the methodology of studies that analyze biological images is essential to guarantee reproducibility in research and a more accurate comparison between studies, as well as minimizing inconsistencies related to the object of study and facilitating applicability in clinical routine.

Our study found a non-equivalence between FD measurements performed by the *BoneJ* and *FracLac* plugins, using the box-counting method, which is considered easily accessible and is the most frequently used for fractal analysis [12]. In this method, grids of decreasing size are scanned over a binary image, and the number of boxes containing at least one foreground pixel is counted. As the box size decreases, the proportion of boxes with foreground pixels increases in a fractal structure. In short, the complexity of a structure is assessed by the change in detail with scale. While *BoneJ* measures pixels in a binary image, which can have one of two possible values (background or foreground/black or white), *FracLac* uses the differential box-counting method to measure pixels in grayscale images, which can have one of many possible values. Thus, *FracLac* finds the FD and lacunarity in grayscale images by counting the average pixel intensity per box, based on the relationship between the change in average intensity and the change in grid caliber [38]. We recommend standardizing FD measurements using the *ImageJ* software, as it is more widely used and better-established in the literature, and *BoneJ*, as it is a well-established plugin that brings together other important tools for assessing bone histomorphometry, such as trabecular thickness, trabecular separation, trabecular number, bone volume fraction, bone surface fraction, osteoclast activity, and degree of anisotropy, which allows quantitative evaluation of bone microarchitecture, bone formation, and bone remodeling [39–41].

Fractal analysis has been used to evaluate bone tissue in a wide variety of studies, such as bone functional adaptation [3,4,9,21,23,26,42,43], bone quality [10], osteopenia [44], osteoporosis [8,45–48], osteoarthritis [1,2,17], rheumatoid arthritis [49], osteointegration [50,51], bone failure analysis [16], and fibrous dysplasia [52]. Therefore, fractal analysis uses resources accessible to veterinarians, such as radiographs, to provide information with potential clinical application, diagnostic and prognostic value, which can help in the understanding of clinical cases, decision-making, therapeutic planning and evaluation of the evolution of treatment, as well as enabling early evaluation of microscopic alterations that precede late clinical manifestations.

We believe that our research can contribute to the emergence of new studies in the area of equine orthopedics that use fractal analysis for bone assessment in the species, such as for the assessment of bone quality, changes in the trabecular structure related to physiological and pathological conditions, clinical treatments and surgical procedures, standardization of research groups, assessment of bone adaptation to exercise, and prediction of fracture risk, among others. Our study evaluated the trabecular bone of the proximal phalanx of horses without clinical, radiographic or ultrasound alterations in this anatomical region, and the data obtained can be used for comparison with other individuals, provided that the same methodology is applied.

The use of radiographic images to evaluate the trabecular bone is among the limitations of this study. Despite the advantage of non-invasiveness and the high availability of retrospective data, radiographs are two-dimensional images that represent a three-dimensional trabecular structure and, therefore, are not accurate representations of the trabecular structure due to limitations imposed by this imaging modality, such as the resolution [3,6]. As the amount of information obtained is greater as image quality increases, studies suggest that 3D images are more suitable for fractal characterization of trabecular bone [7,53]. However, unfortunately, equipment for obtaining 3D images, such as computed tomography and magnetic resonance imaging, is still not widely available in the routine of equine veterinarians, which is why we used radiographic images as the object of study.

In conclusion, breed and sex have no significant influence on the values of fractal dimension, BA/TA, and lacunarity of the trabecular bone of the proximal phalanx of horses. The exercise routine to which the horse is subjected is assumed to have a higher influence on the trabecular bone structure than the breed. New studies evaluating other breeds, age groups, horses from different sports, and 3D images can be considered. Fractal dimension quantification using the *BoneJ* and *FracLac* plugins of the *ImageJ* software are not equivalent. Standardizing the measurement of fractal dimension values using the *BoneJ* plugin and detailing the methodology used in future studies are recommended, as these procedures have an important influence on the values of FD, lacunarity, and BA/TA.

Supplementary Materials

The tables of descriptive analysis of FD, lacunarity, and BA/TA values separated by breed and sex are available as **Supplementary Materials**.

Authors' Contributions

Literature review: L.O.P.; Methodology: A.F.S., D.R.A.S., and L.O.P.; Data collection: L.O.P.; Statistical analysis: A.F.S.; Writing: L.O.P.; Review: A.F.S., A.L.M.Y., D.R.A.S., and A.L.V.Z. All authors read and approved the final version of the manuscript.

Data Availability

Data supporting the conclusions of this study are available upon request from the corresponding author.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical approval

Ethical approval was not required to conduct this study as retrospective data was used.

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