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Comparison of conventional and imaging methods to obtain morphometric measurements of the Nordestino breed horse

Comparação do método convencional e de imagem para obtenção das medidas morfométricas em cavalos da raça Nordestina

Neila Lidiany Ribeiro[1]*, Geovergue Rodrigues de Medeiros[2], Maria Luíza Coelho Cavalcanti[1], José Henrique Souza Costa[1], Severino Guilherme Caetano Gonçalves dos Santos[1], George Vieira do Nascimento[1], Maria Norma Ribeiro[3]

ABSTRACT: Characterization studies must be prioritized to allow the maintenance of the racial standard to guarantee the conservation of their genes of origin. The Nordestina breed is a small animal, not exceeding 150 cm for males and 145 cm for females, with a body weight of approximately 280 kg in both sexes. The objective of this research was to compare the conventional method and the imaging method (ImageJ® software) in the performance of morphometric measurements of horses of the Nordestina breed. The morphometric measurements performed were withers height (WH), croup height (CH), body length (BL), neck length (NL), side height (SH), pelvic girdle height (PGH), shin perimeter (SP), chest circumference (CC), face length (FL) and face width (FW). Head length (P=0.045), head width (P=0.001), and shin perimeter (P<.0001) showed a significant difference between the two methods evaluated, these measurements presented higher values when performed by image, but did not present a coefficient of variation greater than 8%. The total canonical correlation between the sets of variables X and Y were 0.99, and the first pair of canonical variables absorbed 85% of the total variation, whose Wilk's and F test (P<0.001) were significant for all canonical pairs, which indicates a strong correlation between the measurements obtained by the conventional method and by imaging. The evaluation of photographs by the ImageJ® software proved to be a viable alternative to the conventional method of taking morphometric measurements in horses of the Nordestina breed.

KEYWORDS: Alternative methodology; image analysis; ImageJ software; morphologic

RESUMO: Estudos de caracterização devem ser priorizados para permitir a manutenção do padrão racial e garantir a conservação de seus genes de origem. A raça Nordestina é um animal de pequeno porte, não ultrapassando 150 cm para os machos e 145 cm para as fêmeas, com peso corporal de aproximadamente 280 kg em ambos os sexos. O objetivo com esta pesquisa foi comparar o método convencional e o método de imagem (software ImageJ®) na realização de medidas morfométricas de cavalos da raça Nordestina. As medidas morfométricas realizadas foram altura da cernelha (AC), altura da garupa (AG), comprimento do corpo (CC), comprimento do pescoço (CP), altura do costado (ACO), altura do vazio subesternal (AVZ), perímetro da canela (Pc), perímetro torácico (PT), comprimento da cabeça (CCA) e largura da cabeça (LCA). O comprimento da cabeça (P=0,045), largura da cabeça (P=0,001) e perímetro da canela (P<0,0001) apresentaram diferença significativa entre os dois métodos avaliados, essas medidas apresentaram valores maiores quando realizadas por imagem, mas não apresentaram coeficiente de variação superior a 8%. A correlação canônica total entre os conjuntos de variáveis X e Y foi de 0,99, sendo que o primeiro par de variáveis canônicas absorveu 85% da variação total, cujos testes de Wilk e F (P<0,001) foram significativos para todos os pares canônicos, o que indica uma forte correlação entre as medidas obtidas pelo método convencional e por imagem. A avaliação de fotografias pelo software ImageJ® mostrou-se uma alternativa viável ao método convencional de tomada de medidas morfométricas em cavalos da raça Nordestina.

PALAVRAS-CHAVE: Metodologia alternative; análise de imagem; Software ImageJ; morfologia.

[1]Pesquisador bolsista PC/CNPq do Instituto Nacional do Semiárido – INSA, Campina Grande, Paraíba, Brasil
[2]Pesquisador do Instituto Nacional do Semiárido – INSA, Campina Grande, Paraíba, Brasil
[3]Professora do Departamento de Zootecnia da Universidade Federal Rural de Pernambuco – UFRPE, Recife, Pernambuco, Brasil
*Corresponding author: (neilalr@hotmail.com)
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INTRODUCTION

Characterization studies must be prioritized to allow the maintenance of the racial pattern to guarantee the conservation of their genes of origin. The Nordestina breed is small animals, not exceeding 150 cm for males and 145 cm for females, with a body weight of approximately 280 kg in both sexes, and coats tortillas, chestnuts, and bays the most common (Melo et al., 2013). Through measurement, data are obtained for the preparation of reviews, studies of body regions, verification of ethnic characters, biometric studies, weight assessment, verification of growth, the study of proportions and aptitude assessment indices, and, finally, as a resource assists the visual training of the judge (Torres & Jardim, 1985).

Phenotypic characterization is one of the basic steps in animal genetic resource conservation programs (Mariante et al., 2009). The evaluation of domestic animals based on shape and proportions and to verify of the qualities and defects of each region and the animal as a whole can be recognized by two methods: the empirical one, called "gap of view," and the objective, through the measurements (Camargo; Chieffi, 1971).

The morphologic structure is of great importance in equine genetic improvement programs, not only because of its economic interest but also because of its functional relationship with the performance of animals in their different activities, requiring evaluations of linear and angular measures that are indicative of the quality of movements and performance (Mcmanus et al., 2005; Godoi et al., 2013; Mariz et al., 2015). Moreover, the typological classification of an equid, as to its suitability for specific jobs, can be done by analyzing the existing relationships between the different body measurements of the animal (Astiz, 2009).

The morphometric evaluation is practiced by measuring the body using metric instruments, such as the hypsometer and measuring tape. It can be performed in different species of zootechnical interest, requiring the evaluator to have prior knowledge of anatomy, especially osteology, and myology, which facilitates the process of identifying structures that serve as a reference for obtaining the most diverse measurements (Zamith, 1946; Miranda et al., 2006; Melo et al., 2013; Oliveira et al., 2014; Mariz et al., 2015).

The method of morphometric evaluation by image analysis has been used to reduce the time to obtain measurements (Van Weeren; Crevier-Denoix, 2006), and the risk of accidents (Santos et al., 2017), reduce animal stress, and increase the levels of reliability of the data obtained, highlighting this aspect the use and development of specific software for evaluating animal images (López et al., 2000; Mariz et al., 2015; Andrialovanirina et al., 2020; Freitag et al., 2021). The development of this software is time and labor-intensive, so the use of free software for image analysis may be beneficial. However, there is still scant research on feasibility proof (Mariz et al., 2015; Andrialovanirina et al., 2020).

Few studies still evaluate alternative forms of morphometric evaluation, especially with horses (Lage et al., 2009; Mariz et al., 2015; Santos et al., 2017; Freitag et al., 2021). However, the use of software developed for use in image analysis in general, without considering characteristics of the species and race evaluated, showed results that point to the potential use of the computational language used to evaluate morphometric characteristics (Mariz et al., 2015).

ImageJ software has been efficiently used to measure fish (Andrialovanirina et al., 2020) and to evaluate linear measurements of high-intensity sports horses (Mariz et al., 2015). Therefore, the objective of this research was to compare the conventional method and the imaging method (ImageJ® software) in the performance of morphometric measurements of horses of the Nordestina breed.

MATERIAL AND METHODS

This study was conducted according to the ethical principles of animal experimentation and under the approval of the Animal Ethics Committee of the National Institute of the Semiarid (protocol number 0002/2022), Brazil.

The study was carried out in Garaci, Itaporanga, Parari, Patos, São Mamede, Sapé, Sumé, São João do Cariri, Baraúna, Nova Palmeira, Picuí, Paulista, Gurjão, Campina Grande, Cabaceiras, Pedra Lavrada municipalities, Paraiba State, Brazil (Figure 1).

A total of 136 adult animals of both sexes were evaluated, on which ten morphometric measurements were made by the conventional method with the aid of a hypsometer and tape measure and by imaging. The five observers were trained before data collection. In addition, the observers' records were tested for inter-observer reliability.

The animals were measured by the conventional method and by image. In the conventional method, measurements were performed by 5 (five) different evaluators, and in the imaging method, only one evaluator measured using the program.

The measurements performed were: Height of the withers (HW) corresponding to the highest point of the interscapular

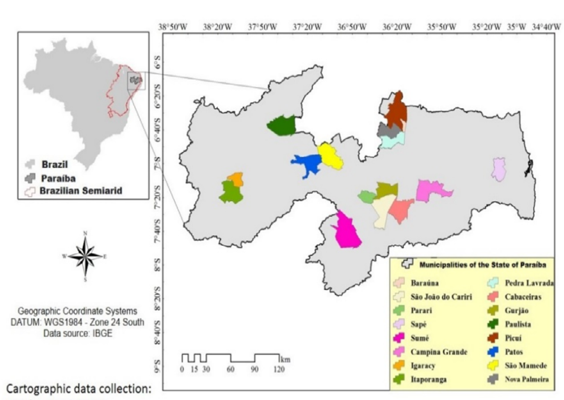


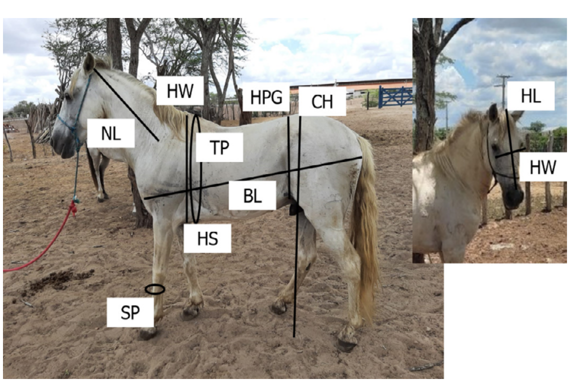
Figure 1. A localization municipalities where morphometric collections on the Nordestino horse was performed.

region to the ground; Croup height (CH) from the highest point of the sacral tuberosity of the ileum to the ground; Body length (BL) traced between the greater tubercle of the humerus and the ischial tuberosity; Neck length (NL) distance between the nape of the neck and the superior junction with the withers; side height (SH) vertical distance between the highest point of the interscapular region and the xiphoid cartilage perpendicularly to the ground; pelvic girdle height (PGH) vertical distance between the highest point of the croup and the flank crease perpendicular to the ground, shin perimeter (SP) measured by the circumference of the left metacarpal bone in its middle third; thoracic perimeter (TP) circumference of the chest traced along the line of the spinous apophysis of the 7th-8th thoracic vertebra to the corresponding lower sternal region, at the level of the olecranon tuberosity; head length (HL) corresponds to the distance from the occipital protuberance to the most rostral point of the upper lip and head width (HW) is the distance between the free part of the right supraorbital border and the left border (Figure 2).

After measurements were carried out by the conventional method, the animals were photographed (Smartphone Samsung A71) in frontal, lateral, and caudal views. The photographs were taken at about 2.0 meters from the animal, facilitating the framing of the entire animal in the image. Assessments by photographic images were performed using ImageJ® 1.46r software (National Institute of Mental Health, USA). In this study, the object used to calibrate the software was a hypsometer measuring 1.50 cm in height, placed fifty centimeters from the animals before obtaining the image.

The variables obtained with the two methods were compared using the Student test at 5% probability (PROC GLM, Statistical Analysis, version 9.3). Finally, the model was applied, including the method (1=conventional and 2= image) as a fixed effect in the analysis.

Pearson's correlation (PROC CORR, Statistical Analysis System, version 9.3) was performed between the variables obtained by the conventional method and by imaging.



HL= head length; HW=head width; NL=neck length; HW=height at the withers; CH=croup height; BL=body length; TP=thoracic perimeter; SP=shin perimeter; HS= height at side; HPG=height at the pelvic girdle.

Figure 2. Morphometric measurements performed on Nordestino horses

Canonical correlation analysis was performed to examine the association between the two sets of variables. Developed by Hotelling in 1935, canonical correlation analysis has as its primary objective the study of the association between sets of variables X and Y through the analysis of their linear combinations (W_k V_k) so that they are strongly correlated with each other. Hence, linear combinations are also called canonical variables.

At each stage of the analysis, two linear combinations are constructed, one concerning the variables of X and another concerning the variables of group Y, that is, at each stage of the analysis a pair of canonical variables was constructed, which were defined by: $V_k = a'_k X_p$ and $W_k a = b'_k Y_q$, being p = 10 biometric measurements obtained by a conventional method, q = 10 biometric measurements obtained by photographic method k = 1, 2, ..., min (p, q) e a'_k and factors with plica vectors x1, respectively, called canonical coefficients and chosen so that the correlation between the variables V_k and W_k be maximized on the set of linear combinations of X and Y. These linear combinations have variances equal to 1 and are uncorrelated with the other canonical variables. The correlations between the variables V_k and W_k are called canonical correlations (r) and were obtained to quantify the associations between the sets of variables X and Y. So, by definition, we have that: $r_{V_k W_k} = \frac{Cov(W,V)}{\sqrt{Var(W)}\sqrt{Var(V)}}$ The squared canonical correlation (r^2) was also calculated to obtain the proportion of shared variance between the canonical variables.

Statistical analyzes were performed using the canonical procedure of STATISTICA version 14. (Trial). The procedure uses four Wilks Lambda statistical tests (likelihood ratio), Pillai Test, Hotelling-Lawley Trace, and Roy Test to test linear hypotheses in multivariate analyses **(Khattree; Naik, 2000)**. Therefore, the likelihood ratio test, whose statistic is called Wilks' Λ (lâmbda), was used to verify the significance of the canonical correlation between the respective canonical variables. Thus, the hypotheses were defined as H_0: all canonical correlations are equal to zero; H_a: the canonical correlations are non-zero.

The ten characteristics obtained by the conventional method (hypsometer) were grouped as variable X, and the variables obtained by the photographic method as variable Y (Figure 3).

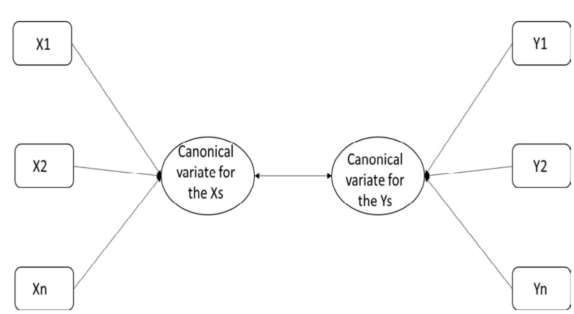


Figure 3. Organization structure of the groups of X and Y variables for the construction of canonical variables.

RESULTS AND DISCUSSION

The measurements of head length (P=0.045), head width (P=0.001), and shin circumference (P<.0001) showed significant differences between the two methods evaluated. These measurements presented higher values when performed by image but did not present a coefficient of variation greater than 8%. The other variables showed no significant difference between the measurement methods of the animals (P>0.05). The height of the posterior was the variable that presented the highest coefficient of variation value. In the conventional method, the coefficient of variation was 15.76%, and in the imaging method, it was 14.73% (Table 1).

The measurement of withers height, croup height, body length, and thoracic perimeter showed greater homogeneity between the methods evaluated, which can be explained by the fact that the height variables are easy to measure since the anatomical reference points for these are under less effect. of the volume of musculature that covers the skeletal base point.

Mariz et al. (2015) evaluated withers height, croup height, body length, and thoracic perimeter and observed a coefficient of variation below 5% between the imaging and conventional methods (with a similar hypsometry to those of the present study). **Santos, 1981)** observed that height at the withers, among all linear measurements, is one of the safest, as it shows, more minor variations resulting from the measurement instrument or errors made by the measurer.

The shin perimeter is related to the bone quality of the skeleton and the functionality of the anatomical region, determining, together with the thoracic perimeter, the carrying capacity of the animal **(Berbari Neto, 2005)**. In addition, the proper proportion between the length of the forearm and the shin is essential, as the shin being short, will present short tendons that suffer less tension than the long ones, and the forearm muscles, being more elastic than the tendons, deal better with stress (**Thomas, 2005).**

Differences were observed between the evaluators. Evaluators 1 and 2 had similar measurements, as did evaluators 3 and 5. If the measures of evaluator four differed from these two groups, three groups were formed (Figure 4).

Even with this difference between the evaluators, Pearson's correlation demonstrates a high correlation between the imaging method and the evaluators, with correlation values above 0.97 (Table 2).

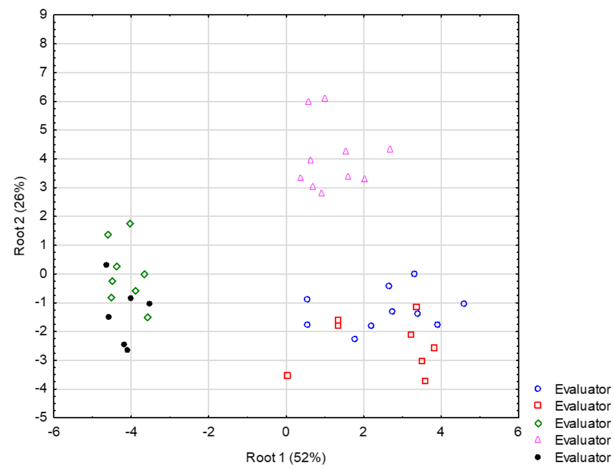


Figure 4. Two-dimensional graph of root 1 and 2 of the grouping of evaluators.

Table 2. Pearson's correlation between measurements obtained by conventional method and by imaging (r^2) for the different evaluators

Evaluators	Pearson's Correlation
1	0.98*
2	0.99*
3	0.98*
4	0.97*
5	0.98*

*Significant Pearson correlations (P< 0.001)

Table 1. Morphometric measurements (cm) in horses by the conventional method and by imaging

Method	HL	HW	NL	HW	CH	BL	TP	SP	HS	HPG
Conventional										
Mean±standard deviation	50±3.83b	18±1.50b	58±6.22	139±6.59	138±6.86	141±9.81	158±9.51	18±0.92b	67±7.75	53±8.44
CV (%)	7.68	8.34	10.66	4.73	4.98	6.97	6.00	5.00	11.49	15.76
Max.-Min	55-42	21-15	70-49	153-129	151-126	160-118	174-140	20-17	76-51	69-41
Image										
Mean±standard deviation	52±4.05a	20±1.16a	60±6.08	141±6.03	140±6.16	142±9.11	160±9.17	20±0.73a	69±7.48	55±8.11
CV (%)	7.75	5.95	10.07	4.27	4.40	6.41	5.73	3.61	10.79	14.73
Max.-Min	58-44	21-17	72-51	154-131	151-129	159-120	176-143	21-19	78-50	70-43
P-value	0.045	0.001	0.281	0.305	0.373	0.650	0.566	<.0001	0.434	0.554

Different letters in the column differ from each other by the Student test at the 5% probability level; CV= Coefficient of variation; HL= head length; HW=head width; NL=neck length; HW=height at the withers; CH=croup height; BL=body length; TP=thoracic perimeter; SP=shin perimeter; HS= height at side; HPG=height at the pelvic girdle.

Body size and conformation are important for horse breeds and are subject to intense selection pressure. Breeder associations typically select horses based on functional criteria and encourage breeding animals with body structures best suited to function, with correct skeletal conformation being a key determinant of body type **(Brooks et al., 2010).**

Muscle development in the anterior portion of the body is characteristic of the evaluated breed, being associated with the control of the action of the forelimbs (**Santiago et al., 2014)**. Therefore, in breeding and selection programs, such variables are essential to be observed, guiding mating **(Costa et al., 2001**; **Lucena et al., 2015)**, taking into account the functionality of interest (saddle, sport, and traction) **(Brooks et al., 2010).**

When comparing the measures between the methods, it is observed that the correlation was above 80%. The variables Neck length, wither height, croup height, body length, thoracic perimeter, and side height showed a correlation of 99% comparing the conventional and imaging methods (Table 3). The total canonical correlation between the sets of variables X and Y were 0.99, and the first pair of canonical variables absorbed 85% of the total variation, whose Wilk's and F test ($p<0.001$) were significant for all canonical pairs (ViWi), which indicates a strong correlation between the measurements obtained by the two methods studied.

It is worth mentioning that the small oscillation in the results is inherent to the standard zoometric evaluation with the use of a hypsometer since it is subject to interference from the type of terrain where the animal is evaluated, animal immobility, pole parallelism, in addition to the effect of the evaluator **(Pimentel** et al., 2011). Thus, the variation obtained in the proposed image evaluation method can be considered acceptable, in addition to the fact that the ImageJ automatic method is low cost and easy to install, and the accessibility of the ImageJ software is open source, which can be run on any computer (**Shafait** et al., 2017).

A factor to be considered in this aspect concerns the form of joint evaluation of the morphometric measurements considered in this study since the differences in depth in some areas of the image concerning the calibration object placed next to the animal may interfere with the results (**Pinto** et al. al., 2005).

Mariz et al. (2015) evaluated the method for conventionally collecting morphometric measurements, by image and with stickers and concluded that the evaluation of photographs by the ImageJ® software, without the use of indicator stickers, is a viable alternative to the conventional method of taking measurements. morphometric measurements in Quarter Horse horses.

The proportion of the shared variance between the two canonical variables for the first pair, given by the squared canonical correlation coefficient (r^2), was 0.99 (Figure 5). That is, 99% of the variation of V_1 was explained by the variation of W_1, which indicates a high association between the sets of variables X and Y.

The standardized canonical coefficients for the first canonical pair "V_1" and "W_1" are linear (canonical) functions and are described below:

$V_{1=}$-0.16FL+0.007FW-0.007NL-0.03WH+0.06CH+0.035BL+1.00CC+0.01SP+0.02SH+0.005PGH

W_1=-0.16FL1+0.004FW1-0.007NL1+0.002WH1+0.03CH1+0.05BL1+0.99CC1-0.007SP1+0.01SH1-0.014PGH1

The thoracic perimeter was observed to have the maximum contribution in the canonical pair formation V_1 W_1. Therefore, the photographic method can be used as a substitute for measurements taken directly on the animal with maximum precision, which can be explained by the high total canonical correlation between the sets of variables X and Y and the high variation shared between the variables estimated by the two methods. (99%). As a result, fieldwork can be simplified and developed with minimal resources applied, reducing costs and risks.

The correlation between W_1 and V_1 was 0.99; this canonical pair is orthogonal and not correlated with the other pairs

Table 3. Pearson's correlation between the variables obtained by the conventional method and by imaging

Variable (cm)	Pearson's Correlation
Head lenght	0.93*
Head width	0.82*
Neck lenght	0.99*
Height Withers	0.99*
Croup height	0.99*
Body length	0.99*
Thoracic perimeter	0.99*
Shin perimeter	0.86*
Height at side	0.95*
Height at the pelvic gird	0.99*

*Significant Pearson correlations (P< 0.001)

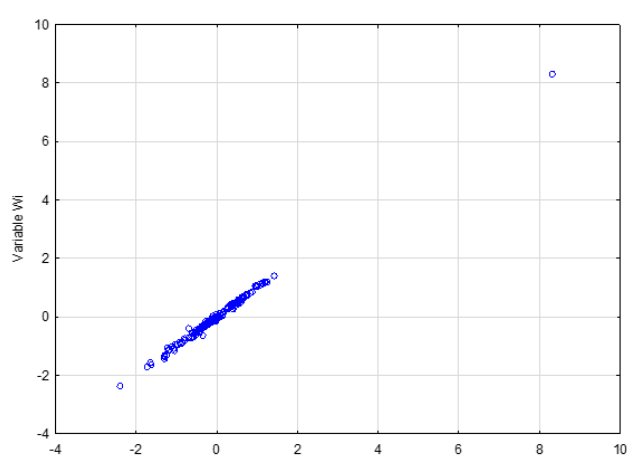


Figure 5. Plotting the roots obtained for the first canonical pair (V_1W1)

obtained. The canonical coefficients' magnitude indicates each variable in obtaining the maximum correlation between the sets of variables X and Y (DALLO, 2014). **Al-Kandari & Jolliffe (1997)** state that the canonical coefficients are analogous to the beta of multiple regression analysis and indicate the contribution of a variable in the formation of its respective canonical variable based on the total variance. Thus, the original variables with higher standardized canonical coefficients have a, more significant contribution to forming their respective canonical variable.

CONCLUSION

The evaluation of photographs by the ImageJ® software proved to be a viable alternative to the conventional method of taking morphometric measurements in horses. Therefore, we recommend the software to obtain measurements in Nordestina horses.

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