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Preliminary Analysis of the Development of the Breeding Program of Peruvian Paso Horse under Field Conditions

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Simple Summary: The Peruvian Paso Horse is considered one of the best saddle horses worldwide due to the gait that makes this horse perform one of the smoothest walks. The overreach, term and acuteness are the most characteristic movements in the gait, making them the most desirable attributes in its breeding and reproduction. There is no report on genetic parameters of these traits, which leads to a poor selection process in the breeding program. The present study calculates the heritability, repeatability, and genetic correlations of these traits under field conditions in a population of 137 animals, providing an important information for the breeders to make a breeding program of their Peruvian Paso Horse.

Abstract: Genetic parameters of overreach, term and acuteness in Peruvian Paso horses (PPH) have not been determined to date. It is important to estimate these parameters for application in PPH breeding, therefore, the aim of this study was to estimate the heritability, repeatability, and genetic correlations under field conditions of overreach, term, and acuteness of PPHs. The study included records of up to 137 stallions and mares. All measurements were recorded in MP4 video format with a resolution of 1920 x 1080 megapixels and at 60 frames per second. All traits were measured three times (once per stride), and each trait was analyzed. KINOVEA software version 0.9.5 was used to analyze the measurements. A multivariate repeated measures animal model with sex effect was used to estimate the variance components for each trait using WOMBAT software. The results showed heritability of 0.411, 0.476 and 0.405, for the traits of overreach, term, and acuteness, respectively. Repeatability was high in all traits (> 0.78). Genetic additive correlations ranged from -0.30 to 0.49. It can be concluded that overreach and term have high heritability values, which allows these traits to respond better in a selection process, unlike acuteness which has a moderate heritability value.

Keywords: Peruvian Paso horse; heritability; genetic correlations; repeatability; overreach; term; acuteness

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1. Introduction

The Peruvian Paso horse (PPH) is a native equine breed in Peru. The National Association of Peruvian Paso Horse Breeders and Owners (ANCPCPP) of Peru have made great efforts for the conservation, breeding and selection of this breed since 1947 [1]. This breed is considered a gaited horse with a symmetrical, four-beat rhythm and lateral sequence of football [2] with limb placements as follows: left hind limb; left forelimb; right hind limb; right forelimb during paso llano gait [3,4]. The ANCPCPP [5] defines paso llano as the PPH flats when the horse breaks the ambling gait on the sides in 4 steps. Other breeds present similar gaits, albeit with some differences, including classic fino, curly rack, coon rack, fox trot, marcha picada, mountain pleasure rack, rocky mountain rack, road gait, sobreandando, and toelt [4]. In PPH, the smoothness and harmony of movement arises from the combination of the mechanisms of movement during the "paso llano" (or "andadura rota")

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and the correct degrees of execution of the overreach with the adornments [2]. The main traits involved are term and acuteness [6]. Term is the forelimb trim that leaves the vertical line while moving and is represented by a frontal estimation of the maximum abduction angle formed by the vertical line and the lateral wall of the hoof [6–9]. This trait is unique in this breed and should not be confused with paddling [2]. Acuteness is the highest elevation of the knee observed laterally and estimated by the angle formed by the vertical line and the maximum elevation of the knee during protraction displacement [6,7]. Overreach [10] is foot placement and timing between the hindfoot and forefoot of the same side during lateral displacement and is estimated by measuring the distance between footfalls of its ipsilateral limbs, being considered positive if the hind football is ahead of the front football, on the same side [11–13] (Figure 1).

These movement characteristics are very relevant for achieving a smooth gait [7]. However, the measurement of movement as a functional trait needs to be developed in the breeding process. Traits measured by the human eye may be challenging when evaluating horses, due to subjectivity and limited accuracy, making it difficult to identify candidates that can be used in a breeding plan [9,14]. Therefore, the use of objective measurements using kinematic parameters is necessary. Several studies have focused on kinematics in horses, identifying changes in sporting performance, health, and equine sports medicine [8,15,39]. Therefore, it is recommended to measure the functional traits of a horse by the estimation of quantifiable kinematic variables [17]. The current phenotype of this horse breed should be improved and standardized, including the estimation of heritability traits to achieve genetic gains that are very important for the breed [7]. To date no study has estimated the genetic parameters of functional traits in PPHs. The selection is mainly phenotypic under non controlled conditions, based on the number of successful competitions and subjective observation of movement. Therefore, the aim of this study was to estimate the heritability, repeatability, and genetic correlations under field conditions of overreach, term, and acuteness in PPHs. This is the first report to establish objective selection criteria to enhance a PPH breeding plan.

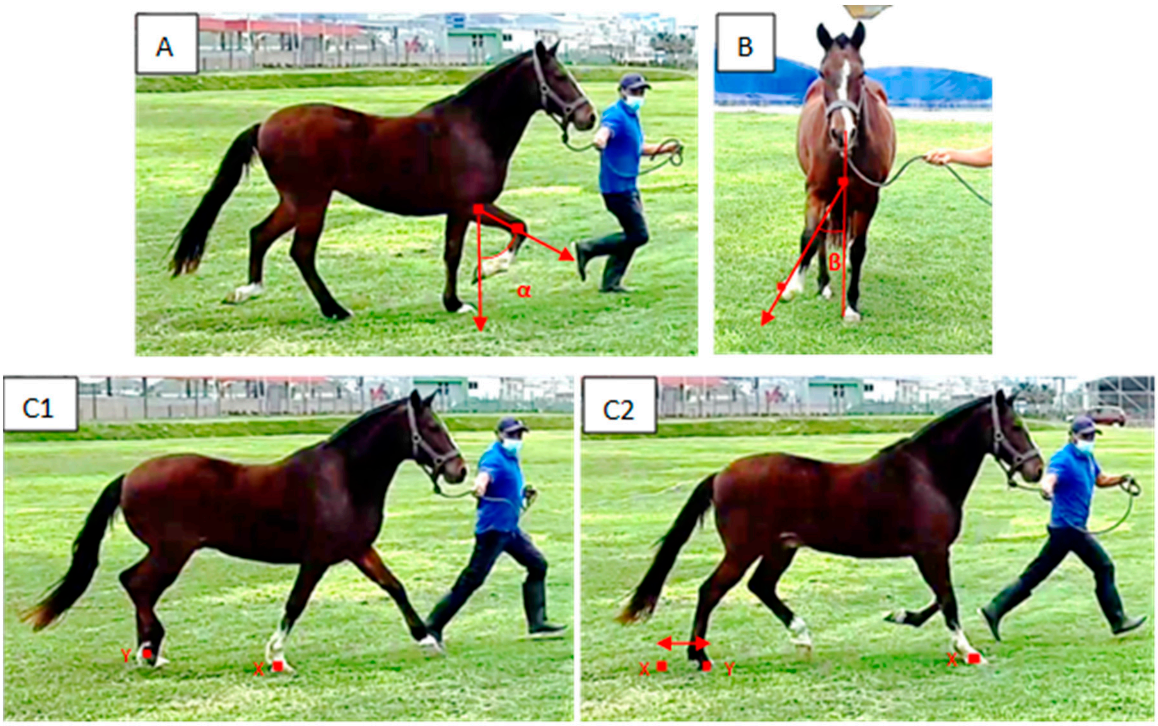

Figure 1. A: Representation of the maximum α angle of the acuteness of the right forelimb measured from the orientation connecting the knee to the elbow with respect to the vertical, in the sagittal plane [9]. B: representation of the maximum β angle of the term of the right forelimb measured from the lateral hoof wall with respect to the vertical in the coronal plane of the horse [9]. C1 and C2: representation of overreach, measured as the distance between X and Y, where X is the footfall of the right forelimb hoof position during maximum protraction and retraction into the ground and Y is the

footfall of the right hind limb hoof position during maximum protraction and retraction into the ground when X is exceeded in the next stride.

2. Materials and Methods

2.1. Animals

One hundred forty animals were phenotyped, and of these, only records that could be analyzed were used. Records not considered in the analysis were discarded due to recording problems related to the video recorder lens. Horses that did not move parallel to the camera, that limped, had a very slow speed, handler blocking camera view or mares with foals at their sides during the gait that prevented the identification of the marks during the "paso llano", were not considered after debugging the videos. Records of 134, 137, and 134 animals, with a higher proportion of females than males (80% and 20%, approximately), were used to study the traits of overreach, term, and acuteness, respectively. The mean age of the horses was 7.67 ± 2.61 years, ranging from 5 to 11 years. The same animals were used for the analysis of the three traits. A total of 1308 individuals that could be traced back 21 generations were included in the database. The generational interval of the entire tracked population was 8.76 ± 4.53 years.

All procedures and handling of the animals was performed considering their welfare without any harm to the animal. This work was approved by institutional Committee of Ethics in Research with Animals and Biodiversity of the Universidad Cientifica del Sur (Cod. 028-2021-PRO99) and permission was obtained from the owners of the animals for data collection. The horses were evaluated as being sound and healthy based on clinical examination by a registered veterinarian. Only healthy animals, with no signs of lameness in one or more legs, were included in the study.

2.2. Measurements

All animals were evaluated in their breeding facilities. These consisted of a flat, dry, unobstructed grassy field. A start and end point was determined which were perpendicular to the location of the camera lens, through which each animal moved during the recording of overreach and acuteness. For the term recording each horse travelled a straight distance of 50 meters from a start to a finish point located in front of the camera lens. Each horse was evaluated on different days, with groups of three to ten horses assessed per day, depending on the availability of the breeders. Each breeder used an experienced handler to record the video recordings.

To identify the reference points for measuring each trait, a 4 x 4 cm tape was attached to the areas marked in Figure 1. All measurements were recorded in video MP4 format with a resolution of 1920 x 1080 megapixels and at 60 frames/s [18]. The animals were placed on a flat surface and were pulled by an operator at a "paso llano" with an approximate speed of between 2.5 and 4 m/s, covering 50 meters. The speed of each animal was calculated from the distance the horse covered during the gait time, as determined by the frame rate using KINOVEA software. Records of animals with travel speeds that did not comply with the "paso llano" gait were not considered. The performance of the horse was recorded by filming with video camera on a tripod with a fixed position positioned horizontally (confirmed with a level) at a height of 1.3 meters and 12 meters from the middle of the line of motion, recording the movement of each animal laterally. All traits were measured by three technical replicates (once per stride) and were included in the model for analysis. KINOVEA software version 0.9.5 (http://www.kinovea.org/) was used to analyze the measurements [19].

2.3. Statistical Analysis

All traits were subjected to descriptive statistical analysis and normality analysis using the Anderson Darling test with p>0.05. JASP software was used for these analyses. Heritability expresses the proportion of the total variance that is attributable to differences in breeding values and is defined as the ratio of the additive genetic variance to the phenotypic variance, $\boldsymbol{h^2} = \boldsymbol{\sigma_a^2}/\boldsymbol{\sigma_p^2}$ where $\boldsymbol{\sigma_a^2}$ is the additive genetic variance, and $\boldsymbol{\sigma_p^2}$ is the phenotypic variance [20]. The phenotypic records of three

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traits were fitted to the repeated measures multivariate animal model with a fixed sex effect to estimate the variance components of each of the three traits using the average information (AI) algorithm for restricted maximum likelihood [21]. WOMBAT software was used for all procedures (http://didgeridoo.une.edu.au/km/wombat.php) [22]. The model used is expressed as:

$$Y_{ijk} = \mu + Sex_i + Animal_j + Horse_k + e_{ijk}$$

Being $\boldsymbol{Y_{ij}}$ the phenotypic value for each trait, μ the population mean, $\boldsymbol{Sex_i}$ the fixed effect of sex (2 levels); $\boldsymbol{Animal_j}$ the random effect of the jth animal $\sim\mathbf{ND}(\mathbf{0}, \mathbf{A}\boldsymbol{\sigma}_a^2)$, $\boldsymbol{A}$ denotes the numerator relationship matrix among animals and $\boldsymbol{\sigma_a^2}$ the additive variance; $\boldsymbol{Horse_k}$ is the random effect of the kth individual $\sim\mathbf{ND}(\mathbf{0}, \mathbf{I}\boldsymbol{\sigma}_{pe}^2)$ **where** I is the identity matrix, $\boldsymbol{\sigma_{pe}^2}$ is the permanent environment variance; and $\boldsymbol{e_{ijk}}$ the residual random effect $\sim\mathbf{ND}(\mathbf{0}, \mathbf{I}\boldsymbol{\sigma}_e^2)$.

Repeatability (R) was calculated from:

$$R = \left(\sigma_a^2 + \sigma_{pe}^2\right) \Big/ \left(\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2\right)$$

For calculation of repeatability, three records per animal per trait were used to estimate variance components. Phenotypic and additive genetic correlations were calculated with the same records used for the heritability calculations.

3. Results

The descriptive statistic of each trait is shown in Table 1. The pedigree structure for each trait is shown in Table 2. Heritability, repeatability and correlations with standard errors results for each trait are shown in Table 3. All traits were subjected to the Anderson-Darling normality test and showed normal distribution with a significant value of p>0.15 for all traits; therefore, the use of the proposed animal model is appropriate.

Table 1. Descriptive statistics per trait.

		Overreach	Term	Acuteness
Animals	Stallions	28	28	28
	Mares	106	109	106
Median		25.9	25.3	72
Mean		25.338	25.111	71.512
Records		500	481	500
Std. Error of Mean		0.909	0.288	0.308
95% CI Mean Upper		29.119	25.675	72.117
95% CI Mean Lower		25.556	24.546	70.907
Std. Deviation		20.33	6.315	6.902
Coefficient of variation		0.744	0.251	0.097
Skewness		0.219	0.177	-0.298
Kurtosis		-0.445	0.038	0.087
Minimum		-18.080	6.800	49.000
Maximum		86.180	44.200	88.600
p-value Anderson Darling Test		0.881	0.814	0.897

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CI. Confidence interval

Table 2. Pedigree structure for each trait.

		Overreach	Term	Acuteness
Number of animals in pedigree file		1615	1641	1615
Number of animals with records		134	137	134
Animals with	3 records	90	100	86
	4 records	2	12	8
	5 records	42	25	40
Number of animals with	unknown sire	79	81	79
	unknown dam	211	211	211
	both parents unknown	56	57	56
Number of animals without offspring		123	126	123
Number of animals with offspring		1234	1257	1234
Number of animals with offspring and records		11	11	11
Number of sires		458	467	458
Number of sires with progeny in data		51	52	51
Number sires with records and progeny in data		3	3	3
Number of dams		774	788	774
Number of dams with progeny in data		102	104	102
Number of dams with records and progeny in data		8	8	8
Average inbreeding coefficient (%)		5.42	5.43	5.44
amongst inbreed animals (%)		8.51	8.41	8.45
Average inbreeding coefficient amongst animals phenotyped (%)		8.31	8.29	8.30

Table 3. Estimates of heritability (h^2) with repeatability (R) (on diagonal), phenotypic correlations (bellow diagonal) and additive genetic correlations (above diagonal). Standard error in brackets.

	Overreach	Term	Acuteness
Overreach	**h^2 = 0.411 (0.199)** **R = 0.856 (0.020)**	-0.697 (0.374)	0.493 (0.360)
Term	-0.213 (0.079)	**h^2 = 0.476 (0.197)** **R = 0.783 (0.029)**	-0.301 (0.432)

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Acuteness	0.189 (0.081)	0.183 (0.081)	**h^2 = 0.405 (0.224)** **R = 0.854 (0.019)**

4. Discussion

The current investigation accurately assessed the heritability, repeatability, and genetic correlations of three distinctive traits in PPH: overreach, term, and acuteness. While there is a considerable body of scientific literature regarding the heritability of functional traits across various equine breeds [10,23–35], our study stands out as a pioneering approach towards PPH. It is pivotal to highlight that while similar evaluations have been conducted in other breeds, they generally rely on competition data, performance tests, or subjective sports evaluations. In contrast, our methodology leverages a quantitative and objective field evaluation, comparable to investigations using a treadmill [10]. Based on the studies published to date, the authors believe that the use of objective kinetic methods, rather than visual methods that depend on the experience of the assessor, are recommended for the evaluation of the performance of horses under field conditions during the "paso llano".

Overreach was found to have high [36] heritability, in line with the findings of Molina et al. [28] for stride length. However, it is imperative to acknowledge the substantial difference between these two traits. In a contrasting finding, Sole et al. [10] reported an overreach heritability in the Lusitano horse that was considerably lower than our observation. Notably, in that breed, the value was deemed negative (-11 cm adjusted for speed). In addition, we observed that overreach in our study significantly differed from that reported in other studies, particularly in the trot gait [10]. However, studies such as those by De Sousa et al. [12] and Miro et al. [13] reported positive overreaches in Andalusian horses, suggesting the need for additional research on PPH, taking variables, such as speed and gait type, into account.

The heritability of term, a trait we believe to be unique to the PPH breed, also proved to be high, opening an intriguing field for future research. Regarding acuteness, its high heritability was similar to that found by Sole et al. [10] in Lusitano horses, albeit for a related, yet different, trait. It is important to consider methodological differences and study populations when comparing these values. The discrepancy in results compared to Molina et al. [28] could be attributed to factors, such as the horse's training level [31] and the omission of the sex effect in their study, since they only assessed males.

As could be observed, the heritability values of all the traits analyzed were high (greater than 0.40), which allows a panoramic discussion on all three traits. These values can be explained by the non-inclusion of an external factor that allows the free gait of the horse, such as the inclusion of a rider that could alter the rhythm and movement of the animal during the flat gait [37]. These movements are performed freely and are more homogeneous without the external effect and are therefore more heritable [38]. Other factors, such as the training of each horse, type of feed or the number of competitions in which they participate, can be useful for inclusion in the animal model for the estimation of genetic parameters [38]. Another reason that might explain the high heritability values is that there may have been more specialization in PPH contests [38], or better use of the selection process in the breed [36], although it may also be because this population was more homogeneous due to the higher number of mares (~80%) analyzed in this study and only adult animals (5 to 11 years old) were included [38].

One way to achieve greater genetic progress may be with a higher selection intensity, as well as a higher heritability value [39]. Therefore, a reliable estimate of heritability is very important in a PPH breeding plan [38]. Furthermore, it should also be taken into account that improvement in these traits is the result of a complex combination of conformational, physiological and behavioral traits [40]. Efficiency in genetic selection for bio-kinematic variables can be more efficient than selection based on animal performance and this can be translated into higher heritability values [41]. However, it should also be noted that evaluation by this method requires trained personnel, investment in

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materials, equipment and the availability of animals that are not being prepared for regional or national competitions, which takes more time and can increase costs.

Due to the limited amount of research conducted on these traits in PPH, it is difficult to discuss genetic correlations in detail and therefore comparisons cannot be made directly. As a criterion for categorizing correlations, the Quinnipiac University scale [42] was used to classify correlations less than or equal to 0.20 as weak, greater than 20 and less than 0.40 as moderate and greater than 0.40 as strong [38,43,44]. The additive genetic correlations found in the present study ranked between absolute values of 0.213 and 0.697 considered as between moderate and strong, similar to other studies conducted under field conditions [41], and the phenotypic correlations ranked between absolute values of 0.183 and 0.213 considered to be between weak and moderate.

The positive phenotypic correlation of overreach with acuteness seems consistent since a horse with higher acuteness elevates the forelimb more than horses with lower acuteness and, thus, overreach is greater. However, overreach has a negative phenotypic correlation with term, which means that more overreach induces a lesser angle to extend the forelimb away from the middle line of the horse. Acuteness and term are positively correlated, which means that the higher the acuteness the more the foreleg moves away from the midline, which gives the handling characteristic of the PPH.

The values of the additive genetic correlations were higher than the phenotypic correlations and in the same direction, except for the correlations between term and acuteness, which were negative. This is because genetic correlations are generally higher than phenotypic correlations and are not always consistent with each other [43]. Genetically, overreach has a positive and favorable genetic correlation with acuteness, showing that animals with higher acuteness can have a higher overreach which increases the distance travelled during gait and maintains comfort for the rider and the horse, unlike other breeds, such as Lusitano, Menorca, Purebreed Spanish and Dutch Warmblood, in which overreach is negative [41]. However, overreach is negatively correlated with term, showing that achieving the goal of improving overreach and term cannot be easily achieved.

Although it is true that genetic correlations provide information about the relationship between traits, they are not always as useful as phenotypic correlations at the time of evaluation during performance [43]. Their main utility can be applied to the construction of selection indices or to predict correlated response to selection [45], although their efficiency also depends on the genetic variation of these traits [40]. The additive genetic correlations found were mostly high indicating good predictability in performance during the flat passage [38]. Similar results were observed in other breeds for gait traits, with values ranging from moderate to high for dressage horses [40].

Repeatability values for all the traits were greater than 0.78 which is considered excellent [46]. Similar results in kinematics traits were found in trained dressage and bullfighting horses (over 0.50), and in Swedish Warmblood horses in scored gaits with values between 0.75 and 0.77 [47]. These results demonstrate that previous training can influence the regularity of the gait, in agreement with the present study, in which only adult animals that have already had previous training were evaluated [10]. The low standard error values close to 0.02 indicate high precision in all traits (0.904, 0.855 and 0.903 for overreach, term and acuteness, respectively), being defined as the variance of the mean of n measurements as a percentage of the variance of one measurement [20], indicating that this parameter has little effect on the temporal environment of the three traits.

These results are higher than those described by Sepulveda et al. [46] although it should be noted that the traits studied were different. Taking into account the methodology of Sepulveda et al. [46], which found higher repeatability values in daily than in weekly observations, it is possible to suppose that observations made with minute differences can be even higher, as found in this study. This can be corroborated in a study conducted in horses of different breeds [48] in which the repeatability of head and pelvis position asymmetry presented values between 0.89 and 0.95, which are considered as high values, measured in consecutive strides.

Furthermore, our results corroborate that traits with high repeatability require few measurements (3 in this study) to obtain higher precision, and an increase in the number of measurements may be irrelevant for parameter estimation. In a breeding plan this high precision may be due to an increase in the additive genetic variance, indicating that two or more measurements have higher heritability than a single measurement, allowing a better breeding value estimate to be obtained [20]. Taking into account the high repeatability values found, this parameter can be used as an indicator of how effective the selection process can be, considering its relationship with heritability, as repeatability values are higher than heritability, due to the inclusion of permanent environmental variance (within-individual variance) in its estimation [20,49]. High values also indicate that individuals tend to have consistent performance, and therefore, obtaining several measures for evaluation may be impractical [49].

We wish to highlight certain considerations and limitations in our research. While our heritability values are promising, the overall precision of the traits was limited, likely due to the small sample size. The complexity of performing the kinematic measurements and the time it took to move between different hatcheries were the main reasons for the small sample size. This limitation was accentuated due to the restrictions of the COVID-19 pandemic in Perú. Despite the small number of animals phenotyped, the estimation of genetic parameters are justified as preliminary values and are useful for use in subsequent references, as has occurred in other studies including sample sizes similar to our study (100 to 362) [40]. The population of animals included in this study was small, however the average total inbreeding coefficient (~5.43 %) was similar to the studies of Larrea et al. [50] and Montenegro et al. [51] (5.97 and 5.44 %, respectively), and thus, the results obtained can be interpreted as a reference for the total PPH population. All horses were evaluated with the same device under field conditions and the possible bias is the same for all horses [43]. The low number of horses evaluated could affect the results and obtain different values, and therefore, further research with more animals could solve this issue. It is relevant to consider factors that might influence the results, such as the movement speed of the animal and camera position as external stimuli, or the behavior of the handler [52].

5. Conclusions

It can be concluded that overreach, acuteness, and term have high heritability values in PPH, which makes them traits that can respond better in a selection plan. Nonetheless, the high standard error values may be due to the limited number of animals evaluated. The genetic correlations found were moderate to high, although the phenotypic correlations were weak to moderate. The positive genetic correlations found between overreach and acuteness allow a selection process aimed at increasing both traits, however, the opposite was found between overreach and term, in which the genetic correlations were negative. The high repeatability values found with high precision indicate that the number of measurements can be reduced for all three traits. Finally, the results of this study will contribute to the development of breeding plans for PPH under field conditions, although further research involving a larger number of animals and factors is recommended.

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Informed Consent Statement: Not applicable

Data Availability Statement: The datasets presented in this article are not readily available because the data are part of an ongoing study. In addition, the data is not published due to privacy restrictions.

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Conflicts of Interest: The authors state that records of eight animals owned by José Dextre were used. It is also stated that José Dextre was Chairman of the Board of the Universidad Científica del Sur during the development of the methodological phase of this research.

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