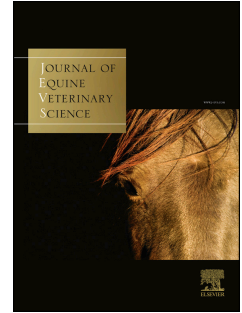


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Genetic diversity of Lusitano horse in Brazil using pedigree information

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1 **Genetic diversity of Lusitano horse in Brazil using pedigree information**

2

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34 **Abstract** – This study aimed to evaluate population parameters and to describe  
35 the genetic diversity of the Lusitano breed in Brazil using pedigree data. Two  
36 populations evaluated: total population (TP) containing 18,922 animals, and  
37 reference population (RP) composed of a part of TP containing 8,329 animals,  
38 representing the last generation. The generation interval ( $10.1 \pm 5.1$  years) was  
39 in the range for horse populations. Pedigree completeness in RP shows almost  
40 100% filling in the three most recent generations, indicating improvement in the  
41 pedigree data and accuracy of the results. The inbreeding coefficient (4.46%)  
42 and average relatedness (5.97%) for RP, indicating control on the part of  
43 breeders. The effective population size was 89 (TP) and 90 (RP). The effective  
44 number of founders ( $f_e$ ) of 33 and 29, effective number of ancestors ( $f_a$ ) of 30  
45 and 26, and effective number of founder genomes ( $f_g$ ) of 19 and 15 for TP and  
46 RP, respectively, indicating a reduction of genetic variability in the last  
47 generations. The total number of ancestors that explains 100% of the genetic  
48 diversity in the Lusitano breed in Brazil was 427 (TP) and 341 (RP). The  
49 reproductive parameters, probabilities of gene origin showing loss of variability  
50 in the last generations and the genetic contributions of ancestors suggest the  
51 need to monitor genetic diversity over time in breeding programs in order to  
52 allow control of the next generations and to increase their variability.

53

54 **Keywords:** ancestors; demographic characterization; effective population size;  
55 founders; inbreeding.

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## 68 1. Introduction

69 The Puro Sangue Lusitano (PSL), also known as the Lusitano horse,  
70 represents a breed with a rich and ancient history, originated in southern  
71 Portugal on the Iberian Peninsula. Its evolution is essentially due to their use for  
72 working cattle and cutting bulls [1]. Although records date back to 1824, the  
73 studbook of the Lusitano breed was officially established in 1967 [2], a time  
74 when the main Iberian breeds, Portuguese and Spanish, were separated and  
75 received the denomination PSL in Portugal and Pura Raza Española (PRE) in  
76 Spain.

77 The main studbook of the Lusitano breed is managed in Portugal by the  
78 Portuguese Lusitano Horse Breeder Association (APSL in the Portuguese  
79 acronym), which has the largest number of birth records of the breed in the  
80 world [2]. Brazil is the country with the second largest number of animal records  
81 of this breed. This statistic was determined after the reciprocity partnership  
82 between APSL and the Brazilian Lusitano Horse Breeder Association (ABPSL  
83 in the Portuguese acronym) was established in 1991. This agreement permitted  
84 all Lusitano horses registered in the Brazilian studbook to be equally registered  
85 in the Portuguese studbook, which contains the birth records of all countries.

86 Studies investigating population parameters of the Lusitano breed have been  
87 conducted only with data from the APSL studbook (Portugal) for all Lusitano  
88 horses registered in the world [2,3]. Thus, data on the population statistics and  
89 genetic diversity of the Lusitano breed in Brazil are lacking.

90 Parameters such as pedigree completeness [4], generation interval,  
91 inbreeding coefficient [5], and probability of gene origin [6] are important to  
92 design strategies for the selection and improvement of animals. The data  
93 generated permit to verify genetic diversity and its changes over time [7]. The  
94 complete or partial results of genetic diversity and population parameters are  
95 reported in the literature for various horse breeds, including Andalusian [8] ,  
96 Holstein [9], Paint-Horse [10], Hanoverian [11], Spanish Arab Horse [12],  
97 Lipizzaner [13] and Old Kladruber [14]. In Brazil, studies were conducted on the  
98 Pantaneiro [15], Mangalarga [16] Campolina [17], Brazilian Sport Horse [18]  
99 and Quarter Horse [19] breeds.

100 This study aimed to evaluate the population parameters of the Lusitano horse  
101 in Brazil in order to observe the genetic diversity and to contribute to the

102 knowledge and development of this breed in Brazil and to compare our results  
103 with the ones obtained by different authors for the worldwide population [2,3],  
104 providing the information necessary to implement a breeding program of the  
105 breed in Brazil.

106

## 107 **2. Material and methods**

### 108 **2.1. Data and computer programs**

109 The pedigree file of the Lusitano breed containing information about the  
110 animal's name, sire and dam, sex, date of birth and origin was provided by  
111 ABPSL. Animals born and registered in Brazil between 1912 and 2012 were  
112 considered and made up the total population (TP) of 18,922 animals (48.1%  
113 males, stallions and geldings) and the reference population (RP) consisting of  
114 8,329 animals (51.0% males, stallions and geldings), corresponding to the birth  
115 records from 2003 to 2012. RP was used as the reference of the active  
116 population, representing the last generation, which was equal to one mean  
117 generation interval (10.1 years) and contained animals (stallions and mares)  
118 that potentially could transmit their genes to the next generation.

119 Data preparation and statistical analysis were performed with the MEAN and  
120 FREQ procedures of the SAS program [20]. The population and reproductive  
121 parameters, probability of gene origin and genetic diversity were obtained with  
122 the ENDOG V4.8 program [21].

123

### 124 **2.2. Reproductive parameters and Generation interval**

125 The existence of imported animals registered in the studbook of ABPSL  
126 permitted to observe their use as breeding stock and to compare it with that of  
127 sires and dams born in Brazil. The coefficients of variation for the mean number  
128 of offspring, age at first and last progeny and time in reproduction obtained for  
129 stallions and mares explain the high standard deviation between the different  
130 parameters analyzed. These parameters were not calculated for RP because of  
131 the small number of imported breeding animals during this period and because  
132 the population is still in reproduction.

133 The generation interval (GI) was obtained based on the mean age of the  
134 parents at the birth of offspring that reproduced [22], and was calculated for the

135 four different paths of selection: father-son, father-daughter, mother-son and  
136 mother-daughter and total parent-offspring.

137

### 138 **2.3. Quality of pedigree data**

139 The quality of the information in the ABPSL studbook is reported in two ways:

140 1) pedigree completeness summarizes the proportion of known ancestors in  
141 each ascending generation per descendant and was calculated as proposed by  
142 MacCluer et al [4], in which ancestors without progenitors in the pedigree were  
143 considered founders [21]; 2) based on the mean number of generation  
144 equivalents (GE), computed over the sum of all known ancestors by calculating  
145  $(\frac{1}{2})^n$ , where  $n$  is the number of generations between the animal and each  
146 known ancestor [7].

147

### 148 **2.4. Parameters related to the inbreeding coefficient**

149 The inbreeding coefficient ( $F$ ) defines the probability that an individual has  
150 two identical alleles by descent and was calculated using the algorithm  
151 proposed by Meuwissen and Luo [23].

152 The average relatedness (AR) coefficient of each animal was described by  
153 Gutiérrez and Goyache [21] as the representation of a given animal in the  
154 pedigree and was obtained based on the probability that a randomly selected  
155 allele of the population belongs to a given animal.

156 The increase in inbreeding ( $\Delta F$ ) for each generation was obtained as follows:

157  $\Delta F = (F_t - F_{t-1}) / (1 - F_{t-1})$  (1) where  $F_t$  and  $F_{t-1}$  are the average inbreeding  
158 in generations  $t$  and  $t - 1$ , respectively.

159 According to Falconer and Mackay [24], the effective size of a population ( $N_e$ )  
160 is defined as the number of individuals of a population with a non-ideal structure  
161 that would give origin to a certain rate of consanguinity if its structure were ideal  
162 (e.g., equal number of males and females, absence of selection, random  
163 matings, etc.). Using  $\Delta F$ ,  $N_e$  was estimated considering  $N_e = 1/2\Delta F$ , which  
164 represents the number of animals that equally contribute to the next generation  
165 and that would promote a similar increase in inbreeding in the population  
166 studied [21]. In addition,  $N_e$  was also calculated based on the individual  
167 increase in inbreeding as suggested by Gutiérrez et al [25] and was used for the  
168 calculation of genetic drift.

169

## 170 **2.5. Probability of gene origin and genetic drift**

171 The effective number of founders ( $f_e$ ) is obtained by measuring the  
172 contributions of the most influential founders. Lacy [26] defines  $f_e$  as the  
173 expected number of founders that contribute equally and produce the same  
174 genetic diversity in the population studied. This parameter was calculated using  
175 the formula

176  $f_e = 1 / \sum_{k=1}^f q_k^2$  (2) where  $q_k$  is the probability of gene origin of founder  $k$ .

177 The effective number of ancestors ( $f_a$ ) represents the minimum number of  
178 ancestors (founders or not) necessary to explain the full genetic diversity of the  
179 population [6]. This parameter was calculated as

180  $f_a = 1 / \sum_{j=1}^f p_j^2$  (3) where  $p_j$  is the marginal contribution of ancestor  $j$ . The

181 marginal contribution is the additional genetic contribution made by an ancestor  
182 that was not explained by another previously chosen ancestor [6].

183 The effective number of founder genomes ( $f_g$ ) is defined as the number of  
184 sires and dams that contribute equally to the population structure and produce  
185 identical genetic diversity without the loss of alleles [27]. This parameter was  
186 estimated as proposed by Caballero and Toro [28] using the formula

187  $f_g = 1/2C$  (4) where  $C$  is the average coancestry between individuals of the

188 population.

189 Genetic drift is the random change in allele frequencies in a population,  
190 which occurs at a higher intensity when the population undergoes a drastic  
191 reduction in its effective size [24]. According to Sørensen et al [29], a ratio of  
192  $f_e/N_e > 0.5$  in a population indicates the occurrence of changes in genetic drift.  
193 One approach to evaluate genetic drift is the observation of bottleneck effects.  
194 The  $f_e/f_a$  ratio should be close to 1 if important bottlenecks have not occurred in  
195 the population [6]. Stabilization of genetic drift in a population can be observed  
196 when  $f_e$  is close to  $N_e/2$ , suggesting a greater representation of founders [28].

197

## 198 **2.6. Genetic conservation index**

199 The number of founders represented in the contributions received by an  
200 individual and the balance between them were evaluated by computing the  
201 genetic conservation index (GCI) of all animals [30]. It is supposed that higher

202 indices correspond to animals with a more balanced representation from a large  
203 number of founders, i.e., greater genetic conservation of the breed. The GCI  
204 was computed for each animal using the formula

205  $GCI = 1 / \sum P_i^2$  (5) where  $P_i^2$  is the proportion of genes of founder animal  $i$  in  
206 the pedigree [21]. The mean of the populations was then calculated.

207

### 208 **3. Results**

#### 209 **3.1. Data analysis and distribution of birth records**

210 In TP, 87.3% of the animals were born in Brazil after 1967 and 4.0% were  
211 imported. The remaining animals are ancestors of both origins, which are  
212 registered only as parents in the database of ABPSL. In RP, 99.6% of the  
213 animals studied were born in Brazil and the remaining 0.4% are derived from  
214 imported animals and ancestors.

215 The first birth registered by ABPSL and recognized by APSL was the female  
216 Azambuja in 1967 and the first male was Zapata in 1969, both half-sibs of the  
217 mare Zaza. Between 1967 and 1985, the births of only 305 animals were  
218 recorded (Figure 1). A constant increase was observed after 1986 and the  
219 number of registered births per year reached three digits for the first time.  
220 Stabilization of the growth trend occurred in 2002, with reduced oscillations  
221 (approximately 5%) in the following eight years. The largest number of  
222 registered births was observed in 2006 (966 animals). A marked decline in the  
223 number of births was seen in the last three years (2010 to 2012) of the study  
224 (Figure 1).

225 Births were observed in all months but there was wide oscillation. In TP, only  
226 4.6% of births occurred in the ideal month (July). The months with the largest  
227 number of births were October (16.5%), September (15.7%), and November  
228 (15.3%). The first, second and last quarter of the year concentrated 17%, 5%  
229 and 45% of births, respectively.

230

#### 231 **3.2. Reproductive parameters and Generation interval**

232 In the database, there were 16,511 (50.4% males, stallions and geldings)  
233 Lusitano horses born in Brazil (national) and 781 (32.9% males, stallions and  
234 geldings) imported animals (e.g., Portugal and Mexico). Of the 8,319 national  
235 males, only 10.1% had offspring, while 257 (72.2%) of the imported males were

236 used as stallions. Of the 8,192 national females, 37.7% had offspring and  
237 96.2% of the 524 imported females were used as mares.

238 All animals born in Brazil (16,511) were sired by 1,115 stallions; 57.5% of the  
239 offspring had a sire of Brazilian origin, 40.8% had an imported sire, 1.6% were  
240 obtained by artificial insemination, and the sire was unknown in 0.1%. Among  
241 the 3,594 breeding mares that contributed to the Brazilian population, 77.2% of  
242 the offspring had a dam of Brazilian origin, 22.6% had an imported dam, 0.1%  
243 were obtained by embryo transfer, and the dam was unknown in the remaining  
244 0.1%.

245 There was a greater utilization of females, with a ratio of 3.1 mares (4,644)  
246 per stallion (1,507). This ratio was 4.5 mares per stallion (210 stallions and 946  
247 mares) in the most productive year (2006). The mean number of offspring  
248 (Table 1) considering only breeding animals was 12.3 and 4.0 for stallions and  
249 mares, respectively. Considerable differences were observed in the maximum  
250 number of offspring, with stallion Afiançado de Flandes having 419 offspring  
251 registered, the largest number for the whole breed worldwide.

252 The mean age of stallions at the birth of their first progeny was 7.0 years and  
253 mares had their first foal at 5.9 years (Table 1). The mean age at the birth of the  
254 last progeny was similar in stallions and mares, with a difference of 0.2 years  
255 (Table 1). The difference between the birth of the first and last offspring,  
256 indicating the time in reproduction, was  $3.7 \pm 5.0$  years for stallions and  $4.6 \pm$   
257  $5.1$  years for mares.

258 High standard deviations were observed for all reproductive parameters  
259 analyzed (Table 1). A standard deviation higher than the mean was found for  
260 the number of offspring per stallion, which was almost the double of the mean  
261 value. The coefficients of variations indicated a high level of dispersion, which  
262 were 199.1% (number of offspring), 54.3% (age at first progeny), 53.3% (age at  
263 last progeny) and 135.1% (time in reproduction) for stallions, and 85.0%  
264 (number of offspring), 52.5% (age at first progeny), 50.5% (age at last progeny)  
265 and 110.9% (time in reproduction) for mares.

266 The overall mean GI was  $10.1 \pm 5.1$  and  $10.2 \pm 5.0$  years for TP and RP,  
267 respectively (Table 2). When the four paths of selection were considered,  
268 shorter GIs were observed for the mother-offspring paths in TP. The same trend  
269 was not found in RP, with longer and shorter GIs between fathers and their

270 offspring. The standard deviations were high (Table 2) and an approximate  
271 dispersion of the results was observed in both populations studied.

272

### 273 **3.3. Quality of pedigree data**

274 The quality of the information of the ABPSL studbook based on average  
275 pedigree completeness (Figure 2) was close to zero from the 15<sup>th</sup> (oldest) to the  
276 11<sup>th</sup> generation in both populations and in the three most recent generations  
277 (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>), with pedigree completeness of 97.7%, 95.4% and 92.2% in  
278 TP, respectively, and of 99.8%, 99.5% and 98.2% in RP. The number of GE  
279 was  $5.7 \pm 1.4$  and  $6.4 \pm 0.7$  for TP and RP, respectively.

280

### 281 **3.4. Parameters related to the inbreeding coefficient**

282 There were 14 matings between full-sibs, 607 between half-sibs, and 149  
283 between parents and offspring. The F value was  $4.06\% \pm 4.94\%$  in TP and a  
284 slight increase of  $0.40\%$  to  $4.46\% \pm 4.34\%$  was observed in RP. The AR  
285 coefficient was  $5.41\% \pm 1.69\%$  in TP and increased by  $0.56\%$  to  $5.97\% \pm$   
286  $1.20\%$  in RP. The  $\Delta F$  per generation was  $0.96\% \pm 2.09\%$  in TP, while a lower  
287 value ( $0.85\% \pm 0.90\%$ ) was observed in RP (Table 3). The percentage of  
288 animals with F different from zero was 88.7% in TP and 98.0% in RP (Table 3).  
289 Only two non-inbred animals were born in the last year evaluated (2012).

290 The  $N_e$  obtained based on  $\Delta F$  is the most common parameter in the literature  
291 and was 89 and 90 in TP and RP, respectively. Using the individual increase in  
292 inbreeding,  $N_e$  values of 52 (TP) and 59 (RP) were obtained.

293

### 294 **3.5. Probability of gene origin and genetic drift**

295 The genetic variability of the populations demonstrated by the probability of  
296 gene origin (Table 3) indicated an effective number of founders ( $f_e$ ) of 33,  
297 ancestors ( $f_a$ ) of 30, and founder genomes ( $f_g$ ) of 19 in TP. These values  
298 decreased to 29, 26 and 15, respectively, in RP.

299 Analysis of possible genetic drift in the populations showed an  $f_e/N_e$  ratio of  
300 0.64 in TP and of 0.49 in RP (Table 3). When the absence of bottleneck effects  
301 was evaluated,  $f_e/f_a$  ratios of 1.10 (TP) and 1.12 (RP) were obtained. The  $f_e \sim$   
302  $(N_e/2)$  values were 33 ~ 26 and 29 ~ 30 for TP and RP, respectively.

303

### 304 **3.6. Genetic conservation index**

305 The GCI indicated a mean number of founders per individual of  $9.6 \pm 2.9$  and  
306  $9.7 \pm 2.9$  in TP and RP, respectively, with a maximum GCI of 22.1 founders. In  
307 RP, GCI values less than 7.7 and higher than 11.7 corresponded to the 25<sup>th</sup> and  
308 75<sup>th</sup> percentiles, respectively.

309

### 310 **3.7. Genetic contributions**

311 The total number of founders (Table 3) of the Lusitano breed in Brazil  
312 represents 2.7% (TP) and 5.4% (RP) of each population and the total number of  
313 ancestors 2.3% (TP) and 4.1% (RP). The cumulative genetic contribution (Table  
314 3) of the 10, 50 and 100 most influential founders exhibited a reduced increase  
315 in RP by 0.8%, 3.9% and 3.0%, respectively, compared to TP. The cumulative  
316 marginal genetic contributions of ancestors were also slightly increased in RP  
317 by 5.4%, 2.6% and 1.4% for the 10, 50 and 100 major ancestors (Table 3).

318 Figure 3 shows the genetic contributions of the 10 most influential founders  
319 of the Lusitano Breed in Brazil since the first birth registered (1967) until 2012,  
320 which are Agareno, Destinado IV, Príncipe VIII, Primoroso, Cartujano,  
321 Jamonero III, Innato, Mejicano, Habanero VIII, and Carocha III.

322 The marginal genetic contributions of the 15 most influential ancestors,  
323 founders or not, responsible for the presence of the breed in Brazil are shown in  
324 Table 4 for TP and RP. The ancestors that increased their marginal  
325 contributions in RP were Agareno, Príncipe VIII, Afiançado de Flandes,  
326 Estribilho, Whisky, Bailador, Quimono, and Sultão I. Ancestors Cartujano and  
327 Viscaina were replaced in RP by Hucharia and Guizo.

328

## 329 **4. Discussion**

330

### 331 **4.1. Data analysis and distribution of birth records**

332 In RP, only 0.2% of the animals were imported, indicating the sustainability of  
333 Brazilian breeding. These findings show that animals born in Brazil have the  
334 capacity to influence the future of the Lusitano breed in the world, with the  
335 possibility of Brazilian breeders becoming an exporter of Lusitano horses.

336 Similar onsets of birth records have been reported in the literature for the  
337 Brazilian Campolina [17] and Mangalarga Marchador [31] horse breeds. A

338 decline in the registration of births was observed in the 1990s for the Brazilian  
339 horse breeds Mangalarga [16], Campolina [17], Brazilian Sport Horse [18] and  
340 Mangalarga Marchador [31]. According to the last authors, the reduction in the  
341 number of registered births can be explained by the implementation of the  
342 economic plan of the Brazilian government that resulted in a cause-effect  
343 relationship due to the oscillations in the Brazilian economy. In contrast, the  
344 annual number of registered births increased in the Lusitano breed during this  
345 period (1990s) (Figure 1), possibly because it is not a native Brazilian breed and  
346 was in a phase of importation and expansion in the country during that phase.  
347 According to the Anualpec yearbook [32], the number of horses decreased  
348 between 2002 and 2009 in Brazil, with a reduction of 278,375 births, while the  
349 number of Lusitano horses was stable during this period. The decline that  
350 started in 2010 (Figure 1) was the result of the serious economic-financial crisis  
351 in Europe and the United States, which caused a deceleration in horse breeding  
352 after 2008 in various parts of the world. A similar trend has been described in  
353 studies on the Lusitano [2], Old Kladruber [14] and Pantaneiro [15] breeds. The  
354 Lusitano breed in Brazil exhibited resistance to the decrease in equine records  
355 more than once, but the downward trend that started in 2010 (Figure 1) has  
356 become a matter of concern for the evolution of the breed. However, it is  
357 possible that some of the animals born in 2012 had not yet appeared in the  
358 database when it was consulted for the present study.

359 The importance of the month of birth is related to the fact that July is  
360 considered the ideal month for the birth of sporting animals in the southern  
361 hemisphere. Competitions start in July, i.e., animals born in this month have a  
362 competitive advantage over animals born in subsequent months because they  
363 are older and therefore exhibit greater physiological and sport development.  
364 This applies particularly to the classes of younger animals. Studies involving  
365 animals born in the southern hemisphere indicated the same trend observed for  
366 Lusitano horses registered in Brazil for the Mangalarga Marchador breed [31],  
367 with a concentration of birth (> 85%) between September and March. In the  
368 Mangalarga breed [16], a peak was found in November (17.4%) and a higher  
369 concentration between September and January (78.5%). A better but far from  
370 desired distribution was observed in Thoroughbreds [33], with 20.2% of births in  
371 the ideal month (July) and a larger number of births in August (24.1%), followed

372 by September (23.0%). The distribution of births over the year in Brazil can be  
373 explained by the abundance of feed (pastures) throughout all quarters of the  
374 year and by the higher nutritional value in the last quarter (spring), a fact that  
375 leads Brazilian breeders to opt for the quality of their pastures at the expense of  
376 the ideal month. Since the Lusitano horse is mainly a sporting animal, the best  
377 choice is that a larger number of births should occur in the third quarter of the  
378 year. To help comparing the results and their discussion with several different  
379 horse breeds a supplementary table can be consulted online (Supplementary  
380 material - Table A ).

381

#### 382 **4.2. Reproductive parameters and Generation interval**

383 The differentiated values of breeding animals that left descendants  
384 (progenies) by national and imported origins are also observed in the literature.  
385 In the study of Cervantes et al [12] on Spanish Arab Horses, the contribution of  
386 imported individuals was high, corresponding to 47.9% of all descendants of the  
387 breed's studbook. Koenen et al [34], providing an overview of the breeding  
388 objectives for sport horses, reported percentages of mares covered by an  
389 imported stallion of 74% for Danish Warmblood, 62% for Swedish Warmblood,  
390 32% for Irish Horse Board, 31% for Royal Dutch Sport Horse, and 6% for Selle  
391 Français. The percentage of use of imported Lusitano breeding animals among  
392 those imported to Brazil can be explained by the choices of breeders who  
393 purchased these animals from foreign populations mainly for breeding, with  
394 dams being used almost exclusively for this purpose. Despite the greater  
395 utilization within the origin of imported animals, the percentage of offspring with  
396 Lusitano parents born in Brazil was higher, demonstrating the reproductive  
397 sustainability on the American continent of the responsibility of Brazilian horse  
398 farms.

399 The percentages of breeding animals that left descendants are 3.3%  
400 (stallions) and 22.6% (mares) for the Mangalarga Marchador breed [31], and  
401 8.3% (stallions) and 22.2% (mares) for the world Lusitano population [3]. These  
402 values are lower than those obtained in the present study, indicating greater  
403 utilization of breeding animals in the formation of the Lusitano population in  
404 Brazil and consequent higher genetic diversity. The smaller number of stallions  
405 in all studies reported may be due in part to the greater difficulty of approval in

406 the studbook and their reproductive superiority, in which a single stallion can  
407 have dozens of offspring while a natural limit exists for mares. The low breeding  
408 stallion-to-mare ratio reduces the intensity of selection, which may result in less  
409 genetic progress of the population [35].

410 For the world Lusitano population, Vicente et al [2] indicated that the ratio of  
411 5 mares per sire (lower than in the present study) impairs selection of the  
412 Lusitano breed. The small number of mares per stallions observed in the  
413 present study, similar to Quarter Horse in Brazil [19], should be taken into  
414 consideration by the association and by breeders, which can be modified and  
415 improved, increasing genetic progress to the levels (mares per stallion) of other  
416 breeds such as Thoroughbreds in New Zealand (43 to 1) [36] and Hanoverians  
417 (45 to 1) [11].

418 Vicente et al [2] reported mean numbers of offspring similar to those of the  
419 present study (Table 1) for the Lusitano breed, with a mean number of offspring  
420 of 13.1 for stallions and of 4.0 for mares, suggesting identical reproductive  
421 guidelines on Brazilian horse farms and in the remaining countries where  
422 Lusitanos are reared. The numbers of offspring observed in the literature were  
423 different (stallions) and similar (mares) compared to those of the Lusitano  
424 population in Brazil (Table 1), with 22.2 (stallions) and 3.1 (mares) offspring for  
425 the Campolina breed [17], 10.7 (stallions) and 3.7 (mares) offspring for the  
426 Quarter Horse [19], and 23.8 (stallions) and 4.4 (mares) offspring for the  
427 Mangalarga breed [16]. Reproductive inequalities between sexes in horses can  
428 be seen in all breeds (Supplementary material - Table A ), indicating greater  
429 participation of dams in the maintenance of the genetic diversity of each breed.

430 The age at birth of the first foal indicates the onset of reproductive life and,  
431 considering that horses are able to breed at 2 years of age [37], this did not  
432 influence the age at first foal since horses exhibit a late mean age at birth of  
433 their first progeny [2,38]. The main causes of this event as reported in various  
434 studies include differences in reproductive strategies between breeds and farms  
435 (high standard deviations in the results of Table 1), after functional performance  
436 and credits established in their sports career. Another factor are the regulations  
437 of the associations, which only permit the registry of animals in the studbook at  
438 certain ages and riding classes, a fact resulting in the approval of animals at  
439 older ages. Other factors include hormonal disorders, genital and parasite

440 infections, and inadequate management practices before the breeding season,  
441 interfering with the onset of reproductive life of horses. An almost identical  
442 mean age at first progeny (Table 1) was reported for stallions of the world  
443 Lusitano population [2], with a difference of only 0.3 years. The delay in the age  
444 at first progeny in the Lusitano breed may be explained by the regulations of  
445 their association, which only permit the application of stallions after 4 years of  
446 age and of recommended or merit stallions (animals recommended by APSL for  
447 breeding can have an unlimited number of offspring) at a minimum age of 6  
448 years. Many breeders who wish their mares covered by these stallions wait to  
449 perform matings or use the semen of these animals, consequently increasing  
450 the age at birth of the first foal. However, the mean age of mares (Table 1) of  
451 the present study was not far from the ideal proposed by Davies Morel et al  
452 [39]. These authors described the ideal age of mares to start breeding to be  
453 between 5 and 6 years, a period when they will have reached the final mature  
454 size.

455 The age at birth of the last progeny (Table 1) was similar to that reported by  
456 Vicente et al [2], who estimated a mean age of 10.5 years for sires and dams.  
457 The end of reproductive life of horses has been little studied and the age at last  
458 progeny is less reported than the age at first progeny. Further studies are  
459 necessary to understand the reasons for at age last progeny.

460 The coefficients of variation for all reproductive parameter indicate high  
461 dispersion of the results among animals, which was greater in stallions for all  
462 parameters, demonstrating an imbalance in the utilization of males and females.  
463 The results suggest different objectives within the Lusitano breed in Brazil, with  
464 the observation of sires and dams with a small or large number of offspring, late  
465 age at first progeny, and a reduced mean number of dams per sire. The values  
466 observed are common among some equine breeds, but are in contrast to the  
467 balanced values of cattle for which studies have already determined, for  
468 example, the ideal number of cows per bull [40].

469 Scientific studies have not shown the same influence on horse breeds as  
470 studies involving other livestock species (e.g., cattle, pigs, and small ruminants).  
471 The difficulty in designing the same experiments is mainly due to the  
472 peculiarities of horses and to the fact that, unlike in the case of other livestock  
473 species, the access of the scientific community to horse farms is limited,

474 impairing the approximation between researchers and breeders. Breeders of  
475 the Lusitano horse in Brazil should increase and balance the number of dams  
476 per sire (genetic evolution), begin the reproductive life of animals earlier  
477 (decrease in GI), and collaborate more actively with researchers to establish  
478 new selection programs, which would permit to observe reproductive  
479 parameters that contribute to the genetic evolution of horses as observed for  
480 other livestock species.

481 Vicente et al [2], evaluating Lusitano horses using data collected throughout  
482 the world, obtained a GI that was similar to (TP) and the same as (RP) that of  
483 Lusitano in Brazil, indicating a possible influence of animals born in Brazil on  
484 the values of the world population. Although long, the GI is similar for all breeds  
485 in Brazil, such as Mangalarga (9.5 years) [16], Campolina (8.7 years) [17],  
486 Brazilian Sport Horse (10.8) [18] and Quarter Horse (9.6 years) [19]. The GI  
487 obtained in Europe for Andalusian (10.1 years) [8], Holstein (10.3 years) [9]  
488 and Old Kladruber (11.3 years) [14] was the same as those estimated for the  
489 Lusitano horse (Supplementary material - Table A ) . A long GI is the result of  
490 different factors such as the late selection of stallions and mares and long  
491 reproductive life. Consequently, since the GI is present in the denominator of  
492 the formula used to calculate genetic gain, it will act inversely proportional,  
493 reducing the expected response to selection. The reduction in genetic gain due  
494 to a long GI in the Lusitano breed is supported by the high standard deviations,  
495 indicating that some breeders are concerned about the GI of their populations,  
496 while a large number of breeders who are not concerned are responsible for  
497 decelerating the evolution of the Lusitano breed in Brazil.

498

#### 499 **4.3. Quality of pedigree data**

500 The mean values of pedigree completeness (Figure 2) tended to increase  
501 over time and the loss of data was lower when compared to a study on Spanish  
502 Arab horses [12], which reported percentages of 92.0%, 86.6% and 80.8% for  
503 the three most recent generations. For Lipizzan [40] and Old Kladruber [14], the  
504 authors indicated high values of completeness around 100% for the last five  
505 and six generations, respectively. In the study of the world Lusitano population,  
506 the percentages of pedigree completeness obtained for the whole population of  
507 98.8%, 97.8% and 97.1% [2] indicate that the APSL data are slightly more

508 complete than the whole data of ABPSL, with more in-depth pedigree  
509 knowledge. The estimation of pedigree completeness is important since the  
510 parameters related to the inbreeding coefficient, probability of gene origin and  
511 genetic drift of a given individual depends on how much of his ancestry is  
512 known. The moderate values of the present study can be improved, the greater  
513 this knowledge, the more reliable will be the estimated values in relation to the  
514 base population studied.

515 When compared to the literature, the number of GE (5.7 in RP) can be  
516 considered low, but was higher than that reported in studies on Pantaneiro (0.7  
517 GE) [15], Brazilian Sport Horse (1.7 GE) [18], Paint-Horse (4.8 GE) [10],  
518 Quarter Horse (5.1) [19] and equal to Holstein (5.6 GE) [9]. However, the GE  
519 was lower than in Purebred Lusitano (9.9 GE) [2] and Old Kladruber (15.1 GE)  
520 [14]. Similar to the literature (Supplementary material - Table A), the increase in  
521 the amount of pedigree data in RP indicates improvement in the quality of  
522 pedigree information in the studbook of ABPSL. However, we must always  
523 analyze and compare these results with care since they depend on the quality  
524 and degree of completion of the pedigrees, so the values obtained for GE may  
525 have a direct influence on inbreeding,  $f_e$  and  $f_a$  estimates.

526

#### 527 **4.4. Parameters related to the inbreeding coefficient**

528 The main effect of F (Table 3) is an increase in homozygosity as a result of  
529 more closely related animals. A higher inbreeding coefficient (9.4%) has been  
530 reported by Vicente et al [2] for the whole population of Lusitano horses  
531 registered in the world when a period of 5 years (2005 to 2009) was analyzed.  
532 An even higher value (11.3%) was obtained by the authors for a reference  
533 population. An inbreeding coefficient (F) of 4.6% was observed exclusively for  
534 Lusitano born in Brazil and for the same period (2005 to 2009). Considering the  
535 values reported by Vicente et al [2], Lusitano horses born in Brazil contribute to  
536 an overall decrease of inbreeding in the breed. This fact may indicate that farms  
537 have been introducing different lineages of less related Lusitano horses in Brazil  
538 over the years. On the other hand, this reduction in the inbreeding coefficient of  
539 animals reared in Brazil may also be explained in part by the lower level of  
540 pedigree knowledge compared to the Lusitano studbook of APSSL. The  
541 maintenance of a low level of inbreeding in Brazilian Lusitano horses may

542 contribute in the future to the export of breeding animals in order to reduce  
543 inbreeding on Lusitano horse farms around the world. Other authors reported  
544 higher value of F for Andalusian (8.5%) [8] Lipizzaner (10.8%) [13] and Old  
545 Kladruber (13.0%) [14]. Low levels of F are observed for Brazilian breeds,  
546 including Campolina (1.3%) [17], Brazilian Sport Horse (0.24%) [18] and  
547 Mangalarga Marchador (5.7%) [31] (Supplementary material - Table A). Low  
548 levels of F are an indicator that the breeds are organized in an open studbook  
549 system, which permits the utilization of sires and dams from other breeds with a  
550 consequent reduction in inbreeding.

551  $\Delta F$  was reduced (Table 3), with a small decrease in RP, despite the  
552 observation of a larger number of mating among related animals compared to  
553 TP. An increase in consanguinity was observed among annual births, finishing  
554 2012 with 99.5% of births of related animals. The standard deviations obtained  
555 (Table 3) are not of major concern since they remained within acceptable levels  
556 for inbreeding parameters. High standard deviations were also observed in the  
557 study of Vicente et al [2]. Despite the lower dispersions in the present study, the  
558 results should not be overlooked because the small number of Lusitano horses  
559 compared to other breeds or species may, at any time, change the F values to  
560 less desired levels.

561 Values of  $N_e$  have been reported for Hanoverian (372) [11], Spanish Sport  
562 Horse (226) [41], Brazilian Sport Horse (223) [18], Holstein (55) [9], and Old  
563 Kladruber (53) [14] breeds. Studies that obtained high values of  $N_e$  suggest, by  
564 definition, reduced inbreeding levels because of the direct relationship between  
565  $N_e$  and inbreeding rate [24]. This is obviously also related to the fact that most  
566 of these breeds have open studbooks, thus permitting the introduction of new  
567 genes and a consequent reduction in inbreeding. The small increase of  $N_e$  in  
568 RP (Table 3) is related to the fact that the increase in inbreeding was lower in  
569 the last generation, and possibly to the introduction of new lineages and  
570 different utilizations of major ancestors, either founders or not (Figure 3; Table  
571 4). However, and according to Caballero and Toro [42], the comparison of  
572 different  $N_e$  values is problematic since the true value is unknown and the  $N_e$   
573 obtained depends in part on the effect of changes in mating policies (level of  
574 inbred animals).

575 For the world Lusitano population, Vicente et al [2] reported that the  $N_e$  of 41  
576 obtained for a period of 5 years (2005 to 2009) is a matter of concern for the  
577 maintenance of genetic diversity. The  $N_e$  observed in the present study for the  
578 same period and using the same method as those authors (individual increase  
579 in inbreeding) was 57. Considering the minimum  $N_e$  (50) recommended by the  
580 FAO [43], the value obtained in this study is less worrisome for the maintenance  
581 of genetic diversity, and Lusitano horses born in Brazil may therefore assist in  
582 maintaining the diversity of the world population.

583

#### 584 **4.5. Probability of gene origin and genetic drift**

585 Lower probabilities of gene origin are reported in the literature for the  
586 Lusitano breeds ( $f_e$  of 28,  $f_a$  of 12 and  $f_g$  of 6) [2], Holstein ( $f_e$  of 50,  $f_a$  of 29 and  
587  $f_g$  of 17) [9] and Old Kladruber ( $f_e$  of 93,  $f_a$  of 17 and  $f_g$  of 5) [14] and, while  
588 higher values were found for Paint-Horse ( $f_e$  of 561,  $f_a$  of 208 and  $f_g$  of 139) [10],  
589 Brazilian Sport Horse ( $f_e$  of 466,  $f_a$  of 274 and  $f_g$  of 224) [18], Quarter Horse ( $f_e$   
590 of 1,045,  $f_a$  of 156 and  $f_g$  of 105) [19] and Spanish Sport Horse ( $f_e$  of 963,  $f_a$  of  
591 407 and  $f_g$  of 254) [41] (Supplementary material - Table A ). Higher values  
592 suggest the use of a larger number of animals in the formation of the breeds.  
593 The genetic variability of breeds observed in the literature, as demonstrated by  
594 the results of the probability of gene origin, is a matter of concern in horse  
595 breeding. However, the variation in the three parameters of gene origin  
596 probability obtained in the present study was lower than that described in the  
597 literature. The effective number of founders in both populations, comparing the  
598 number of founders (Table 3), indicates the preferential use of certain lineages  
599 of founders. Since each population has its own characteristics, comparison with  
600 the literature should be done with caution and within each breed, considering  
601 the three parameters for each population.

602 Genetic drift is approaching stability in the Brazilian Lusitano breed (Table 3),  
603 with a progressive increase in the representation of founders [28,29] in RP as  
604 indicated by  $f_e/N_e > 0.5$  and  $f_e \sim (N_e/2)$ , which are close to the desired. The  $f_e/f_a$   
605 ratio (Table 3) confirms the absence of worrisome bottleneck effects in the  
606 population despite the increase of 0.02 in RP. Different results are observed in  
607 the literature, with the loss of genetic variability due to the utilization of lineages  
608 with a reduced number of founders and worrisome bottleneck effects; for

609 example,  $f_e/f_a$  ratios of 1.69 for the Brazilian Sport Horse [18], 1.72 for the  
610 Holstein [9], 2.34 for Lusitano using world data [2], 2.37 for the Spanish Sport  
611 Horse [41], 2.70 for the Paint-Horse [10], 5.47 for the Old Kladruber [14], and  
612 Quarter Horse 6.70 [19].

613 . The persistence of the founder lines is observed in several breeds  
614 throughout the world, being lower in the present study. It should be noted that,  
615 the values of each breed may vary due to different qualities of Studbook data  
616 information (Pedigree completeness and GE).

617

#### 618 **4.6. Genetic conservation index**

619 In the literature, mean GCIs of 1.3, 9.5 and 14.8 and maximum values of 7.2,  
620 19.2 and 31.2 have been reported for the Pantaneiro [15] Lusitano [2] and  
621 Quarter Horse [19] breeds, respectively (Supplementary material - Table A ).  
622 The small differences between the world Lusitano population and the present  
623 study are visible in the maximum values and are explained by the  
624 heterogeneous importation of genetic lineages of Lusitano into Brazil.  
625 Consequently, different sires and dams were used over time, which resulted in  
626 slightly higher GCI values.

627 Animals with higher indexes exhibit greater conservation, i.e., a greater  
628 balance in the number of founders, and should be used (preferentially in the  
629 choice of breeding animals) in genetic selection programs to maintain the genes  
630 transmitted by founders [30]. Brazilian farms are a good choice for breeding  
631 animals of the Lusitano breed or of world breeds with origin in the Lusitano  
632 horse, with the Mangalarga Marchador, Campolina, Andaluz Brasileiro or  
633 Iberian breeds (e.g. Portuguese and Spanish Sport Horses), in order to  
634 introduce founder genes of the Lusitano breed.

635

#### 636 **4.7. Genetic contributions**

637 A slight increase in the genetic contributions explained by the 10, 50 and 100  
638 most influential founders was observed from TP to RP (Table 3), indicating a  
639 similar use of lineages as the major founders in RP. This change contributed to  
640 maintaining the genetic diversity of the Lusitano breed in Brazil over the last 10  
641 years (2003-2012) evaluated. The marginal genetic contributions of ancestors,  
642 with a small increase in RP (Table 3), suggest that ancestors contributed

643 equally in TP and RP, demonstrating the maintenance of alleles of ancestral  
644 sires and dams in the last generation.

645 The marginal genetic contribution of the 15 most influential ancestors in the  
646 present study (Table 4) was higher than that reported for Hanoverians (34.9%)  
647 [11]. Considering the 10 most influential ancestors with the greatest marginal  
648 genetic contributions to the world Lusitano population reported in the study of  
649 Vicente et al [2], half of these animals were also ancestors of the Brazilian  
650 population (Table 4), represented by Agareno, Príncipe VIII, Destinado IV,  
651 Primoroso and Cartujano, at different proportions and maintaining only the order  
652 of the marginal genetic contribution of the most influential ancestor (Agareno).  
653 Although these authors studied the same breed as in the present study, the  
654 data used refer to the period from 2005 to 2009, with a much larger sample of  
655 data and the approaches taking place in different populations. Consequently,  
656 the observation of differences in the parameters between the two studies was  
657 expected.

658 In the study on the world Lusitano population [2], the number of founders was  
659 14 and 99, corresponding to 50% and 90% of the genetic contribution of  
660 founders, respectively. The Brazilian Lusitano population exhibited variations in  
661 founder lineages (Figure 3) and, consequently, greater genetic diversity of the  
662 animals born on Brazilian farms. These differences may help explain the lower  
663 inbreeding and greater genetic diversity of Lusitano horses born in Brazil.

664

## 665 **5. Conclusion**

666 The inbreeding and relatedness coefficients demonstrate some control on the  
667 part of Brazilian breeders, suggesting that Lusitano horses born in Brazil can be  
668 introduced as breeding animals in the world Lusitano population in order to  
669 increase the genetic diversity of the breed. The long GI obtained indicates a  
670 delay in annual genetic gain and the lack of speedy decision-making about the  
671 appropriate use of sires and dams. Although the absence of bottleneck effects  
672 is evident and positive, the low probabilities of gene origin and the high genetic  
673 contribution of a small number of animals suggest that Brazilian breeders need  
674 to monitor the genetic diversity of the Lusitano horse in next generations  
675 through breeding programs.

676

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**Figure captions**

Figure 1. Number of births (males, females, and all animals) registered in the Studbook of the Lusitano horse breed in Brazil by year of birth.

Figure 2. Completeness of pedigree information per generation for both populations (total and reference) of Lusitano horses in Brazil (15<sup>th</sup> generation of older animals).

Figure 3. Evolution of the genetic contribution of the 10 most influential founders of the Lusitano breed in Brazil from the first record in 1967 to 2012, during five different periods.

## Tables

**Table 1.** Summary descriptive statistics of reproductive parameters of Lusitano stallions and mares in Brazil

Item	Total population	
	Stallion	Mare
<b>Offspring</b>		
Total (n)	18,515 <sup>a</sup>	18,436 <sup>b</sup>
Mean $\pm$ SD (n)	12.3 $\pm$ 24.5	4.0 $\pm$ 3.4
Maximum (n)	419	20
<b>Stallions/mares</b>		
Total (n)	1,507	4,644
Age at first progeny (years)	7.0 $\pm$ 3.8	5.9 $\pm$ 3.1
Age at last progeny (years) <sup>c</sup>	10.7 $\pm$ 5.7	10.5 $\pm$ 5.3

N number of the respective observation; SD, standard deviation. <sup>a</sup> offspring with known father (independently of knowing the mothers). <sup>b</sup> offspring with known mother (independently of knowing the father). <sup>c</sup> Only calculated for animals with two or more offspring.

**Table 2.** Generation interval (in years) for the four paths of selection between parents and offspring that reproduced for Lusitano horses in Brazil

Path of selection	Total population		Reference population	
	n	Mean $\pm$ SD	n	Mean $\pm$ SD
Father-son	1,393	10.9 $\pm$ 6.0	146	9.4 $\pm$ 4.7
Father-daughter	4,409	10.8 $\pm$ 5.5	679	11.4 $\pm$ 6.0
Mother-son	1,374	9.5 $\pm$ 4.5	144	10.2 $\pm$ 4.7
Mother-daughter	4,350	9.2 $\pm$ 4.5	679	9.6 $\pm$ 4.4
Overall mean	11,526	10.1 $\pm$ 5.1	1,648	10.2 $\pm$ 5.0

n number of observations; SD standard deviation.

**Table 3.** Summary statistics of parameters related to inbreeding, probability of gene origin, genetic drift and genetic contributions of founders and ancestors of the Lusitano breed in Brazil

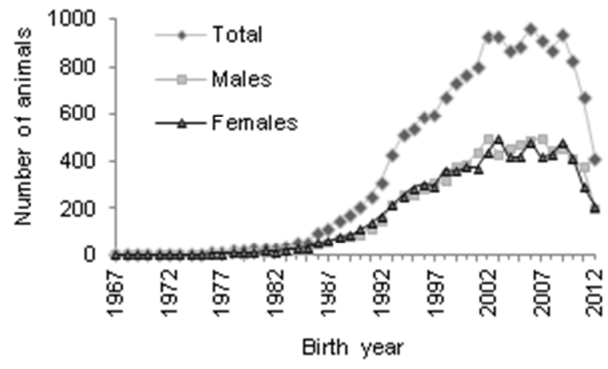
Item	Total population	Reference population
Parameters related to inbreeding		
Average inbreeding coefficient <sup>a</sup> , F (%)	4.06 ± 4.94	4.46 ± 4.34
Average related coefficient, AR (%)	5.41 ± 1.69	5.97 ± 1.20
Increase in inbreeding <sup>a</sup> , ΔF (%)	0.96 ± 2.09	0.85 ± 0.90
Effective population size <sup>b</sup> , N <sub>e</sub> (n)	89	90
Effective population size <sup>c</sup> , N <sub>e</sub> (n)	52	59
Number of animals with inbreeding coefficient different from zero (n)	16,777	8,160
Probability of gene origin		
Effective number of founders, f <sub>e</sub> (n)	33	29
Effective number of ancestors, f <sub>a</sub> (n)	30	26
Effective number of founder genomes, f <sub>g</sub> (n)	19	15
Genetic drift		
f <sub>e</sub> / N <sub>e</sub> <sup>c</sup>	0.64	0.49
f <sub>e</sub> / f <sub>a</sub>	1.10	1.12
f <sub>e</sub> ~ (N <sub>e</sub> / 2) <sup>c</sup>	33 ~ 26	29 ~ 30
Contributions of founders and ancestors		
Total number of animals per population	18,922 <sup>d</sup>	8,329 <sup>d</sup>
Total number of animals with both parents known (n)	18,406 <sup>e</sup>	8,315 <sup>e</sup>
Number of founders (n)	516	453
Genetic contributions of the 10 most influential founders (%)	42.7	43.5
Genetic contributions of the 50 most influential founders (%)	74.3	78.2
Genetic contributions of the 100 most influential founders (%)	85.7	88.7
Number of ancestors (n)	427	341
Genetic contributions <sup>d</sup> of the 10 most influential ancestors (%)	46.8	52.2
Genetic contributions <sup>d</sup> of the 50 most influential ancestors (%)	82.0	84.6
Genetic contributions <sup>d</sup> of the 100 most influential ancestors (%)	91.7	93.1

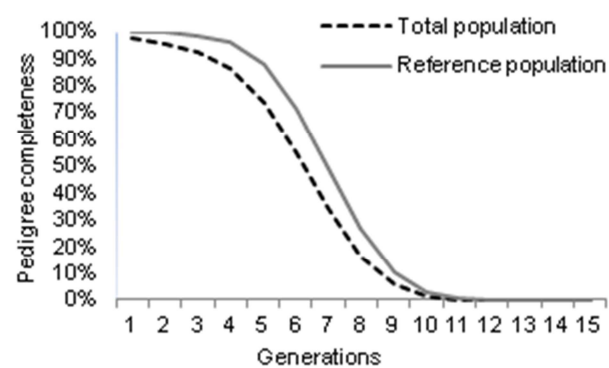
<sup>a</sup> All animals in the population studied. <sup>b</sup> According to Falconer and Mackay (1996). <sup>c</sup> According to Gutiérrez et al. (2009). <sup>d</sup> total animal studies in each population. <sup>e</sup> total of animals with known father and mother in each population. <sup>f</sup> Marginal genetic contributions.

**Table 4.** Marginal genetic contributions (in %) of the 15 most influential ancestors (founders or not) to the Lusitano breed in Brazil

No.	Name	Year of birth	Sex	Breeder	Marginal contribution (%)
Total population					
1	Agareno	1931	S	Manuel Tavares Veiga	10.62
2	Príncipe VIII	1943	S	D. Francisco C Navarro	7.12
3	Destinado IV	1940	S	D. Francisco C Navarro	6.35
4	Primoroso	1927	S	Hermanos Dominguez	4.61
5	Cartujano	1928	S	D. António Perez Tinao	4.09
6	Bailador	1962	S	Manuel Tavares Veiga	3.20
7	Estrilho	1963	S	M Assunção Coimbra	2.85
8	Jamonero III	1953	S	D. Isabel M V Terry	2.70
9	Viscaina	1933	M	D. M Romero Benitez	2.64
10	Afiançado de Flandes	1982	S	Quinta de Flandes	2.60
11	Whisky	1947	M	F Sommer D'Andrade	2.38
12	Babel	1965	S	L J Ortigão Costa	2.32
13	Quimono	1974	S	A J Fonseca Alcobia	2.23
14	Innato	1962	S	D. J Domeq de La Riva	1.94
15	Sultão I	1942	S	Manuel Tavares Veiga	1.78
All 15					57.45
Reference population					
1	Agareno	1931	S	Manuel Tavares Veiga	12.48
2	Príncipe VIII	1943	S	D. Francisco C Navarro	8.58
3	Destinado IV	1940	S	D. Francisco C Navarro	4.73
4	Afiançado de Flandes	1982	S	Quinta de Flandes	4.20
5	Estrilho	1963	S	M. Assunção Coimbra	3.85
6	Whisky	1947	M	F Sommer D'Andrade	3.84
7	Bailador	1962	S	Manuel Tavares Veiga	3.44
8	Primoroso	1927	S	Hermanos Dominguez	3.33
9	Quimono	1974	S	A J Fonseca Alcobia	2.95
10	Hucharía	1943	M	Estado Português CN	2.74
11	Babel	1965	S	L J Ortigão Costa	2.04
12	Sultão I	1942	S	Manuel Tavares Veiga	2.04
13	Jamonero III	1953	S	D. Isabel M, V Terry	2.00
14	Innato	1962	S	D. J Domeq de La Riva	1.69
15	Guizo	1947	S	Manuel Tavares Veiga	1.69
All 15					59.87

No. number; S Stallion; M mare.







**Highlights**

- The Brazilian Lusitano horse has the capacity to influence the future of the Lusitano breed in the world.
- The low level of inbreeding in Brazilian Lusitano represents an export market for breeding animals.
- Maintenance of genetic diversity in the last population.
- Absence of bottleneck effects and genetic stability of the Lusitano breed in Brazil.