

Estimates of genetic parameters and cluster analysis for worm resistance and resilience in Santa Inês meat sheep

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Abstract – The objective of this work was to estimate genetic parameters for traits related to resistance to endoparasites in Santa Inês sheep, for a possible inclusion in sheep breeding programs, as well as to evaluate the additive genetic pattern of the animals using cluster analyzes, in order to assess whether there are groups within the population that can be used as candidates for selection for resistance to endoparasites. The studied traits were: Famacha score, fecal egg counts (FEC), packed cell volume (PCV), total plasma protein (TPP), body condition score (BCS), and body weight (BW). Heritability estimates ranged from 0.17 (0.05) for TPP to 0.31 (0.07) for BCS. The cluster analyzes based on the breeding values identified four clusters. Famacha and BCS can be recommended as selection criteria for resistance to hematophagous endoparasites because they can respond to selection and are easy to be measured. Animals from cluster 1 have great potential as candidates for selection because of their additive genetic pattern, taking into account body weight and endoparasite resistance due to higher breeding values for BW, BCS, PCV, and TPP and lower breeding values for Famacha and FEC.

Index terms: cluster analysis, Famacha, genetic correlation, heritability, k-means, multivariate analysis.

Estimativas de parâmetros genéticos e análise de agrupamento para resistência e resiliência à verminose em ovinos de corte Santa Inês

Resumo – O objetivo deste trabalho foi estimar parâmetros genéticos para características relacionadas à resistência a endoparasitas em ovinos Santa Inês, para uma possível inclusão em programas de melhoramento genético de ovinos, bem como avaliar o padrão genético aditivo dos animais por meio de análises de agrupamento dos valores genéticos, para verificar se há subgrupos dentro da população que possam ser utilizados como candidatos à seleção para resistência a endoparasitas. As características estudadas foram: score Famacha, contagem de ovos fecais (FEC), volume globular (PCV), proteína plasmática total (TPP), escore de condição corporal (BCS) e peso corporal (BW). As estimativas de herdabilidade variaram de 0,17 (0,05) para TPP a 0,31 (0,07) para BCS. As análises de agrupamento com base nos valores genéticos identificaram quatro grupos. Famacha e BCS podem ser recomendados como critérios de seleção de resistência a endoparasitas hematófagos, uma vez que podem responder à seleção e são facilmente medidos. Os animais do grupo 1 apresentam grande potencial como candidatos para seleção em razão do seu padrão genético aditivo, ao se levar em consideração peso corporal e resistência ao endoparasita devido aos maiores valores genéticos para BW, BCS, PCV e TPP e aos menores valores genéticos para Famacha e FEC.

Termos para indexação: análise de agrupamento, Famacha, correlação genética, herdabilidade, k-means, análise multivariada.

Introduction

The number of sheep, distributed worldwide throughout all continents, was approximately 1.2 billion in 2014 (FAO, 2018), with an annual growth rate of 1.5% in the last five years (Monteiro et al., 2018). Among countries, Brazil concentrates the eighteenth largest sheep herd (FAO, 2015). The data compiled by Martins et al. (2016) shows that Brazilian sheep production is an opportunity to make domestic products more competitive in the external market, but also that it presents challenges regarding the stagnation of production in an economic scenario of oscillations. In this context, improvements in animal health are important considering losses in meat sheep due to infections caused by gastrointestinal nematodes.

These infections stand out among the main factors that hinder sheep production. In locations with warm weather, *Haemonchus contortus*, a blood-feeding parasite that can cause anemia (Kenyon et al., 2009), is the most important species, resulting in losses in the production of sheep (Kenyon et al., 2009; Molento et al., 2009; Cornelius et al., 2014; Torres-Acosta et al., 2014). It should be noted that the prophylaxis of this endoparasite has been compromised by the indiscriminate use of anthelmintic chemotherapy, leading to the appearance of nematodes with resistance to these drugs (Cornelius et al., 2014; Rose et al., 2015).

Fecal egg counts and packed cell volume are traits commonly used for the identification of resistant animals; however, they are costly to measure because they require expensive equipment that are not feasible for many producers (Riley & Van Wyk, 2009; Van Burgel et al., 2011). An alternative is the Famacha method, which evaluates the degree of anemia of an animal based on the color of the conjunctiva of the eye. This technique can be used to identify animals that are resistant, resilient, or susceptible to hematophagous endoparasites, especially *H. contortus*, and does not require laboratory resources because it can be carried out by trained technicians. The body condition score is another trait that can be used to help in the identification of parasitized animals, by monitoring feed conversion, dehydration and/or anorexia (Molento et al., 2011) sheep and goats, and body weight (Stafford et al., 2009).

However, there are few known studies in the scientific literature about the estimates of genetic parameters for traits related to resistance to endoparasites in Santa Inês sheep. These traits could be included as selection goals

in sheep breeding programs, in order to obtain genetic gains for resistance to endoparasites. With this, the genetic selection of sires and dams that are resistant or resilient to gastrointestinal infections, i.e., of parasitized animals with the ability to maintain production, would be possible (Lôbo et al., 2009; Riley & Van Wyk, 2009, 2011) and could reduce chemotherapeutic treatments (Molento et al., 2011).

The multivariate cluster analysis is a powerful statistical tool to be applied to explore information from large datasets, i.e., when multiple variables are evaluated simultaneously. The aim of this analysis is to group individuals based on the similarity of the characteristics used as criteria to group them (Hair Jr. et al., 2009). However, this method is still not widely applied in animal breeding studies to group animals based on additive genetic information detected through the estimated breeding values of important economic traits. Among these researches, stands out that of Savegnago et al. (2016), who identified additive genetic patterns of Holstein cows using breeding values for milk production, in order to select animals that met the selection goals of the breeding program. Cruz et al. (2016) adopted the same methodology to identify additive genetic patterns for persistency of lactation in Guzera cattle.

The objective of this work was to estimate genetic parameters for traits related to resistance to endoparasites in Santa Inês sheep, for a possible inclusion in sheep breeding programs, as well as to evaluate the additive genetic pattern of the animals using cluster analyzes, in order to assess whether there are groups within the population that can be used as candidates for selection for resistance to endoparasites.

Materials and Methods

The dataset contained records of fecal egg counts (FEC) to identify the proportion of *H. contortus* by coproculture, packed cell volume (PCV), total plasma protein (TPP), Famacha score based on conjunctival color, body weight (BW), and body condition score (BCS) from 769 Santa Inês sheep born between 2002 and 2014. The animals, from different herds, showed kinship among each other and were obtained from six farms located in the municipalities of Cravinhos (21°20'25"S, 47°43'46"W), Jardinópolis (21°01'04"S, 47°45'50"W), Nova Odessa (22°46'39"S, 47°17'45"W),

Pontal (21°01'21"S, 48°02'14"W), Serrana (21°12'41"S, 47°35'44"W), and São Carlos (21°01'03"S, 47°53'27"W), in the state of São Paulo, Brazil. These municipalities are characterized by tropical climate, with two well-defined seasons, a rainy summer from November to April and a dry winter from May to October.

The fecal samples from each animal were collected directly from the rectum. FEC was carried out using the technique of McMaster, modified by Gordon & Whitlock (1939). The ten samples with the highest FEC per management group were subjected to the culture of infective larvae for the identification of parasite genus (Roberts & O'Sullivan, 1950).

The blood samples were collected by puncture of the jugular vein into 5-mL vacuum tubes containing ethylenediaminetetraacetic acid (EDTA). PCV was determined by microhematocrit centrifugation, and TPP was evaluated by refractometry (Schalm et al., 1975). The protocol shown in Table 1 was adopted to calculate the percentage of correct Famacha assessments by the examiner in the field compared with the percentage of PCV obtained by the laboratory analysis.

The Famacha score was evaluated by trained technicians using the chart for diagnosis, i.e., by comparing the different conjunctiva colors on a scale from 1 to 5, in which: 1, red; 2, pink-red; 3, pink, 4, white; and 5, pale white. The animals were also weighed on an electronic scale, and BCS was obtained by visual assessment and by palpation of the dorsal-lumbar region of the spine, determining the amount of fat and muscle found in the angle formed by the spinous and transverse processes of the vertebrae. A score from 1 to 5 was then attributed to the animals, in which: 1, cachectic; 2, lean; 3, normal; 4, stout; and 5, obese.

Table 1. Protocol⁽¹⁾ to calculate the percentage of correct assessments of Famacha scores, compared with the percentage of packed cell volume (PCV).

PCV	Famacha score	
	1 or 2	3, 4, or 5
≥ 23%	Correct	Wrong
< 23%	Wrong	Correct

⁽¹⁾Adapted from Reynecke et al. (2011) but these properties give no objective guidance for setting the optimum threshold at which anthelmintic treatment should occur. The aim of this work was to use Receiver Operating Characteristic (ROC).

The Famacha scores were grouped into three classes according to the number of records: score 1, 77.22%; score 2, 18.60%; and scores 3, 4, and 5, 4.18%; the last scores were grouped into a single class due to their low percentages, i.e., 4.1, 0.04, and 0.04% for scores 3, 4, and 5, respectively. Because it did not have normal distribution, FEC was transformed using $FEC = \log(FEC + 1)$.

The threshold-linear multi-trait animal model was used to estimate the genetic parameters for the studied traits. For all traits, the model included fixed, direct additive genetic, permanent environmental, and residual random effects. The fixed effects of the model, for each trait, were chosen using the least squares method by the GLM procedure of the SAS software (SAS Institute Inc., Cary, NC, USA). The fixed effects for all traits were: sex; contemporary group, including herd, month, and year of collection; production system, which could be extensive, semi-feedlot, or feedlot; and age class of the animal at measurement – 1, lambs from 1 to 150 days; 2, young lambs from 151 to 550 days; and 3, adult, above 550 days. For Famacha, PCV, TPP, and FEC, the fixed effects also included the effect of deworming, i.e., whether or not the animal had received anthelmintics up to 30 days prior to data collection.

The (co)variance components were estimated by the threshold-linear multi-trait animal model using Bayesian inference through THRGIBBS1F90 (Tsuruta & Misztal, 2006). The prior distributions for these components were noninformative inverse Wishart distributions for all random effects. The process of stochastic simulation resulted in a single chain of 250,000 iterations, considering a burn-in period of 50,000 iterations to minimize the effect of initial values on the sampling algorithm. A thinning interval of 25 iterations was used to avoid serial self-correlation between samples. Therefore, 8,000 samples were used to estimate posterior means and standard deviations of the parameters of interest. Convergence was verified by the visual inspection of the chain of each parameter through trace graphs. Posterior estimates were obtained using POSTGIBBSF90 (Tsuruta & Misztal, 2006).

The hierarchical and nonhierarchical cluster analyzes were performed using the predicted breeding values (EBVs) of the traits FEC, PCV, TPP, Famacha, BW, and BCS, in order to group animals based on

the similarities of their breeding values and then to evaluate the additive genetic pattern of the groups formed within the studied population. The hierarchical cluster analysis was used to determine the number of clusters in which the population could be previously divided into. The Euclidean distance was adopted as a measure of similarity between the animals, and the clustering algorithm, to form the groups, based on the method of Ward Jr. (1963). According to Hair Jr. et al. (2009), the use of this method results in groups of approximately equal sizes due to the minimization of internal variation within them. After defining the number of clusters, the nonhierarchical cluster analysis by the k-means method (Hartigan, 1975; Hartigan; Wong, 1979) was used to explore the additive genetic pattern of the clusters, based on the breeding values of the evaluated traits. The hierarchical cluster analysis was performed using the `hclust` function of the `stats` package, whereas the nonhierarchical cluster analysis was carried out with the `k-means` function of the `mclust` package, both of the R software (Scrucca et al., 2016).

Results and Discussion

The mean Famacha score was 1.27 ± 0.52 (Table 2), indicating that the animals of the evaluated flocks

were not anemic. According to studies by Van Wyk & Bath (2002), only animals with scores 3, 4, and 5 should receive treatment. For TPP, the mean observed was 6.51 ± 0.74 g dL⁻¹ (Table 2), considered inside the normal range of 6.0 to 7.5 g dL⁻¹ for sheep (Garcia-Navarro, 2005).

The mean FEC was $605.15 \pm 1,404$ (Table 2). According to the literature, based on these results, it would not be necessary to apply anthelmintics to the studied animals. Coutinho et al. (2015), for example, administered anthelmintics to animals only when the mean FEC reached 800 eggs per gram. Medina-Pérez et al. (2015), in sheep, and Torres-Acosta et al. (2014), in goats, showed the classical over-dispersion distribution of the GIN fecal egg excretions (FEC) used a threshold of >750 eggs per gram for deworming animals.

The obtained results suggest that most animals were resistant or resilient to gastrointestinal parasites (Table 2). Although parasitized, as shown by FEC, no negative alterations were observed in the parameters related to infections with gastrointestinal nematodes, i.e., Famacha, PCV, TPP, and BCS.

The heritability estimates ranged from 0.17 (0.04) to 0.31 (0.07), which are considered of low and moderate magnitude. The heritability estimate for Famacha was 0.21 ± 0.04 (Table 3), indicating the existence of

Table 2. Number of animals, as well as mean, standard deviation (SD), and minimum and maximum values for Famacha score, body condition score (BCS), number of *Haemonchus contortus* in fecal egg counts (FEC), total fecal egg counts (TFEC), packed cell volume (PCV), body weight (BW), lamb body weight (BWL, 1 to 150 days), young lamb body weight (BWY, 151 to 550 days), adult body weight (BWA, above 550 days), and total plasma protein (TPP) of Santa Inês sheep.

Parameter	Number	Mean	SD	Minimum	Maximum
Famacha score	2,489	1.27	0.52	1.0	3.0
BCS	2,516	2.72	0.84	1.0	5.0
FEC (eggs per gram)	1,569	1,028.00	2,479.00	0.0	45,400.0
PCV (%)	2,365	30.00	4.00	11.0	46.0
BW (kg)	2,487	49.03	19.28	3.0	136.0
TPP (g dL ⁻¹)	2,352	6.51	0.74	2.0	9.0

Table 3. Posterior mean (standard error) estimates of additive genetic variance (σ^2_a), permanent environmental variance (σ^2_{pe}), residual variance (σ^2_e), heritability (h^2), and repeatability (r) for Famacha score, body weight (BW), packed cell volume (PCV), total plasma protein (TPP), fecal egg counts (FEC), and body condition score (BCS) of Santa Inês sheep.

Parameter	Famcacha	BW	PCV	TPP	FEC	BCS
σ^2_a	0.25 (0.05)	26.61 (7.50)	0.50 (0.10)	0.06 (0.02)	0.22 (0.04)	0.20 (0.05)
σ^2_{pe}	0.21 (0.04)	103.96 (8.55)	0.30 (0.09)	0.07 (0.02)	0.09 (0.02)	0.18 (0.04)
σ^2_e	0.72 (0.07)	16.75 (0.59)	0.80 (0.02)	0.24 (0.01)	0.81 (0.02)	0.25 (0.01)
h^2	0.21 (0.04)	0.18 (0.05)	0.30 (0.06)	0.17 (0.04)	0.19 (0.03)	0.31 (0.07)
r	0.39 (0.05)	0.89 (0.07)	0.47 (0.05)	0.36 (0.03)	0.28 (0.03)	0.60 (0.07)

genetic variability and, therefore, that genetic gains can be obtained if this trait were included as a selection criterion. Riley & Van Wyk (2009) reported a lower heritability of 0.13 ± 0.05 for Famacha score in Merino sheep.

In the present study, the heritability estimate for FEC was 0.19 ± 0.03 . For this same trait, Riley & Van Wyk (2009) found heritabilities of 0.22 ± 0.06 for Merino sheep, while Lôbo et al. (2009) reported values ranging from 0.01 to 0.51 in the first and second exposures of Santa Inês sheep to the parasite. The later authors attributed this variation to the fact that animals challenged for the first time are not yet resistant to endoparasites; as they grow older, they become less susceptible to the pathogenic effects of nematodes.

The heritability estimates for PCV and BCS were 0.30 ± 0.06 and 0.31 ± 0.07 , respectively (Table 3). Riley & Van Wyk (2009) found an estimated heritability of 0.25 ± 0.06 for PCV and of 0.17 ± 0.05 for BCS. The heritability estimates for PCV and BCS show the existence of additive genetic variability, indicating that selection for these traits could allow a moderate genetic progress in worm resistance.

The repeatability estimates ranged from 0.28 (0.03) to 0.89 (0.07). For Famacha, FEC, and TPP, the obtained values were of low to moderate magnitude, indicating that selection for these traits at an early age is not recommended, although successive measurements in the same animal are required to obtain a higher prediction accuracy of its future performance. For PCV, BW, and BCS, the estimates ranged from moderate to high, indicating that a single measurement of the animal could represent its true production capacity.

The genetic correlations between all studied worm resistance traits ranged from -0.23 (0.15) to 0.84 (0.08) (Table 4). Between Famacha and BCS, the obtained value was 0.59 (0.11), considered moderate

and favorable, indicating that selection for worm resistance will have a favorable effect on the genetic growth potential of the assessed animals. While studying Merino sheep in South Africa, Riley & Van Wyk (2009) reported favorable genetic correlations of -0.18 between Famacha and BCS and of -0.17 between Famacha and BW. Working with the same sheep, Woolaston & Piper (1996) found estimates of genetic correlation of 0.48 between PCV and FEC, and Nieuwoudt et al. (2002) of 0.84 (0.06) between PCV and FEC, besides heritability estimates of 0.24 (0.02) for FEC and of 0.14 (0.02) for PCV. Coltman et al. (2001), evaluating Soay sheep, obtained estimates of genetic correlations ranging from -0.39 (0.19) to -0.05 (0.04) between BW and FEC.

The estimates of the genetic correlations of Famacha with PCV, FEC, and BCS, as well as of BCS with PCV and TPP, indicate that both Famacha and BCS can be used as selection criteria in Santa Inês sheep destined for meat production. Selection for Famacha and BCS is very interesting mainly because of the low cost and ease in obtaining these measurements when compared with PCV and FEC, besides the fact that examiners can be easily trained for the evaluation of the Famacha score.

The sheep population was divided into four clusters using the hierarchical cluster analysis, and the additive genetic pattern of the breeding values for each cluster was calculated using the nonhierarchical cluster analysis (Figure 1). The number of animals in each cluster was 200 in cluster 1, 1,262 in cluster 2, 183 in cluster 3, and 642 in cluster 4, totaling 1,291 animals in the pedigree.

The animals in cluster 1 had EBVs below the population average for Famacha and FEC, but above the average for BW, BCS, PCV, and TPP. Therefore, the additive genetic pattern of this group shows that the

Table 4. Estimates of posterior means for genetic (above diagonal) and phenotypic (below diagonal) correlations, with standard errors in parentheses, between Famacha score, body weight (BW), packed cell volume (PCV), total plasma protein (TPP), fecal egg counts (FEC), and body condition score (BCS) of Santa Inês sheep.

	Famacha	BW	PCV	TPP	FEC	BCS
Famacha		-0.40 (0.17)	-0.63 (0.09)	-0.23 (0.15)	0.77 (0.09)	-0.59 (0.11)
BW	-0.10 (0.04)		0.59 (0.15)	0.68 (0.14)	-0.27 (0.17)	0.84 (0.08)
PCV	-0.39 (0.04)	0.16 (0.04)		0.66 (0.14)	-0.57 (0.12)	0.73 (0.11)
TPP	-0.11 (0.03)	0.14 (0.04)	0.29 (0.04)		-0.55 (0.10)	0.51 (0.16)
FEC	0.28 (0.03)	-0.03 (0.03)	-0.27 (0.03)	-0.17 (0.03)		-0.32 (0.15)
BCS	-0.25 (0.04)	0.30 (0.05)	0.31 (0.05)	0.18 (0.05)	-0.09 (0.03)	

animals have great potential as candidates for selection taking into account BW and endoparasite resistance due to higher breeding values for BW, BCS, PCV, and TPP and low breeding values for Famacha and FEC.

The animals from cluster 2 presented below-average breeding values for Famacha, BW, PCV, TPP, and BCS and above-average values only for FEC, indicating that they do not have acceptable body conditions to be used as breeders for the next generation. In addition, breeding values for physiological conditions, i.e., PCV and TPP, were also not favorable for selection, since the low breeding values for these traits could indicate that the animals are susceptible to endoparasites. The same discussion is valid for animals from cluster 3.

The main difference in the genetic pattern of animals from cluster 2 and cluster 3 was related to the average of the breeding values for Famacha and FEC. The animals from cluster 2 had below-average breeding values for Famacha and above-average values for FEC, indicating resistance, that is, potential to eliminate worm infestation and absence of anemia; however, the values obtained for the other traits were below the population average.

The animals from cluster 3 showed mean breeding values for Famacha above the population average

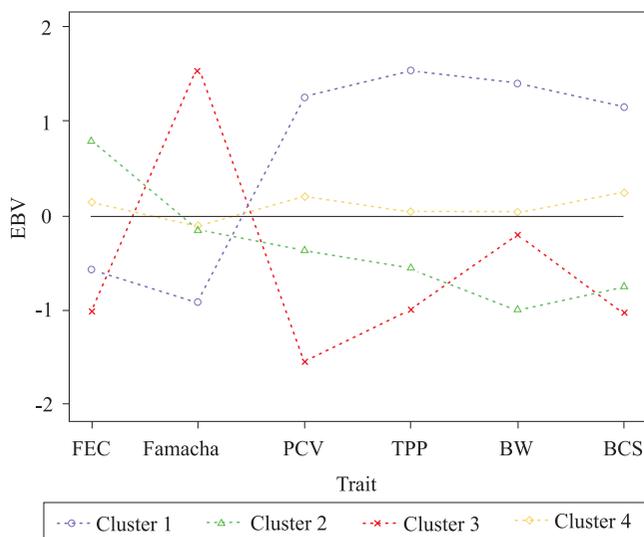


Figure 1. Standardized means of the breeding values (EBVs) for the traits used to divide Santa Inês sheep into four clusters by the k-means method. FEC, fecal egg counts; Famacha, Famacha score; PCV, packed cell volume; TPP, total plasma protein; BW, body weight; and BCS, body condition score.

and EBVs for FEC below the average, indicating susceptibility to endoparasites, since the additive genetic component favors the retention of more eggs, which are little eliminated with feces, favoring infestation by endoparasites and, consequently, anemia. The animals from cluster 4 presented breeding values close to the population mean, for all traits, showing no genetic differential in their additive genetic pattern to be used as candidates for selection.

Conclusions

1. Famacha and body condition scores can be recommended as selection criteria for resistance to hematophagous endoparasites in Santa Inês sheep.

2. The animals from cluster 1 show great potential as candidates for selection because of their additive genetic pattern, taking into account body weight and endoparasite resistance due to higher breeding values for body weight, body condition score, packed cell volume, and total plasma protein and to low breeding values for Famacha score and fecal egg counts.

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