University of São Paulo
"Luiz de Queiroz" College of Agriculture

Feed efficiency traits in Santa Inês sheep under genomic approaches

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Dissertation presented to obtain the degree of Master in Science. Area: Animal Science and Pastures

Piracicaba
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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **GERSON BARRETO MOURÃO**

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1. Associação genômica ampla 2. Consumo alimentar residual 3. Desequilíbrio de ligação 4. Modelos de regressão Bayesianos 5. Seleção genômica I. Título

DEDICATION

I dedicate my dissertation work to my family. A special feeling of gratitude to my loving parents, Luiz Cláudio Alvarenga and Andreia de Lima Botelho Alvarenga; my sister Larissa Botelho Alvarenga; my grandfather and grandmother, Milton e Rosária; and to my boyfriend, Guilherme Madureira.

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SUMMARY

RESUMO

Eficiência alimentar em ovinos da raça Santa Inês sob abordagem genômica

A seleção com base nos valores genéticos genômicos preditos pode aumentar substancialmente a taxa de ganho genético em animais por meio do aumento da acurácia de predição e redução do intervalo de gerações, especialmente para características de difícil e/ou onerosa mensuração, como eficiência alimentar. A eficiência alimentar é uma das características mais importantes na produção animal devido principalmente aos seus impactos econômicos e ambientais. Muitas métricas representam a eficiência alimentar, por exemplo: a relação do ganho de peso e consumo alimentar (**EA**), a proporção do consumo alimentar e ganho de peso (**CA**) e o consumo alimentar residual (**CAR**). Em ovinos, nenhum estudo com o objetivo de buscar variantes genéticas ou verificar a acurácia do valor genético genômico estimado para eficiência alimentar foi publicado. Adicionalmente, antes de aplicar a informação genômica, é necessário compreender e caracterizar a estrutura da população, como por meio do desequilíbrio de ligação (**LD**). O estudo de associação genômica (**GWAS**) e seleção genômica (**GS**) consideram o LD entre marcador e a mutação causal. Com base nas considerações acima, o objetivo deste estudo foi mapear o LD em ovinos, caracterizado pela raça ovina Santa Inês; localizar variantes genéticas para as características de eficiência alimentar (EA, CA e CAR) utilizando a abordagem GWAS; e verificar a acurácia da estimação dos valores genéticos genômico para o CAR. No total, foram coletadas 396 amostras (animais) do músculo *Longissimus dorsi*, para posterior genotipagem utilizando o painel de alta densidade (Illumina High-Density Ovine SNP BeadChip®), compreendendo 54.241 SNPs. O banco fenotípico é composto por 387 animais. O LD médio entre marcadores adjacentes para duas métricas de LD, r^2 e |D'|, foram 0,166 e 0,617, respectivamente. O grau de LD estimado foi menor que o relatado em outras espécies e foi caracterizado por blocos de haplótipos curtos. Consequentemente, para as análises genômicas são recomendados painéis de marcadores de alta densidade. No GWAS, foram encontrados muitos marcadores associados aos fenótipos, em especial, à característica CAR. Alguns genes candidatos foram relatados neste estudo, destacando-se o NRF-1 (fator respiratório nuclear 1), que controla a biossíntese mitocondrial, o processo mais importante responsável por grande parte da produção de energia. Finalmente, verificamos a acurácia do valor genético genômico estimado para o CAR usando modelos de regressão Bayesiana, e encontramos baixos valores para acurácia (0,033 a 0,036) o que pode ser explicado pelo baixo grau de relacionamento entre os indivíduos e tamanho reduzido da população de treinamento.

Palavras-chave: Associação genômica ampla; Consumo alimentar residual; Desequilíbrio de ligação; Modelos de regressão Bayesianos; Seleção genômica

ABSTRACT

Feed efficiency traits in Santa Inês sheep under genomic approach

The selection on genetic values predicted from markers could substantially increase the rate of genetic gain in animals by increasing accuracy of prediction and reducing generation interval, especially for difficult to measure traits, such as feed efficiency. Feed efficiency is the most important trait in animal production due to its impacts on cost of production and environmental factors. Many metrics measure the feed efficiency, such as ratio of gain to feed (**FER**), the ratio of feed to gain (**FCR**) and residual feed intake (**RFI**). Nevertheless, in ovine, no study with the aim of understand the genetic variants or the accuracy of genomic estimated breeding value (**GEBV**) for feed efficiency traits was published yet. Moreover, before to apply the genomic information, it is necessary to understand and characterized the population structure, for instance, by linkage disequilibrium (**LD**). Both genome-wide association studies (**GWAS**) and genomic selection (**GS**) leverage LD between marker and causal mutation. Based on the above considerations, the aim of this study was to map LD in ovine, characterized by Brazilian Santa Inês sheep; to search genetic variants for feed efficiency traits (FER, FCR and RFI) through GWAS; and to verify the accuracy of GEBV for RFI. In total, 396 samples (animals) of *Longissimus dorsi* muscle were collect. A high-density panel of SNP (Illumina High-Density Ovine SNP BeadChip®) comprising 54,241 SNPs was used to obtain the genotyping data. The phenotype data was comprised of 387 animals. The average LD between adjacent markers for two LD metrics, r^2 and |D'|, were 0.166 and 0.617, respectively. The degree of LD estimated was lower than reported in other species and it was characterized by short haplotype blocks. Consequently, for genomic analyses, high-density panels of marker are recommended. Many markers were associated to feed efficiency traits in GWAS, mainly to RFI trait. Few candidate genes were reported in this study, highlighting NRF-1 (nuclear respiratory factor 1), which controls mitochondrial biosynthesis, the most important process responsible by a great fraction of the produced energy. Finally, we verified the accuracy of GEBV for RFI using few Bayesian regression models, and we found low accuracy, ranging from 0.033 (BayesB with π=0.9912) to 0.036 (BayesA), which might be explained by the low relationship among animals and small training population.

Keywords: Bayesian regression models; Genome-wide association study; Genomic selection; Linkage disequilibrium; Ovine; Residual feed intake

1. INTRODUCTION

The ovine production is the promising activity of agriculture because the consumption of ovine meat had increased, especially in Brazil. Brazil is a promising producer due to many advantages, such as extension area to production, tropical weather, and important agriculture area for family farmers, due to ovine be small animals, consequently, easy management.

However, Brazil' ovine production does not supply the intern consumption and not highlight in world exportation and production, such as bovine (first position in exportation), chicken (first position in exportation) and pig (fourth position in exportation). The ovine population on the 2014 year comprised of 17.6 million of heads, a smaller population compared with Australia (72.6 million head) and New Zealand (29.8 million head), the two biggest global exporter countries [1]. Techniques to optimize the ovine production might be a workaround to turn the ovine production more attractive to farmers; consequently, afford to increase the sheep production and highlight it in the world trade.

An important trait that affects livestock production is the efficiency to convert the feed intake into the final product. More efficient animals reduce the cost of production because they require less feed (kilograms- **kg**) to yield a same amount of final product, for example, carcass (kg) [2]. Additionally, improving the animal's efficiency might reduce the methane liberation, allowing a more sustainable productive chain by the proper use of natural resources, and consequently, reducing environmental impacts [2].

Many measures can represent the animal efficiency, such as the ratio between the gain of weight and feed intake (feed efficiency- **FER**), the ratio between feed intake and weight gain (feed conversion ratio- **FCR**) and residual feed intake (**RFI**). The FER and FCR traits can result in overestimation of efficiency because they do not account the food needed for maintenance [3]. Moreover, FER and FCR can select more efficient animals concomitantly to heavier animals [4,5]. In animal production, there is a threshold for weight or weight gain that is economically advantageous; up to it might be an unsustainable economic chain. In this context, the RFI was proposed as a feed efficiency trait that is independent of body weight and weight gain [3]. RFI or net feed intake is the difference between observed feed consumption and expected feed consumption, being the expected feed consumption adjusted for average daily gain (**ADG**) and metabolic mid-point weight ($_{\textbf{metabolic}}$**MIDWT**). Consequently, the RFI is a residual, which in the statistical process is independent of predictors (ADG and $_{\text{metabolic}}$MIDWT).

All these efficiency traits had medium to high heritability [3–5], and therefore, it is possible to use animal breeding as a tool to accumulative improve animal's efficiency. However, the use of genetic selection for efficiency traits is limited due to cost and difficulty related to the measurement of such traits [6].

The use of genomic information can help in the genetic evaluation for these difficult to measure traits, as cited above, as well as improve the accuracy due to the incorporation of more information into the model, consequently, 8-38% extra genetic gain [7]. This kind of data allows inference about the genetic structure of the trait and of the population under selection, being applied in the genomic selection (**GS**).

GS is an approach which uses genome-wide markers simultaneously to predict breeding values [8]. During the selection process, alleles favorable to the traits of interest are selected directly or indirectly. In GS, we can select animals observing the favorable polymorphism directly, based on its effect previously estimated. Generally, a genotyping panel is used in GS. Because these commercial panels contain only a sample of the polymorphisms present in the genome, genomic analyses leverage on the linkage disequilibrium (**LD**) between the polymorphism present into the panel (**SNP** - single nucleotide polymorphism) and the causal mutation [9,10]. Therefore, GS works by selecting animals for hundreds of thousands of random markers simultaneously, assuming that the majority of the causal mutations is in LD with such markers [8].

LD is defined as a stochastic dependence between alleles at two loci [11]. Previously to any genomic study, it is important to map the LD of the breed of interest, because it can change due to population structure (selection, migration, recombination rate) [10]. Since LD can be an indicative of the segregation behavior of alleles, it enables to define the density of the genotyping panel necessary to achieve a certain accuracy of prediction and to determine the threshold time that the marker effects should be updated.

From the knowledge about population LD, it is possible to apply genomic information optimally. Before GS, is necessary to estimate genetic marker effects in a genome-wide association study (**GWAS**). GWAS also allows a genetic study about the process that involves the trait under study, its genetic control. This study allows identifying candidate genes related to the trait, through the verification of significantly markers associated with the phenotype. Moreover, GWAS is the first step for other studies that aim to find the causal mutation and genes that control the trait.

For performance the genomic approaches, we had chosen uses the Bayesian regression approach. The first motivation is the increase in accuracy using Bayesian methods.

Using best linear unbiased prediction model an accuracy was 0.73, although using Bayesian methods increased this accuracy to 0.85, even when the prior was not correct [8].

Therefore, despite the importance of the genomic approach as a tool to understand the genetic control of traits of economic interest and also to optimize their genetic evaluation and selection, we were not able to find studies that use genomic information in the animal breeding for feed efficiency in ovine populations. Therefore, the aims in this study were: to map the LD of Brazilian Santa Inês breed by using a genotyping panel of SNP; to develop the first study involving the comprehension of the genetic control of feed efficiency in an ovine population using GWAS approach; and finally, to verify the accuracy of GS for RFI in a small population, as happen to many other breeds or species. The RFI was chosen for GS analysis because it presented in this and other studies high accuracy and more advantages in comparison to the both traits cited. The breed chosen to represent the study was the Santa Inês breed because it is the most important ovine breed farming in Brazil. The Santa Inês is a tropically adapted breed, with desirable characteristics for meat production and reproductive efficiency. Moreover, this breed was formed by the non-systematic crossing of the Somali, Bergamasca, and Morada Nova breeds [12]; potentially allowing inferences based on these results on other breeds.

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2. LINKAGE DISEQUILIBRIUM IN *Ovis aries*, SANTA INÊS BREED

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ABSTRACT

Linkage disequilibrium (**LD**) is defined as a stochastic dependence between alleles at two loci. Quantitative genetics analysis such as genome-wide association studies or genomic selection (**GS**) leverages LD between the marker and the causal mutation. The objectives of this study were to evaluate the extent of LD in ovine, using the Santa Inês breed, and infer the minimum number of markers required to reach reasonable prediction accuracy in GS. In total, 38,168 SNPs and 395 samples were used for analysis. The overall mean LD between adjacent marker pairs measured by r^2 and |D'| were 0.166 and 0.617, respectively. LD values between adjacent marker pairs ranged from 0.135 to 0.194 for r^2 and from 0.568 to 0.650 for |D'| across all chromosomes. The average r^2 between all pairwise SNPs on each chromosome was 0.018. SNPs with distance between 0.10 to 0.20 Mb showed the average r^2 equal to 0.1033. The identified haplotype blocks consisted of two to twenty-one markers. In addition, the estimates of average inbreeding coefficient and effective population size were 0.04 and 96, respectively. We also found a tendency for relationships between the extent of linkage disequilibrium and both effective population size and inbreeding coefficient. The degree of LD estimated in this study was lower than that reported in other species and it was characterized by short haplotype blocks. Overall, our results suggest that the use of a higher density SNP panel is recommended for the implementation of GS in ovine.

Keywords: Effective population size; Haplotype blocks; Medium-density SNP panel; Inbreeding coefficient

2.1. Introduction

Genomic information is currently used in animal breeding programs to enable selection for difficult to measure traits as well as to increase the understanding of genetic and biological causes underlying phenotypic variation. Genomic selection (**GS**) is an approach which uses genome-wide markers simultaneously to predict breeding values [1]. This approach excels in increasing genetic gains when pedigree-based selection is suboptimal [1], which is the

case of traits with low heritability. For instance, GS based on simulated data showed an increase in reliability of breeding values for young animals when using genomic (greater than 60%) versus parent average (32%) information, equivalent to approximately 20 offspring [2]. Furthermore, genetic gain can be increased using genomic information by shortening the generation interval [1].

Alternatively, genetic markers scattered across the genome offers an opportunity to conduct genome-wide association studies (**GWAS**) to characterize genes underlying genetic variation for traits of interest.

The success of GS and GWAS are dependent of linkage disequilibrium (**LD**) or gametic disequilibrium between the markers and causal mutations [3] because generally the former is observed but not the latter. The LD between a marker and a causal mutation can be considered as the proportion of causal mutation variance that can be captured by the marker [4,5]. Through the knowledge of the degree of LD, it is possible to define the density of genetic markers necessary to achieve a certain accuracy of prediction and to determine when the estimates of genetic marker effects should be updated. It has been well documented that simply increasing marker density does not improve prediction accuracies. Although increased marker density improves resolution, it can also decrease power and add noise in the analyses by the use of non-informative SNP. Furthermore, increased marker density can dilute individual marker effects if, for example, two markers are flagging the same QTL and the two markers are in high LD with each other.

LD is defined as a non-random association between alleles at different loci [6], and it is commonly represented by |D'| and r^2 metrics [7]. The extent of LD can vary between and within species due to evolutionary history and population structure mainly characterized by insertions, deletions, chromosomal rearrangements or inversions [4]. Also, the marker and causal mutation association may change by consequence of other genetic factors such as recombination rate and selection[4]. Thus, LD between markers and causal mutations may decay over time requiring the re-estimation of marker effects.

The LD values have been reported for some domestic breeds and crossbreed and wild sheep by using microsatellites and SNP markers [4,8–14]. Nevertheless, there is a lack of studies regarding LD for *Ovis aries* using SNP panels. Moreover, the LD estimation between different breeds can be informative about the overall diversity level in a species and the selection level applied to them. Therefore, the aim of this research was to characterize LD structure in *Ovis aries*, considering its commercial importance in wool, meat, and milk production. The breed chosen to represent the study was the Brazilian Santa Inês breed. This

tropically adapted breed has desirable characteristics for meat production and reproductive efficiency. Moreover, this breed was formed by non-systematic crossing of the Somali, Bergamasca and Morada Nova breeds [15]; potentially allowing inferences based on these results on other breeds.

2.2. Methods

Animal resources, genotyping and quality control

The dataset included the genotypes of 396 animals from Santa Inês sheep breed collected between 2016 and 2017. These weaned animals were reared during 54 to 92 days on average, at four different periods with slightly different alimentary management. Animals had a mean weight (standard deviation) of 38.9 (5.571) kg. This herd is located at the Experimental Farm of São Gonçalo dos Campos, the city of São Gonçalo dos Campos, Bahia, Brazil, and it is associated with the Federal University of Bahia (**UFBA**).

To characterize the Santa Inês sheep population, the relationship between animals was estimated using a genomic relationship matrix **G** as described in VanRaden (2008) [2]. The **G** matrix was constructed by using the PREGSF90 software of the BLUPF90 package [16–18]. The average relationship between animals (standard deviation) was 0.0011 (0.06340), with minimum and maximum values equal to -0.1353 and 0.9341, respectively.

DNA was extracted from samples of the *Longissimus dorsi* muscle tissue collected from the left hemi-carcass and stored in 2.0 milliliter (**ml**) Eppendorf tubes. DNA extraction was performed according to protocols for lysis buffer and RNase. A high-density panel of SNP (Illumina High-Density Ovine SNP BeadChip®) containing 54,241 SNP was used for genotyping. Chromosomal coordinates for each SNP were obtained from the ovine genome sequence assembly, Oar_v3.1.

Quality control (**QC**) of the genomic data was performed by the GenABEL R package [19] for LD analyses (http://www.r-project.org/). The PREGSF90 interface of the BLUPF90 program [16–18] was used to edit the genomic data for F, N_e, MAF, and haplotype analyses. SNPs with a call rate lower than 0.90, minor allelic frequency (**MAF**) lower than 0.05 and p-value lower than 0.1 for the Hardy-Weinberg Equilibrium Chi-square test were excluded. One sample with a call rate lower than 0.9 was also removed. Table 2.1 summarizes the number of SNPs per chromosome before and after QC. We considered only the autosomal chromosomes (OAR1 to OAR26) in this study resulting in 38,168 SNPs.

Table 2.1 The number of SNPs per chromosome before and after quality control.

Chr	Nº SNPs$_i$	Nº SNPs$_f$
1	5931	4392
2	5475	4020
3	5009	3606
4	2681	1976
5	2364	1723
6	2593	1979
7	2253	1664
8	2058	1521
9	2142	1539
10	1739	1319
11	1181	860
12	1724	1245
13	1697	1214
14	1175	836
15	1695	1223
16	1581	1090
17	1421	1070
18	1414	1011
19	1249	887
20	1149	818
21	899	654
22	1098	758
23	1129	835
24	742	524
25	1002	731
26	925	673

Chr: chromosome; NºSNPs$_i$: SNP count before of QC for each chr; Nº SNPs$_f$: SNP count after QC for each chr.

Inbreeding coefficient and effective population size

Inbreeding coefficient (**F**) was calculated as a function of the expected and observed homozygote difference by using the PLINK software [20]. This is given by

$$F_i = \frac{(O_i - E_i)}{(L_i - E_i)} \text{ [2.1]}$$

Where O_i is the number of homozygous loci observed in the i^{ih} animal, E_i is the expected number of homozygous loci and L_i is the number of genotyped autosomal loci [20].

Effective population size ($\boldsymbol{N_e}$) was obtained by the SNeP software [21]. This software provides history of the effective population size, that is, the number of past generations based on the relationship between N_e, LD (r^2) and recombination rate (**c**) by using the following equation.

$$E[r^2] = (1 + 4N_e c)^{-1} \text{ [2.2]} \ ^{22}$$

Therefore, by solving equation [2.2], we have:

$$N_{e(t)} = \left(4f(c_t)\right)^{-1}(E[r^2|c_t]^{-1} - \alpha)$$ [2.3]

Where N_e is the effective population size at generation t, where t=$\left(4f(c_t)\right)^{-1}$ [23]; c_t is the recombination rate in generation t which is proportional to the physical distance between markers, r^2 is LD, and α is the adjustment for mutation rate, where $\alpha = \{1,2,2.2\}$ [24]. The α assumes 1 when $N_e c$ tends towards 0 (no selection or no mutation); when mutation does occur the α can take on values of 2 and 2.2. The value of 2.2 comes from the equilibrium expression value for $\frac{E[(\rho_{AB}-\rho_A\rho_B)^2]}{E[\rho_A(1-\rho_A)\rho_B(1-\rho_B)]}$ that was equal to $\frac{5}{11}$, [24]; following Ohta & Kimura (1971) [24]. Tenesa et al. (2007) proposed α = 2 [25]. The N_e per chromosome was the result of a harmonic mean due to a relatively small number of SNPs per chromosome.

Linkage disequilibrium analysis

The estimation of LD was performed by the function LD in the R package genetics [26] (http://www.r-project.org/). The estimation of LD was between neighboring pairs of SNPs (adjacent SNPs) and for each pairwise combination of SNPs (pairwise SNPs) on each chromosome. The |D'| is a scale of the frequency difference of the allele pairs (or nucleotide) AB, where A is the allele of the marker (or SNP) 1, and B the allele of the marker 2, and the expected frequency of each allele separately. This parameter ranges from -1 to 1 and it is given by:

$$D' = \frac{D}{Dmax}$$ [2.4]

And

$$D = \rho_{AB} - \rho_A\rho_B$$ [27] [2.5]

Where $\begin{cases} D > 0, D_{max} = \min(\rho_A\rho_b, \rho_a\rho_B) \\ D < 0, D_{max} = \max(-\rho_A\rho_B, -\rho_a\rho_b) \end{cases}$

Here ρ_A is the probability of allele A in marker 1, ρ_a is the probability of allele *a* at marker 1, ρ_B is the probability of allele B at marker 2, ρ_b is the probability of allele *b* at marker 2, and ρ_{AB} is a probability of the pair of AB markers. Maximum likelihood was used to estimate ρ_{AB} because genotype AB/ab is not distinguishable from genotype aB/Ab [28].

The squared correlation between the markers, given by r^2 [7], is expressed as:

$$r^2 = \frac{D^2}{(\rho_A\rho_a\rho_B\rho_b)}$$ [2.6]

Haplotype blocks

The haplotype blocks were identified by following the approach suggested by Gabriel et al. (2002) [29] which was implemented via PLINK [20]. Blocks are partitioned according to whether the upper and lower confidence limits on estimates of pairwise |D'| measure fall within certain threshold values estabilished by Gabriel et al. (2002) [29], 0.70. The desired SNP panel density was estimated by the ratio of the mega base pair of all ovine genome and distance between markers that composed the haplotype blocks [equation 2.7].

$$\widehat{n_e} = {}^{s_{genome}}/_{\overline{s_{haplotype}}} \quad [2.7]$$

Where $\widehat{n_e}$ is the estimated number of markers required; s_{genome} is the size (Mb) of ovine genome and $\overline{s_{haplotype}}$ is the average size (Mb) of haplotype blocks formed.

2.3. Results and Discussion

Descriptive statistics

After quality control (**QC**), 38,168 autosomal SNPs remained comprising approximately 53% of the entire panel. The analyzed SNPs spanned a total of 299.63 megabase (**Mb**) of the genome, with a mean (standard deviation) distance between adjacent SNP of 0.07 (0.075) Mb. SNPs were evenly distributed throughout the genome as the distances between adjacent markers ranged from 0.064 to 0.085 Mb. The chromosomes differ in size and SNP quantity, with chromosome 24 being the smallest in size - OAR24 (44.21 Mb) and chromosome 2 the largest - OAR2 (263.11 Mb). The number of SNPs per chromosome was proportional to the size of each chromosome. Descriptive statistics of the SNP and LD (r^2 and |D'|) for each chromosome are presented in Table 2.2.

Table 2.2 Descriptive analyses, MAF, F, N_e and average linkage disequilibrium (r^2 and |D|) between adjacent and all pairwise SNP pairs by chromosome.

Chr	Size (Mb)	Nº $SNPs_f$	Dist. (Mb)	MAF	F	N_e	r^2 pairwise SNP	r^2 adjacent SNP	|D'| pairwise SNP	|D'| adjacent SNP
1	243.8	4392	0.0676	0.2917	0.036(0.0373)	4530	0.010(0.0238)	0.172(0.2190)	0.176(0.1775)	0.625(0.3353)
2	263.1	4020	0.0655	0.2916	0.157(0.0381)	3196	0.011(0.0256)	0.192(0.2416)	0.177(0.1808)	0.639(0.3310)
3	242.5	3606	0.0673	0.2895	0.045(0.0640)	1491	0.011(0.0264)	0.183(0.2306)	0.181(0.1857)	0.650(0.3368)
4	127.0	1976	0.0643	0.2907	0.067(0.0569)	1276	0.016(0.0339)	0.181(0.2324)	0.215(0.2065)	0.639(0.3373)
5	115.9	1723	0.0673	0.2865	0.060(0.0660)	1303	0.015(0.0334)	0.169(0.2236)	0.215(0.212)	0.638(0.3376)
6	129.0	1979	0.0652	0.2862	0.062(0.0642)	1068	0.014(0.0301)	0.155(0.2047)	0.213(0.2072)	0.611(0.3319)
7	108.5	1664	0.0653	0.2934	0.059(0.0544)	1526	0.015(0.0314)	0.167(0.2192)	0.203(0.1984)	0.612(0.3363)
8	97.7	1521	0.0643	0.2920	0.051(0.0473)	1616	0.016(0.0334)	0.165(0.2220)	0.214(0.2062)	0.595(0.3429)
9	100.7	1539	0.0655	0.2879	0.050(0.0519)	1841	0.018(0.0371)	0.166(0.2214)	0.222(0.2094)	0.619(0.3340)
10	94.0	1319	0.0714	0.2872	0.045(0.0415)	3881	0.020(0.0427)	0.191(0.2507)	0.237(0.2203)	0.638(0.3340)
11	66.8	860	0.0778	0.2864	0.043(0.0357)	3409	0.017(0.0358)	0.152(0.2109)	0.230(0.2229)	0.614(0.3382)
12	86.0	1245	0.0692	0.2907	0.042(0.0388)	3742	0.017(0.0361)	0.157(0.2096)	0.221(0.2118)	0.622(0.3341)
13	88.8	1214	0.0733	0.2917	0.041(0.0382)	3707	0.017(0.0351)	0.169(0.2285)	0.213(0.2027)	0.603(0.3407)
14	68.6	836	0.0823	0.2868	0.039(0.0354)	3173	0.017(0.0362)	0.157(0.2090)	0.227(0.2187)	0.609(0.3373)
15	89.8	1223	0.0735	0.2932	0.040(0.0358)	3605	0.017(0.0363)	0.169(0.2246)	0.225(0.2187)	0.636(0.3366)
16	77.0	1090	0.0708	0.2668	0.045(0.0404)	3793	0.022(0.049)	0.194(0.2423)	0.256(0.2329)	0.650(0.3183)
17	78.4	1070	0.0734	0.2918	0.044(0.0409)	3431	0.018(0.0376)	0.155(0.2147)	0.226(0.2133)	0.602(0.3405)
18	71.8	1011	0.0711	0.2835	0.043(0.0410)	3532	0.018(0.0371)	0.160(0.2143)	0.232(0.2201)	0.622(0.3401)
19	64.7	887	0.0731	0.2904	0.042(0.0381)	3302	0.019(0.0384)	0.172(0.2211)	0.236(0.2216)	0.623(0.3284)
20	55.3	818	0.0678	0.2910	0.063(0.0631)	1386	0.022(0.0419)	0.148(0.1893)	0.251(0.2270)	0.620(0.3295)
21	55.0	654	0.0843	0.3001	0.074(0.0768)	1464	0.023(0.0233)	0.157(0.2142)	0.244(0.2223)	0.583(0.3384)
22	54.9	758	0.0725	0.2902	0.049(0.0423)	1638	0.0210.021)	0.173(0.2226)	0.245(0.2300)	0.641(0.3311)
23	66.2	835	0.0794	0.2878	0.049(0.0423)	1113	0.020(0.0203)	0.142(0.1963)	0.236(0.2142)	0.585(0.3329)
24	44.2	524	0.0845	0.2925	0.035(0.0364)	1439	0.020(0.0209)	0.135(0.1972)	0.240(0.2243)	0.568(0.3391)
25	48.0	731	0.0658	0.2890	0.072(0.0690)	1689	0.022(0.0225)	0.166(0.2191)	0.248(0.2233)	0.602(0.3323)
26	49.7	673	0.0740	0.2938	NA	1149	0.022(0.0224)	0.165(0.2138)	0.244(0.2258)	0.611(0.3333)

Chr: chromosome; Size (Mb): size of chr in mega pair base; Nº $SNPs_f$: SNP count after of QC for each chr; Dist. (Mb): mean intermarker adjacent distance; MAF: mean of minor allele frequency on each chr; F: inbreeding coefficient; N_e: effective population size; r^2 pairwise SNP: mean (standard deviation) r^2 estimated for each pairwise combination of SNPs on each chromosome; r^2 adjacent SNP.: mean r^2 between adjacent SNPs; |D'| pairwise: mean (standard deviation) |D'| estimated for each pairwise combination of SNPs on each chromosome; |D'| adjacent SNPs: mean |D'| between adjacent SNPs.

In addition, 35% of the SNPs (18,716) had MAF lower than 0.20, with a mean MAF over all SNPs of 0.35. According to another sheep study, 33% of the SNPs had MAF lower than 0.20 [30]. Extending our comparison to other species, the mean MAF was relatively higher than those found for *Bos taurus indicus*, with values ranging from 0.19 to 0.25 [31,32]. The MAF is important because LD, independent of the metric used, is a function of allelic frequency. Consequently, applying QC for allele frequencies can affect the distribution and extent of LD [6].

Inbreeding coefficient and effective population size

For a better understanding of the population described in this study, inbreeding coefficient (**F**) and effective population size ($\boldsymbol{N_e}$) were estimated for all chromosomes together and for each chromosome separately, using genomic information. The estimate of F was 0.04, a relatively low coefficient for a population that originated from the same commercial herd. Using pedigree information to estimate the inbreeding coefficient, Pedrosa et al. (2010) found values equal to 0.02 in Santa Inês breed[33]. Al-Mamun et al. (2015) found average inbreeding coefficients for Merino, Border Leicester and Poll Dorset breeds equal to -0.013, 0.09 and 0.02, respectively [13]. A recently published study in ovine found average inbreeding coefficients based on excess of homozygosity (SD) of -0.008 (0.031), ranging from -0.079 to 0.301 [12]. Negative inbreeding coefficients occur when the number of observed homozygous loci is lower than the expected, suggesting that the population is more heterogenous than the expected, perhaps due to the composite nature of the breed.

The N_e estimated herein was 96 in the current generation. Pedrosa et al. (2010) also estimated N_e using pedigree information and found a low value (76) [33]. This briefly difference in N_e can be due to number of animals used (395 vs. 17,097) and sources of relationship information (genomics vs. pedigree). Al-Mamun et al. (2015) found values of N_e ranging from 140 (Border Leicester breed) to 348 (Merino breed) [13]. Brito et al. (2017) [12] found values of N_e in the most current generations in multi-breed sheep populations ranging from 125 to 974. We hypothesize that the fewer number of breeds that composed the Santa Inês probably explain the difference of N_e among breeds. Moreover, the Santa Inês breed is mainly used for meat production in Brazil. In contrast, the Merino breed, for example, is used for much broader purposes in multiple countries.

The presence of artificial selection in the population under study was verified through the reduction of N_e over the generations. In this study, N_e ranged from 1,705 to 28,191 in 16th to 296th generations ago, respectively, before the current generation. Brito et al. (2017) [12] reported estimates of effective population size of 5,537 animals 1,000 generations ago to 687 in the most recent generation. We hypothesize that the large difference in N_e between the current and historic generations could be because the breeds that comprise the composite breed of Santa Inês were divergent historically and, thus, these estimates include multiple divergent breeds. The Santa Inês breed is relatively new, having only begun in the 1950s by non-systematic crossing of the Brazilian Somali, Bergamasca and Morada Nova

breeds [15]. This illustrates that the large estimates of historic N_e reflect time points before the formation of the breed, and even before the ovine domestication.

Linkage disequilibrium analysis between adjacent SNPs

The average (standard deviation) r^2 and |D'| values estimated between adjacent SNPs from the 26 autosomal chromosomes were 0.166 (0.2189) and 0.617 (0.3349), respectively. Al-Mamun et al. (2015) reported LD estimates from multiple domesticated sheep (*Ovis aries*) breeds including: Merino (**MER**), Border Leicester (**BL**), Poll Dorset (**PD**) and crossbred populations (i.e., F_1 crosses of Merino and Border Leicester (**MxB**) and MxB crossed to Poll Dorset (**MxBxP**)) [13]. The authors used the same genotyping panel but adopted a different data quality control (MAF $<$ 0.01) and reported a r^2 mean of 0.12 (MER), 0.20 (BL), 0.19 (PD), 0.13 (MxB) and 0.13 (MxBxP); and |D'| mean of 0.52 (MER), 0.72 (BL), 0.69 (PD), 0.54 (MxB) and 0.55 (MxBxP) [13]. A recent study published with multi-breed sheep reported mean (standard deviation) r^2 of 0.26 (0.100) [12]. The estimates of r^2 are relatively consistent across sheep populations, with the exception of larger r^2 values reported by Brito et al. (2017) [12].

Sheep populations have been associated with lower levels of LD in comparison to other ruminant and nonruminant species. Mean values between adjacent SNPs of 0.32 (r^2) and 0.69 (|D'|) were estimated for an Australian Holstein- Friesian cattle population by using 10,000 SNP (1,546 animals) [6]. The r^2 mean for pigs of Landrace (87 animals), Yorkshire (96 animals), Hampshire (78 animals) and Duroc (90 animals) breeds was 0.36, 0.39, 0.44 and 0.46, respectively, estimated from 60,000 SNPs [34].

The average LD (standard deviation) between adjacent SNP within the same chromosome ranged from 0.135 (0.1972) to 0.194 (0.2423) for r^2 and 0.568 (0.3391) to 0.650 (0.3368) for |D'| (Table 2.2). Chromosomes 6, 11, 12, 14, 17, 20, 21, 23 and 24 had lower average LD using r^2 lower than 0.16 as the threshold [32]. Considering r^2 metrics between adjacent SNPs, chromosomes 2, 10 and 16 presented higher level of LD compared to other chromosomes. The high level of LD present on OAR10 was similar to that observed by Al-Mamun et al. (2015) [13].

Linkage disequilibrium analysis among all pairwise SNPs

The average (standard deviation) for r^2 and |D'| estimated between all pairwise SNPs on the 26 autosomal chromosomes were 0.018 (0.032) and 0.225 (0.213), respectively. In a study which used microsatellite markers to evaluate LD in domestic sheep (*Ovis aries*), a

mean (standard deviation) value of 0.211 (0.004) for |D'| was found [10]. Al-Mamun et al. (2015) who also used domesticated sheep (*Ovis* aries), found mean r^2 between all pairwise SNP of 0.007 (MER), 0.013 (BL), 0.018 (PD), 0.009 (BxM) and 0.012 (BxMxP); and mean |D'| of 0.168 (MER), 0.29 (BL), 0.27 (PD), 0.18 (BxM) and 0.19 (BxMxP) [13]. Additionally, Miller et al. (2011) using non-domesticated sheep (*Ovis canadensis* and *Ovis dalli*) and the same genotyping panel but adopting a different QC (MAF $<$ 0.10), reported a mean r^2 (standard deviation) of 0.042 (0.067) [4]. Considering the confidence interval obtained for the estimates presented in this study as well as in the studies previously reported, it is possible to assume that estimates of r^2 and |D'| across all SNP combinations on a chromosome are relatively consistent across sheep populations.

Figures 2.1 and 2.2 illustrate r^2 and |D'|, respectively, as a function of the intermarker distance for chromosomes 1 and 24. Supplementary Figures 2.1 and 2.2 depict r^2 and |D'|, respectively, for the other chromosomes. Overall, the relationship between LD and intermarker distance suggest that as intermarker distance decreases, LD increases. A notable exception is chromosome 1. On this chromosome, r^2 presented secondary high peaks around the interval from 100 to 150 Mb (Figure 2.1). On all chromosomes, |D'| maximum was observed between many SNP pairs with high intermarker distances (Figure 2.2). We contend that this might occur due to the dependence of |D'| on allele frequency. The unexpected increase in LD between some SNP pairs with larger intermarker distances could also be explained by selection. It is possible that favorable alleles for different traits were selected, resulting in a high degree of LD on longer intermarker distance, even extending to inter chromosome pairs of SNP. Another potential reason for high r^2 values when intermarker distance was large is assembling errors, potentially explaining the phenomenon on chromosome 1.

The average (standard deviation) r^2 between all pairwise SNPs contained on the same chromosome with intermarker distance greater than or equal to 0.10 and lower than 0.20 Mb was 0.1033 (0.0807) across all chromosomes. Using LD categories defined by Espigolan et al. (2013), Table 2.3 shows the average intermarker distances between pairwise SNPs exhibiting low LD ($r^2 \leq 0.16$), medium LD ($0.16 < r^2 < 0.70$) and high LD ($r^2 \geq 0.70$) [32]. Higher levels of r^2 (greater than 0.70) were found at distances between markers smaller than 0.768 Mb with 3,296 combinations of SNPs (0.01 % of all combinations). For medium levels of r^2 (0.16 to 0.70), we observed distances lower than 5.277 Mb with 273,659 combinations of SNPs (0.849 %). Considering low levels of r^2 (lower than 0.16) distances found were higher than 15.110 Mb with 31,939,376 combinations of SNPs (99.140 %).

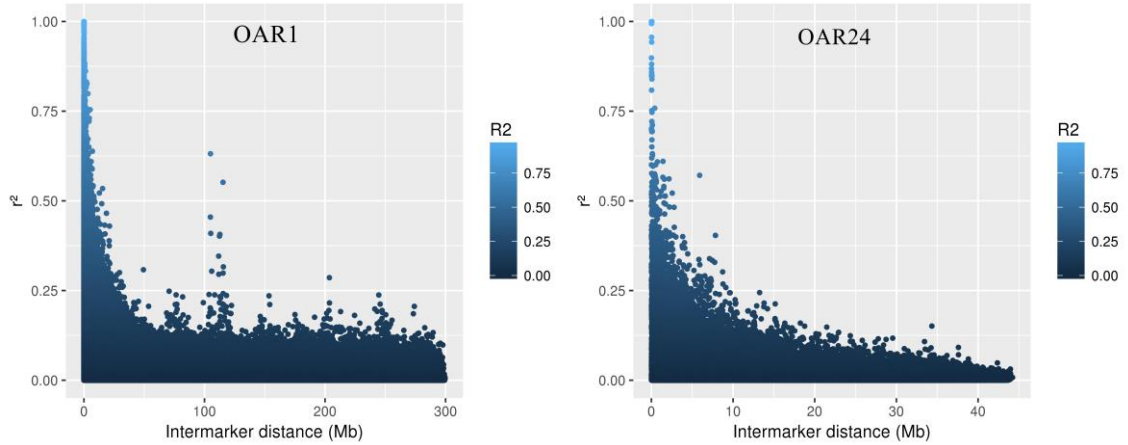

Figure 2.1 Linkage disequilibrium (LD) measured by r² plotted as a function of intermarker distance (Mb) for chromosomes 1 (OAR1) and 24 (OAR24).

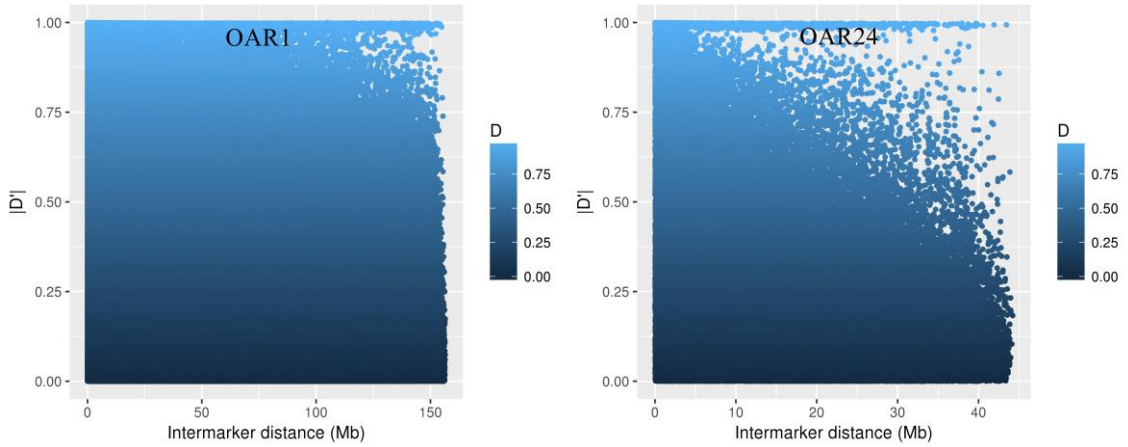

Figure 2.2 Linkage disequilibrium (LD) measured by |D'| plotted as a function of intermarker distance (Mb) for chromosomes 1 (OAR1) and 24 (OAR24).

Table *2*.3 Mean intermarker distance and frequency for each category of linkage disequilibrium (high, medium and low) according to r² metrics.

Chr	High			Medium			Low		
	Mean[1]	Dist[2]	Freq[3]	Mean	Dist	Freq	Mean	Dist	Freq
OAR1	0.847	0.243	0.004	0.240	4.798	0.434	0.009	100.697	99.563
OAR2	0.850	0.463	0.009	0.248	4.518	0.669	0.011	63.832	99.323
OAR3	0.849	0.389	0.013	0.247	3.929	1.010	0.013	41.975	98.976
OAR4	0.847	0.158	0.010	0.244	4.370	0.984	0.014	41.952	99.006
OAR5	0.846	0.146	0.012	0.245	4.001	0.917	0.013	39.375	99.071
OAR6	0.848	0.520	0.007	0.242	3.899	0.724	0.013	42.614	99.270
OAR7	0.860	0.128	0.009	0.241	3.347	0.797	0.013	36.970	99.194
OAR8	0.844	0.171	0.011	0.240	4.007	0.913	0.014	33.116	99.076
OAR9	0.848	0.299	0.013	0.248	4.062	1.172	0.015	34.267	98.815
OAR10	0.842	0.768	0.039	0.259	5.277	1.929	0.018	27.292	98.033
OAR11	0.837	0.264	0.018	0.246	2.573	1.047	0.014	22.343	98.935
OAR12	0.849	0.237	0.011	0.244	3.355	1.129	0.015	28.272	98.860
OAR13	0.855	0.147	0.014	0.242	3.893	1.023	0.014	30.061	98.964
OAR14	0.849	0.119	0.016	0.252	2.588	1.039	0.014	22.174	98.945
OAR15	0.843	0.280	0.017	0.247	3.400	1.094	0.014	29.842	98.889
OAR16	0.813	0.408	0.036	0.268	4.708	2.056	0.016	26.320	97.908
OAR17	0.862	0.142	0.014	0.243	3.605	1.241	0.015	25.775	98.745
OAR18	0.8510	0.204	0.015	0.248	3.041	1.174	0.015	24.634	98.811
OAR19	0.835	0.222	0.019	0.246	2.766	1.238	0.016	21.592	98.743
OAR20	0.826	0.432	0.012	0.244	3.518	1.696	0.018	18.814	98.292
OAR21	0.846	0.104	0.022	0.243	2.980	1.823	0.019	17.715	98.154
OAR22	0.850	0.191	0.027	0.251	3.052	1.575	0.017	18.690	98.398
OAR23	0.873	0.129	0.010	0.235	3.796	1.360	0.017	22.134	98.630
OAR24	0.863	0.054	0.016	0.242	2.281	1.352	0.017	15.110	98.632
OAR25	0.872	0.094	0.022	0.244	2.949	1.697	0.018	16.127	98.280
OAR26	0.834	0.168	0.019	0.252	2.530	1.855	0.017	16.903	98.126

Low LD ($LD \leq 0.16$), medium LD ($0.16 < LD < 0.70$) and high LD ($LD \geq 0.70$), for r². [1] Mean r² estimated to each pairwise combination of SNPs on each chromosome of interval. [2]Intermarker distance for respective category between two by two marker (low, medium or high) (mega pair base- Mb), and [3] Frequency of SNP number in each category, percentage (%).

Relationship between linkage disequilibrium, inbreeding coefficient and effective population size

Table 2.2 also shows the relationship of r², |D'|, MAF, F, and N_e. The mean MAF was similar across all chromosomes. The correlation between the two measures of LD was 0.75 when LD was estimated between adjacent SNP and 0.97 when estimated among all pairwise SNP. Although |D'| tends to overestimate LD values compared to r² as reported by Zhao, Fernando and Dekkers (2009) [35], both LD metrics exhibited the same behavior (Table 2.2). This is expected since these metrics are defined similarly as a function of allele frequency. The differences between the two metrics (r² and |D'|) are related to the weight applied to the allele frequencies. Given |D'| is entirely dependent on the frequency of the

alleles, |D'| possibly inflates LD estimates [35]. On the other hand, the r² proposed by Hill and Robertson [7] aims to reduce this frequency dependence.

According to Hill and Robertson [7], LD (numerator of r²) and F have a linear relationship as shown in the equation below. This expectation is due to selection, for example. In a population under selection, the number of homozygotes tends to increase for many favorable alleles. Consequently, the inbreeding coefficient and LD between these selected alleles increase.

$$E(D^2) = \frac{1}{15} p_0(1-p_0)q_0(1-q_0)[6(1-F) - 5(1-F)^3 - (1-F)^6]$$ [7] [2.7]

Where $D^2 = (\rho_{AB} - \rho_A\rho_B)^2$ is the numerator of r², p_0 and q_0 are the frequency of A and B alleles, respectively, in generation zero or with initial equilibrium. Positive relationship (0.22) was observed between the D² estimated by equation (1) as a function of inbreeding coefficients and the average D² observed between adjacent SNPs on each the chromosome. A possible justification for the low correlation could be the relatively limited number of SNP per chromosome on the panel used in the current study. The SNP contained on the panel used herein covers only 299.6 Mb out of a total of 2,615.52 Mb, equivalent to 11% of the sheep genome. However, a few negative values were observed (-0.08) when estimating the correlation between D² estimated by F (equation (1)) and average D² between all pairwise SNPs on the chromosome. Additionally, equation (1) [7] was derived under the assumption of finite and natural populations.

The expectation of D at time t can be derived from c and N_e [36]. This is given by:

$$E(D_t) = (1-c)\left(1 - \frac{1}{2N_e}\right) E(D_{t-1})$$ [36] [2.8]

Where c is the recombination rate and D_t is the estimate of D at generation t. It is expected a negative correlation between D, which is the numerator of |D'| and r²; and population effective size ($\boldsymbol{N_e}$). Considering N_e as an indicator of selection, lower N_e values are result of high selection pressure, and a consequently reduction in the number of breeding animals and genotyping variability. A negative relationship between average LD between all pairwise SNPs on a chromosome and N_e was observed (-0.16), as expected. However, the correlation between average LD between adjacent SNPs and N_e was positive (0.35). One potential reason for the observed discrepancy is the fact that N_e was estimated based on the LD between all pairwise SNPs rather than LD between adjacent SNPs.

Haplotype blocks

The construction of haplotypes with only two (frequency =1,879) to twenty-one (frequency = 1) markers was consistent with the low LD among pairwise SNP reported in this study. The mean size of haplotype blocks and the frequency of the number of SNPs for each chromosome are reported in Table 2.4. The average distance (standard deviation) between markers that formed the haplotype blocks was 0.04 (0.033) Mb. Considering the size of the sheep genome and the average distance between SNP that formed the haplotype blocks, it was possible to indirectly infer the minimum number of markers needed for genomic analyses, which was 61,415 SNPs. As discussed earlier when the number of SNPs in the genotyping panel is lower than the desired density, incorrect conclusions can be obtained due to the use of small number of markers for the estimation. The opposite can be also true: when a panel contains a large number of SNPs with no genetic signals, there might be many of those SNPs associated with phenotype because the effect of causal mutations will be divided among nearby SNPs. Thus, it will be more difficult to identify causal mutations associated with the phenotype. However, due to the high standard deviation of distance between markers that formed the haplotype, it is important to use this number with caution.

2.4. Conclusions

The extent of LD among adjacent markers for domestic sheep, represented in this study by the Santa Inês breed, resembled those of previously reported results in other breeds of domesticated sheep. The mean LD values between all SNP pairs on each chromosome were consistent with domestic and wild sheep (*Ovis canadensis* and *Ovis dalli*) and they were lower than the estimates reported in other species. Additionally, we found a tendency for the relationship between LD and F or N_e in our commercial population. The findings reported in this study will be useful to determinate the number of markers needed for GS and GWAS in *Ovis*.

Table 2.4 Summary of mean and standard deviation (SD) of intermarker distance in haplotype blocks for each chromosome and frequency of size haplotype blocks.

Chr	Mean blocks size in Mb (SD)	Number of markers on haplotype block										∑
		2	3	4	5	6	7	8	9	10	21	
OAR1	2.278 (0.8138)	235	9	17	6	2		1				270
OAR2	2.516 (1.2153)	220	9	22	18	1	3			2		275
OAR5	2.447 (1.0964)	178	8	15	10	3	1	2				217
OAR6	2.432 (0.8914)	93	5	14	6							118
OAR5	2.367 (0.9296)	91	5	7	3	3						109
OAR6	2.215 (0.6147)	93	7	5	2							107
OAR7	2.241 (0.8413)	97	3	4	3			1				108
OAR8	2.363 (0.9605)	77	4	4	3	3						91
OAR9	2.225 (0.87058)	100	4	5		1			1			111
OAR10	2.798 (2.3260)	72	5	5	8	1	1			1	1	94
OAR11	2.292 (0.7978)	41	2	4		1						48
OAR12	2.325 (0.7425)	66	3	10	1							80
OAR13	2.557 (1.0882)	47	1	7	5	1						61
OAR14	2.317 (0.7225)	33	4	3	1							41
OAR15	2.540 (0.9972)	47	3	8	5							63
OAR16	2.387 (0.9470)	52	1	5	3	1						62
OAR17	2.270 (0.7450)	54	4	2	3							63
OAR18	2.367 (0.9724)	42	1	2	3	1						49
OAR19	2.314 (0.9485)	45		4	1		1					51
OAR20	2.325 (0.7642)	33	2	4	1							40
OAR21	2.344 (0.8273)	26	3	1	2							32
OAR22	2.232 (0.6873)	49	3	2	2							56
OAR23	2.531 (0.9153)	23	2	6	1							32
OAR24	2.960 (1.6452)	16	1	5	2				1			25
OAR25	2.286 (1.0167)	32		1	1		1					35
OAR26	2.167 (0.7071)	17			1							18

Chr: chromosome; SD: standard deviation; ∑: sum of number of markers on haplotype block inside chromosome.

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3. GENOME-WIDE ASSOCIATION AND SYSTEMS GENETIC ANALYSES OF FEED EFFICIENCY TRAITS IN SHEEP

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ABSTRACT

The traits related to feed efficiency such as residual feed intake (**RFI**), feed efficiency ratio (**FER**) and feed conversion ratio (**FCR**) have economic and environmental impacts in livestock production. Therefore, the better understanding of the genetic mechanisms associated with these traits can helps to improve the animal production. The aim of this study was identify genetic variants across the whole genome of ovine specie associated with feed efficiency traits. This is the first genome-wide association study (**GWAS**) performed to feed efficiency traits in this species. In this study, we estimated the genomic heritability for RFI (0.75), FER (0.30), and FCR (0.26); performed a GWAS and identified putative candidate genes associated with the traits of interest. Several association analyses, many markers were classified as significant association with RFI and few for FER and FCR. Two genes that have two significant markers inside can be considered as a candidate: *NRF-1* (nuclear respiratory factor 1) and *MAP3K5* gene (Mitogen-Activated Protein Kinase 5). No significant marker was coincident between RFI and FER or RFI and FCR. One marker was significantly similar between FER and FCR, but the gene that this marker being inside is uncharacterized protein. Finally, the RFI trait is an interesting trait to select due to its definition and high heritability, consequently, due to many SNPs associated.

Keywords: Bayesian regression, Feed conversion ratio, Genetic correlation, GBLUP, Heritability's, Residual feed intake, Santa Inês breed, Candidate genes.

3.1 Introduction

Feed efficiency is an important trait in animal production due to economic and environmental factors, which is related to feed intake. In relation to the economic factors the quantity of food in animal production system represents more than half of the production costs [1]. Therefore, the optimization of the conversion of feed intake kilograms (**kg**) into kg of final products (such as growth in meat production, milk or wool) might reduce the costs.

However, in relation to environmental factors the methane gas from enteric fermentation in ruminant species is a world environmental concern because ruminant species contributes approximately with 12% of anthropogenic greenhouse gas emissions globally[2]. In general, animal methane production depends upon the quantity of feed consumed, feed digestibility and animals digestibility characteristics [3,4]. The feed efficiency traits, on the whole, take into account these variables that influence in methane animal production.

In cattle experiment was observed a positive association between methane production and feed efficiency trait (residual feed intake- **RFI**)[5]. A reduction of 1 kg/day in RFI was associated with a reduction of 13.38 grams/day of methane emission[5], consequently, improve the animal efficiency reduces the methane emission. Additionally, among two animals group [greatest efficiency (**G**) with low values of RFI and lower efficiency (**L**) with high values of RFI] was observed: 1. no difference in average daily gain (**ADG**); 2. reduction of 41% of dry matter (feed intake)/day ingest for G group; 3. reduction of 25% emitted methane daily for G group[5] .

Based on these two main factors that affect the economic traits in ruminant species, some different phenotypic measures were proposed to account the feed efficiency such as ratio of gain to feed (feed efficiency- **FER**), ratio of feed to gain (feed conversion ratio- **FCR**) and RFI, are essential to reduce production costs and environmental impacts associated with animal production systems. The FER and FCR traits can result in overestimation of feed efficiency by not consider the feed used to maintenance [6]. The RFI is adjusted by gain and weight, probably solving the FER and FCR problem [6]. The RFI is the difference between the actual feed intake and expected feed requirements for production (growth in meat production, for example), as well as maintenance of body weight [7].

The understanding of genetic mechanisms underlying traits could be important for identification of candidate genetic variances in genomic selection studies, candidate genes, and consequently, knowledge about the biological process that interferes into trait responses. All these findings could help to drive a new insights to optimize the animal production (animal breeding approach, animal management, and nutrition) [8].

Many species studied the genetic control for feed efficiency trait, in special for RFI. In pigs, significant quantitative trait loci (**QTL**) regions were find, such as on chromosome 1 (markers within *MAP3K5* and *PEX7* gene) and chromosome 13 (*DSCAM* gene), that might be interesting for RFI[9]. In ruminant species, many studies were developed, mainly in bovine. For Nelore breed, two markers were strongly associated with RFI, which were located near to genes that regulates appetite and ion transport [10].

In view of this question, the purpose of this study was to associate the genetic variants across the ovine genome with feed efficiency traits (FER, FCR and RFI) using animals from Brazilian Santa Inês herd, and estimate the genomic heritability and genetic correlation of the traits related to feed efficiency.

3.2 Methods

Estimation of residual feed intake, feed efficiency and feed conversion ratio

All the animals (387) used in this study were of Santa Inês breed from the Experimental Farm of São Gonçalo dos Campos, city of São Gonçalo dos Campos, Bahia, Brazil. The animals were intact males, vaccinated, and evermined and the phenotypic data was collected jointly with Federal University of Bahia (UFBA) during 2 years (2016 to 2017). The animals were received food and water *ad libitum*. These animals were maintained in individual stalls during 54 to 92 days, conducted at four different periods and with an average age (standard deviation) of 4 (2) mouths. The animals received diets ranging from 11 to 16% crude protein among contemporary groups, all of them formulated according to the NRC (2007). Contemporaries groups (n = 27, composed of 9 to 18 animals) were formed based on lots composition and period of confinement.

The feed intake (**FI**) was calculated as the difference between the food provided and leftovers, adjusting for dry matter intake (**DMI**). The measures of food provided and leftovers were done manually.

The weight body gain (**WBG**) was calculated as the deviation of final body weight/animal (**FBW**) from initial body weight (**IBW**), both with 16 hours of fasting. The observed mean and standard deviation for IBW and FBW were 23.5 and 4.543, and 38.9 and 7.050 kilogram (kg), respectively.

The values of DMI and WBG were divided the days in by confinement to obtain the parameters per days, which were used in efficiency metric estimation, resulting in standardized feed intake (**SFI**) and average daily gain (**ADG**).

The feed efficiency (**FER**) was estimated by a function of ADG and SFI [equation 3.1]. The feed efficiency metric, FER, infer by animal efficiency, where high values are equivalent an efficient animal, compared animals of low FER values.

$$FER = {ADG}/{SFI} \quad [3.1]$$

The feed conversion ratio (**FCR**) is inversely proportional to FER, being the function of SFI and ADG [equation 3.2]. Therefore, using this metric, efficiency animal has lower values than a non-efficiency animal.

$$FC = {}^{SFI}/_{ADG} \text{ [3.2]}$$

Finally, the residual feed intake (**RFI**), such as FER and FCR, is an efficiency indicator metric, using to estimated animal RFI value the body weight [equation 3.3]. Consequently, improvement in RFI could affect body size and growth rate [11,12].

$$RFI_{kg} = SFI(kg) - EFI(kg) \text{ [3.3]}^{6}$$

Where the EFI is an expected feed intake. To calculate the EFI, ADG and metabolic mid-point weight (***metabolic*MIDWT**) were used to model daily SFI [11,12]. A model was fitted using the GLM procedure of the SAS Institute, Inc. (1996). The equation resulting were [equation 3.4]:

$$EFI(kg) = -0.15187 + 0.97868 * ADG + 0.08286 * metabolicMIDWT \text{ [3.4]}$$

To use these phenotypes in genomic analysis, it was previously adjusted for fixed effect, contemporaneous group [equation 3.5].

$$y_i = \mu_j + v_{ij} + \varepsilon_i \text{ [3.5]}$$

Where y_i is a phenotype to animal *i*, μ is a phenotype mean to contemporaneous group *j*, v_i is a contemporaneous group *j* where animal *i* being, e ε_i is a residual for animal *i*. The phenotype adjust was estimated by the sum of phenotype and residual estimated from [equation 3.5]. Descriptive analysis for phenotype without fixed effects correct was described in Table 3.1.

Genetic correlation and heritability

The genomic DNA was extracted from *Longissimus dorsi* muscle samples from the left hemi-carcass collected into 2.0 milliliter (**ml**) Eppendorf tube and stored at -20 ºC. The DNA extraction procedure was performed according to protocols using lysis buffer and RNase. A high-density panel of SNP (Illumina High-Density Ovine SNP BeadChip®) comprising 54,241 SNPs was used to obtain the genotyping data. Chromosomal coordinates for each SNP were obtained by sheep genome sequence assembly, Oar_v3.1. The quality control (**QC**) of genotyping data was performed by the PREGSF90 interface of the BLUPF90 program [13–15]. SNPs and samples with a call rate lower than 0.90, MAF lower than 0.05 and p-value lower than 0.1 for Hardy-Weinberg Equilibrium Chi-square test were excluded.

Table 3.1 Mean, standard deviation, minimum and maximum values of the traits studied.

	IBW (kg)	FBW (kg)	SFI (kg)	ADG (kg)	FCR	FER	RFI (kg)
Mean	23.55	38.80	1.10	0.20	5.99	0.18	-0.005
(SD)	(4.543)	(7.050)	(0.233)	(0.059)	(1.884)	(0.050)	(0.150)
Min	12.00	16.60	0.40	0.05	2.66	0.06	-0.72
Max	37.80	62.20	1.76	0.35	16.53	0.33	0.52

SD: standard deviation; Min: minimum; Max: maximum; IBW: initial body weight; FBW: final body weight; SFI: standardized feed intake; ADG: average daily gain; FCR: feed conversion ratio; FER: feed efficiency; RFI: residual feed intake.

The genetic correlation among the feed efficiency traits and the variables that compose them were performed by AIRELMF90 interface into BLUPF90 program [13–15]. The genomic heritability for feed efficiency traits were obtained by GenSel software[16].

Association analyses

The association analysis was performed using a Bayesian regression model to estimate the marker effects. First of all, variance components were estimated using the genomic best linear unbiased prediction approach (**GBLUP**) to attributed as prior in Bayesian model. The GBLUP used additive infinitesimal model, and the relationship matrix based on genomic information, called genomic matrix [17,18]. These methods were chosen to estimate the variance component due it estimation is unbiased from prior information. The model to estimate the variance component was [equation 3.6]:

$$y_{adj} = Za + \varepsilon \text{ [3.6]}$$

Where y_{adj} is the phenotype adjust by [3.5], Z is an incidence matrix, a is vector of effects marker and ε is the residual effects. For this analysis was performed by the AIREMLF90 interface of the BLUPF90 program [13–15].

The bayesian regression model, BayesB [19], was used to estimate the marker effects. The model showed above [3.6] was used to BayesB modelling. In this methods, the prior information assumption identical and independent mixture distribution for marker effects, where each has a point mass at zero with probability π and a univariate-*t* distribution with probability 1- π having a null mean, scale parameter S_α^2 and *v* degrees of freedom [16]. In this methods, each marker has a specific variance, and the effect for *i* marker is written as [3.7]:

$$\alpha_i = \varphi_i \delta_i \text{ [3.7]}$$

Where φ_i has a normal distribution with null mean and locus-specific variance σ_i^2, which in turn has a scale inverse chi-square prior with scale parameter S_α^2 and v degrees of freedom. In this methods, the π is known and for this study were represent by 0.99. Thus, we assume that percentage part of the markers is associated with phenotype. The genome-wide association was performance by the GenSel software [16].

Finally, the marker was classified as significantly association with the phenotype based on bayes factor (**BF**) parameter. The BF was written as:

$$BF_i = \frac{\left(\frac{\rho_i}{1-\rho_i}\right)}{\left(\frac{1-\pi}{\pi}\right)} \text{ [3.8]}$$

Where ρ_i is a posterior frequency of a marker to be in the model for each *i* SNP. The classification for a marker as defined as when BF_i is higher than three, can be considered as suggestive linkage between marker and causal mutation. The BF range (20 to 150) indicates linkage between marker and causal mutation. Finally, BF higher 150 is a very strong evidence of linkage [20].

The finding of candidate genes was performance by ENSEMBL website platform (http://www.ensembl.org/). The variant consequence of significant SNPs by BF was found into variant effect predictor platform of ENSEMBL website (**VEP** - http://www.ensembl.org/info/docs/tools/vep/index.html). Finally, the candidates genes was characterized by gene ontology (GO) terms[21].

3.3 Results

Phenotyping and genotyping data

The average for FCR, FER and RFI were equal to 5.990, 0.180 and -0.005 kg, respectively. Remarkably, RFI presented average close to zero due to it is residual parameter consequently, will have a normal distribution with mean zero.

For association and complementary analyses remained 43,779 SNPs and 386 animals. Descriptive analysis for data quality control was: call rate lower than 0.90 excluded 3,463 SNPs; MAF lower than 0.05, 5,942 SNPs were excluded; and p-value lower than 0.1 for Hardy-Weinberg Equilibrium Chi-square test, 1,057 SNPs were excluded. One sample with call rate lower than 0.9 was also removed.

Genetic correlations and heritability

The genomic heritability for RFI, FER and FCR were 0.75, 0.26 and 0.30, respectively. The genomic heritability estimatives were analyzed for convergence test by Heidelberger & Welch, which all passed for this test. The genetic correlations among feed efficiency traits and feed intake, gain daily weight and life body weight are showed in off-diagonal Table 3.2.

Table 3.2 Genetic correlations of feed efficiency traits and variables that compose them.

Traits	RFI	FCR	FER	ADG	SFI
RFI					
FCR	-0.549				
FER	0.232	0.990			
ADG	-0.037	0.989	0.998		
SFI	0.021	0.997	0.996	-0.999	
$_{metabolic}$MIDWT	-0.493	0.999	0.998	0.972	0.999

RFI: Residual feed intake; FCR: Feed conversion ratio; FER: feed efficiency ratio; ADG: average daily gain; SFI: standardized feed intake; $_{metabolic}$MIDWT: metabolic mid-point weight.

Genome-wide association

After quality control, 43,779 SNPs and 386 animals remained in this study, which were used in association analysis. Four SNPs were strongly associate with the efficiency metrics (BF>20), and one 126 SNPs were classified as suggestive association (BF>3; Table 3.3) according to Bayes factor estimatives. The Figure 3.1 shows the representative bayes factor (BF) of each SNP per chromosome for the three feed efficiency traits: RFI, FER and FCR.

Comparing the efficiency traits, a higher number of significative SNP were observed for RFI. Moreover, no associated SNP to RFI was common with other traits. We initially expected this, because the RFI is independent of body weight and weight gain [11,12], consequently, its theory is to selected efficient animals without select animals with high weight and rate gain, different of FER and FCR.

Table 3.3 Number of SNP classify as suggestive (BF > 3) or strongly (BF > 20) association for residual feed intake, feed efficiency and feed conversion ratio.

Category	RFI	FER	FCR	Coincident SNPs		
				RFI:FER	RFI:FCR	FE:FCR
Suggestive	104	4	17	0	0	1
Strongly	4	0	0	0	0	0

RFI: residual feed intake; FER: feed efficiency ratio; FCR: feed conversion ratio.

Between FER and FCR was possibly verify one commonly SNP. The SNP OAR11_8626515.1, located on OAR11, and it being inside of ENSOARG00000011009 gene with uncharacterized protein. Our initially hypothesis is that at least one SNP will be similar between this traits, because they are a function of the same variable, being only inversely proportional.

The Table 3.4 presents the SNPs that are located inside of gene probably associated with the phenotype (direct or indirect), chromosome, position, SNP effect and bayes factor. Other SNPs were presented in Supplementary tables 3.1.

A total of 80 SNPs (55 %), which were significant, located into intergenic variant; 35 SNPs (31 %) located into intron variant; 9 SNPs (6 %) located into non-coding transcript variant; 6 SNPs (4 %) located into downstream gene variant; 3 SNPs (2 %) located into upstream gene variant; and 1 SNP (1 %) located into splice region variant (Figure 3.2).

3.4 Discussion

The understood of genetic mechanisms underlying traits could be important for identification of candidate genetic variances in genomic selection studies, candidate genes, and consequently, knowledge about the biological process that interferes into trait responses. All these findings could help to drive new insights to optimize the animal production (animal breeding approach, animal management, and nutrition) [8].

Genetic correlations and heritability

The RFI heritability estimated in this study was relatively higher than other. In lambs without genomic information was found the heritability for RFI equal to 0.26[22]. Verifying the heritability without genomic information in another ruminant specie, bovine, the heritability was 0.16 [23]. In pigs, also without genomic information, was found RFI heritabilities values

ranging from 0.34 to 0.40. For FER heritability, the value was similar with the estimation without genomic information in lamb (0.26) [22], in bovine (0.26) [23] and in pig (0.30- 0.32) [24].

It is understood that the genomic heritability might overestimated compared with heritability based on pedigree information. However, the FER heritability was similar to other studies. Moreover, Bolormaa et al. (2013) founded heritability for RFI using genomic information equal to 0.36 [25], established by the overestimation of RFI heritability.

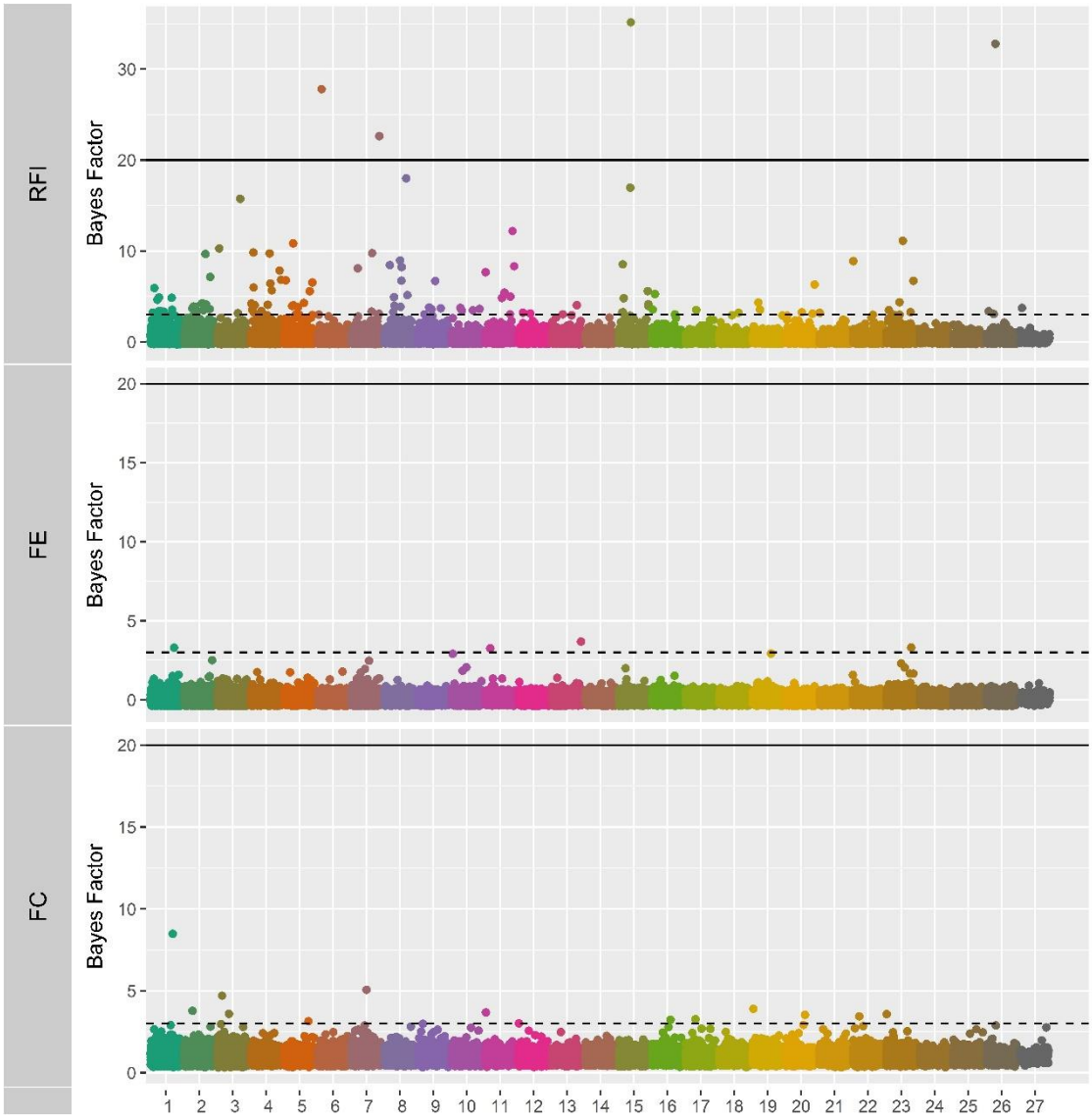

Figure 3.1 Manhattan plot of bayes factor of each SNP per chromosome for three traits of animal efficiency: residual feed intake (RFI), feed efficiency ratio (FER) and feed conversion ratio (FCR).

Table 3.4 SNP associated, respectively gene, chromosome, position, SNP effect and bayes factor.

Trait	SNP	Ensemble (gene)	Gene	Chr:Position	Effect	$\%\sigma_g^2$	Allele	BF
RFI	rs417277672	ENSOARG00000020653	MCF2L2	1:202056238	0.00146	0.0002	[T/G]	4.79534
RFI	rs416195886	ENSOARG00000007440	GRIN3A	2:21252248	-0.0014	0.00018	[A/G]	4.27561
RFI	rs425114550	ENSOARG00000004714	NRF1	4:93569119	-0.0039	0.00042	[T/C]	9.92287
RFI	rs424957575	ENSOARG00000000875	TPM4	5:6669927	0.00109	0.00013	[A/G]	3.0829
RFI	rs409680528	ENSOARG00000000337	MAP3K5	8:61642640	0.00283	0.00035	[A/G]	8.21248
RFI	rs411939214	ENSOARG00000001541	PEX3	8:67841404	-0.002	0.0002	[T/C]	4.53483
RFI	rs420010466	ENSOARG00000009854	EXT1	9:58992455	-0.0012	0.00015	[T/C]	3.27273
RFI	rs413906063	ENSOARG00000011562	YBX2	11:26623188	-0.0027	0.00032	[T/G]	7.47451
RFI	rs414722940	ENSOARG00000015595	BPTF	11:48432323	-0.0015	0.0002	[A/G]	4.57815
RFI	rs398294146	ENSOARG00000004714	NUMA1	15:49881907	0.01061	0.00123	[T/G]	35.4013
FC	rs405741086	ENSOARG00000015353	FAM160B1	22:34265670	-0.0047	0.0005	[T/C]	3.38908

Effect: additive effect of marker into phenotype; $\%\sigma_g^2$ additive genetic variance explained by marker; Allele: nucleotide base variation for marker; BF: bayes factor.

In lamb, the genetic correlation using only pedigree information between FER and FI was high, 0.79, and RFI with FI was 0.77[22]. The genetic correlation between FER and RFI was low (0.23)[22]. In bovine, the correlation between RFI and FER, SFI, ADG, and $_{metabolic}$MIDWT were 0.70, 0.64, 0.09 and 0.93, respectively[23]. Our correlations contrasting with other studies, observing underestimation of our values.

Genome-wide association

Despite of differences in the estimation of efficient animals, the results did not show similar genetic architecture between the traits (number of associated SNPs). Probably, the RFI presents a large number of associated SNPs due to higher heritability (0.75) than other two traits studied herein. Significant SNPs associated with RFI were similarly distributed on the genome, which on chromosomes 14, 18, 21, 24 and 25 did not appear any SNP. The chromosomes with a higher density of association and similarly SNPs between traits were on OAR1 to OAR5, OAR7, OAR11:OAR13, OAR15:OAR17, OAR20, OAR 22, OAR23 and OAR27.

As an introduction of biological mechanisms that might control RFI variation, Richardson and Herd (2004) [26] estimated many physiological mechanisms that contribute, such as protein turnover, tissue metabolism and stress, which was responsible by 37%; activity (10%); digestibility (10%); heat increment of fermentation (9%); body composition (5%); and feeding patterns (2%).

On chromosome one, OAR1, eleven associated SNPs were present, which those three being inside or behind a known gene. The SNP rs417277672 were found at 202.06 mega base pairs (**Mb**) positions (intron variant), with effect and BF equal to 0.0015 and 4.80, respectively. The *MCF2L2* (MCF.2 cell line derived transforming sequence-like 2) gene, located at 201.87 - 202.21 Mb region, might be an interesting candidate gene. This gene was positively correlated with insulin secretion and it was more expressed in the nondiabetic/normoglycemic person[27]. The normal insulin secretion wished for the efficiency use of glucose, mainly responsible by all activities of body. We were not able to find any study correlating feed efficiency and insulin secretion. However, the study with aim to understand the relationship between the insulin concentration and RFI, found a positive genetic relationship among both [26]. Moreover, it was observed that the insulin concentration tended to be higher in the higher-RFI animals [26]. The higher insulin concentration was related mainly with high feed intake and high feeding times, which have negative correlation with RFI [26].

The SNP rs416195886 located on chromosome 2 at 21.25 Mb position (intron variant), with effect and BF equal to -0.0014 and 4.28. This marker being inside of gene *GRIN3A* [glutamate ionotropic receptor N-methyl-D-aspartate (NMDA) type subunit 3A], were comprising the 21.25 - 21.46 Mb position. The NMDA receptor effects on neuronal activity. The *NRF-2* and *NRF-1* mediated this activity, as well as, the energy metabolism activity. The transcription levels of *GRIN3A* increases when the *NRF-2* decreased. On the other hand, the levels of *GRIN3A* decreases significantly on the situation with over-expression of *NRF-2* [28].

Additionally, the SNP rs425114550 (OAR4 at 93.56 MB) appear such as significant on our analyses, being inside of *NRF-1* (nuclear respiratory factor 1) gene delimiting of 93.54 to 93.62 Mb. This gene is an transcription factor, which participate in mitochondrial processes of biosynthesis, more specifically on electron transportation chain [29].

The mitochondria is responsible for production of more than 90% energy used by cells, as well as, the energy dissipated (lost energy) [29], which represents 25% of total basal metabolic rate of animal [30,31]. More efficiency animals (negative RFI) presents lower rates of electron lost than less efficiency animals (positive RFI), and this electron was dissipated on electron transportation chain [32]. Consequently, the higher levels of electron lost, will provide an environment with heat release, more reactive oxygen and less ATP, resulting in more required of feeding [33].

On chromosome 5, the SNP rs424957575, located at 6.67 Mb, being beside from *TPM4* gene (Tropomyosin 4- 6.65-6.67 Mb) of 205-distance basis. On animals group of high-feed efficiency were characterized such as downregulated genes related to major histocompatibility complex cell recognition, stress-related heat shock protein, and most important for the region measure here (OAR5 at 6.67 Mb), several genes associated with muscle fibers or cytoskeletal architecture (e.g. tropomyosin) [34]. The specific allele copy of the SNP nearly from *TPM4* results a negative impact on phenotype.

Another important SNP such as rs425114550 (associated to *NRF-1*) is the rs409680528, located on chromosome 8 at 61.64 Mb position. This SNP being inside of *MAP3K5* gene (Mitogen-Activated Protein Kinase 5) 61.57-61.80 Mb. Studies based on *MAP3K5* related this gene to residual feed intake using molecular cloning, characterization, tissue expression pattern, and copy number variations were did, and found useful information regarding the influence of *MAP3K5* on RFI in pigs [35]. The relative copy number of *MAP3K5* was higher in low RFI Duroc pigs than in high [35].

Moreover, in pig, this gene was cited on genome-wide association study for RFI, and concluded that this gene can be an interesting candidate [36]. The effect of *MAP3K5* (or in

generally, *MAPK*) on controlling feed efficiency traits may be mediated by variety of pathways such as hormones and growth factors that act through receptor tyrosine kinases, cytokine receptors to vasoactive peptides acting through G protein-coupled and seven-transmembrane receptors [36]. Among these pathways, was observed a positive correlation between insulin growth factor 1 (IGF1) (growth factor) concentration and RFI [26].

On OAR9, the candidate gene is *EXT1* (exostosin 1 – from 58.994 to 59.42 Mb) because the rs420010466 SNP beside it (58.992 Mb). This gene was found in a quantitative trait loci (**QTL**) region to carcass weight (**CWT**) and eye muscle area in bovine (**EMA**). In this examples, , this gene was more highly expressed in muscle samples from animals with increasing CWT and EMA [37].

On OAR11, two SNPs were associated with two candidate genes relating to reproductive traits. The first is the rs413906063 SNP located in 26.62 Mb with effect and BF equal to -0.0027 and 7.47, respectively. This SNP is located inside of *YBX2* gene (Y-box binding protein 2; from 26.62 to 26.62 Mb). This gene is an upstream regulator of protamine expression, implicated in male infertility [38]. Supporting this idea, a recent study shows that the percentage of azoospermia in humans change as the YBX2 genotype change [39]. Besides, in the same chromosome, the gene BPTF (Bromodomain PHD finger transcription factor; from 48.38 to 48.47 Mb) is responsible to regulate and signaling pathways of embryos and embryonic stem cells in early mouse [40]. In general, the reproduction and feed efficiency to deposition of meat or milk has negative correlation (Reference). It held due the energy directly to reproduction, needing more consumption to attend this physiology and the production.

On OAR5, the rs398294146 SNP was classified as strong association with phenotype. This SNP presented effect and BF equal to 0.011 and 35.40, respectively, and located in 49.88 where being inside of *NUMA1* gene (nuclear mitotic apparatus protein 1; from 49.85 to 49.88 Mb). This gene was found in a group of differentially methylated gene in small intestine of piglet were directly connected with ubiquitin C to form a network concerned with cancer, cell to cell signaling and interaction and cellular growth and proliferation [41]. In addition, this gene is thought to be associated with the formation of villi of newborn, and villi plays an important role in absorption of nutrients by expanding the surface of small intestine [41]. The absorption able of nutrients could be related with digestibility able, which are negatively correlated with RFI (-0.44) [37].

On OAR22 the rs405741086 SNP (34.27 Mb) being inside of *FAM160B1* gene (family with sequence similarity 160 member B1; from 34.26 to 34.29 Mb). The *FAM160B1*

was downregulated in fat-fed protein kinase C-deficient in mice [42]. On the other hand, this gene was associated with non-obstructive azoospermia (NOA), whereas patients with NOA the expression of *FAM160B1* is lower than patients without NOA [43].

In conclusion, many SNPs inside candidate genes were briefly presented in this study. The main pathways that they involves were mitochondrial biosynthesis, insulin secretion, growth factor, digestibility and reproduction. Moreover, no SNP coincident was observed between RFI and FER or FCR, as except. We initially hypothesis was found few or many SNPs coincident between FER and FCR, however, we found only one.

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4. GENOMIC SELECTION FOR RESIDUAL FEED INTAKE USING A SMALL OVINE POPULATION

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ABSTRACT

The selection on genetic values predicted from markers could substantially increase the rate of genetic gain in animals and plants, especially if combined with reproductive techniques to shorten the generation interval. However, the accuracy of genomic selection (**GS**) might vary depending on several factors, such as the heritability of trait, linkage disequilibrium in the population, size of the training population and relationship between animals in the training and target populations. Consequently, it is important to evaluate the prediction accuracy of genomic estimated breeding value (**GEBV**) to ensure desired response to selection and determine the potential cost/benefit of implementing GS. In this study, we proposed to evaluate the accuracy of GEBV in ovine, characterized by Santa Inês breed, for one of the most economically relevant traits in animal production, feed efficiency represented by residual feed intake (**RFI**). This is the first research in ovine using genomic information for efficiency traits. We used a genotyping data comprising 54,241 single nucleotide polymorphism panel (**SNP**) for 387 animals (Illumina High-Density Ovine SNP BeadChip®). We chose two Bayesian regression models to estimate marker effects (BayesA and BayesB). For BayesB models we compared three different π values (0.99 and 0.9912). Accuracies were characterized by Pearson' correlation between the GEBV and adjusted phenotype, and it was estimated by two-fold cross-validation. Of the other models compared accuracies for GEBV were similar with averages being 0.036 (BayesA), 0.034 (BayesB, π=0.99) and 0.033 (BayesB, π=0.9912). Negative accuracies were estimated in five training-validation groups for all methods. We contend that the poor prediction accuracies were observed due to: low average linkage disequilibrium between adjacent markers in ovine (r^2=0.16), low or null average relationship between animals (0.0011), and the small training population used (193 animals).

Keywords: BayesA, BayesB, Bayesian model regression, Cross-validation, Feed efficiency, Genomic selection, Genomic relationship, Linkage disequilibrium, Ovine.

4.1 Introduction

The ability of animals to convert the feed consumed into final products impacts the costs of production due to improved feed utilization, and also have an environmental impact because of reduced methane emission and decreased resources (e.g., land) needed for production [1,2]. Many traits measure the efficiency of animals in converting feed into products, such as the ratio between weight gain and feed intake, the ratio between feed intake and weight gain and residual feed intake (**RFI**). The ratio between weight gain and feed intake and the ratio between feed intake and weight gain can result in over estimation of efficiency because they do not consider the feed required for maintenance [3]. Koch et al. (1963)[3] proposed RFI, which is feed intake adjusted for differences in feed consumption for average daily weight gain (**ADG**) and metabolic mid-point weight (***metabolic*MIDWT**). RFI is the difference between the feed consumption and the expected feed consumption.

Techniques that maximize feed efficiency traits are important. Changes of feed efficiency through genetic selection is possible due to the medium to high heritability associated with these traits[3]. However, the use of genetic selection for feed efficiency traits is limited because they are expensive and difficult to measure, mainly in extensive production systems. Genomic information can solve this problem because it permits the selection of animals based on the entire genome through an approach called genomic selection (**GS**).

GS is the selection of individuals based on the genome-wide information of hundreds or thousands of markers, such as single nucleotide polymorphisms (**SNP**). In bovine and swine for example, many studies were developed and positive results were obtained. For *Bos taurus* and *Bos indicus* breeds, the average accuracy found (standard deviation) for the genomic estimated breeding value (**GEBV**) across seven breeds was 0.36 (0.14)[4] with a moderate to high accuracy for RFI.

However, no study involving genomic selection for feed efficiency traits in ovine has been previously published. Since the present study is a pioneer research with genomic selection in *Ovis* aries for feed efficiency, the number of animals used is still small. The use of small genotyped and phenotyped population configures a challenge in getting high accuracies, not only in this study, but also for other that have limited access of data. The ovine production is a promising livestock, mainly in Brazil, due to increase consumption of meat sheep during years. Besides, the Brazil does not supply the meat sheep demand within country and the country does not have participation on exportation of meat, such as bovine, pig and chicken. Techniques that may maximize the meat production by herd is interesting to country and world economy, as well as to environmental impacts. Therefore, the aim of this study was to evaluate the accuracy of GEBV for RFI in an ovine population, characterized by Brazilian

Santa Inês breed. A resistant breed, adapting to different conditions, as well as nice reproductive traits performance, characterizes the Santa Inês sheep.

4.2 Methods

Phenotype estimation and population structure

All phenotypic data used in this study are from 387 Santa Inês sheep. All animals were uncastrated, vaccinated, and dewormed, and recorded at the Experimental Farm of São Gonçalo dos Campos, city of São Gonçalo dos Campos, Bahia, Brazil, which is associated with the Federal University of Bahia (**UFBA**), during a period of two years (2016 to 2017). Sheep were given feed and water *ad libitum*. These animals were maintained in individual stalls during 54 to 92 days, conducted at four different periods and with a mean age (standard deviation) of 120 (60) days. The animals received diets ranging from 11 to 16% crude protein among contemporary groups, all of them formulated according to the NRC (2007). Contemporary groups (n = 27, composed of 9 to 18 animals) were formed by animals from the same herd commercial and period of confinement.

Feed intake (**FI**) was measured manually as the difference between the feed provided and leftovers. Feed intake was converted to dry matter intake (**DMI**). The body weight gain (**BWG**) was measured as the deviation of final body weight (**FBW**) from the initial body weight (**IBW**), both with 16 hours of fasting. The mean (standard deviation) for IBW and FBW of 23.5 (4.54) and 38.9 (5.05) kilograms (**kg**), respectively.

Both variables, DMI and WBG, were divided by days in confinement to obtain the parameters per day, which were used to estimate efficiency metrics, resulting in standardized feed intake (**SFI**) and average daily gain (**ADG**).

Residual feed intake (**RFI**) is an efficiency indicator metric, which accounts for body weight in its estimation [equation 4.1]. Consequently, improvement could be made in RFI without affecting body size and growth rate [5,6].

$$RFI_{kg} = SFI(kg) - EFI(kg) \text{ [4.1]}$$ [3]

Where the EFI is an expected feed intake. To calculate the EFI, ADG and metabolic mid-point weight (*metabolic***MIDWT**- equation 4.2) were used to model daily SFI [5,6]. A model was fitted using the GLM procedure of the SAS Institute, Inc. (1996). The resulting equations were [equation 4.3]:

$$_{metabolic}\text{MIDWT} = \left(^{(IBW + FBW)}/_{2}\right)^{0.75} \text{ [4.2]}$$

$$EFI(kg) = -0.15187 + 0.97868 * ADG + 0.08286 * metabolicMIDWT \text{ [4.3]}$$

The phenotypes used in genomic analysis were previously adjusted for the fixed effect of contemporary group [equation 4.4].

$$y_{ij} = \mu_j + v_{ij} + \varepsilon_{ij} \text{ [4.4]}$$

Where y_i is the phenotype of animal *i* of contemporaneous group *j*; μ is the phenotype mean in contemporary group *j;* v_{ij} is the contemporaneous group *j* assigned to animal *i;* and ε_{ij} is the residual effect for animal *i*, ~ N(0,σ_e^2). The adjusted phenotype was estimated by the sum of the actual phenotype and the residual estimated from [equation 4.4].

The genomic relationship between animals was estimated by the G[7] matrix constructed by using the PREGSF90 software of the BLUPF90 package [8–10]. Inbreeding coefficient (F) was also calculated as a function of the expected and observed homozygote difference by using PLINK software [11]. This is given by:

$$F_i = \frac{(O_i - E_i)}{(L_i - E_i)} \text{ [4.5]}$$

Where O_i is the number of homozygous loci observed in the i^{th} animal, E_i is the expected number of homozygous loci and L_i is the number of genotyped autosomal loci [11].

Genotyping and quality control

DNA was extracted from samples of the *Longissimus dorsi* muscle tissue collected from the left hemi-carcass and stored in 2.0 milliliter (**ml**) Eppendorf tubes. DNA extraction was performed according to protocols using lysis buffer and RNase. A high-density panel of SNP (Illumina High-Density Ovine SNPs BeadChip®) comprising 54,241 SNP was used for genotyping. Chromosomal coordinates for each SNP were obtained by ovine genome sequence assembly, Oar_v3.1. Quality control (**QC**) of the genomic data was performed by using the PREGSF90 interface of the BLUPF90 program [8–10]. SNPs with a call rate lower than 0.90 (n= 3,463), minor allele frequency (**MAF**) lower than 0.05 (n=5,942) and p-value lower than 0.1 for the Hardy-Weinberg Equilibrium Chi-square test (n=1,057) were excluded, remaining 43,779 SNPs. One sample with call rate lower than 0.9 was also removed.

Association analysis

The association analysis used a Bayesian regression model to estimate the marker effects. First of all, variance components was estimated using the genomic best linear unbiased prediction approach (**GBLUP**), which were attributed as prior values in the Bayesian model. The GBLUP used additive infinitesimal model, and the relationship matrix

based on genomic information, called genomic relationship matrix [12,13]. These methods were chosen to estimate the variance components because its estimation is more independent from the prior information. The model used to estimate the variance components was [equation 4.6]:

$$y_{adj} = Za + e \text{ [4.6]}$$

Where y_{adj} is the phenotype adjust by [4.4] (n x 1); Z is an incidence matrix of random effects additive genetic; a is the vector of genetic additive effects ~ $N(0, G\sigma_a^2)$; where G is a genomic relationship matrix and σ_a^2 additive genetic variance; and ε is vector of the residual effects, with normal distribution $e|\sigma_e^2 \sim N(e|0, I\sigma_e^2)$. This analysis was performed by the AIREMLF90 interface of the BLUPF90 program [8–10].

The Bayesian regression model was used to estimate the marker effects. The Bayesian method is characterized by a combination of data (likelihood) and additional information (prior). We used two methods initially proposed by Meuwissen et al. (2001)[14] that assume different marker effect variance. In the other words, the BayesA and BayesB have a similar feature characterized by a marker specific variance..

BayesA method uses the prior knowledge that many SNPs have small individual effects on the trait and only a few SNPs have moderate to large effect, consequently, all markers have an effect on phenotype (π equal to zero) [14]. The marker genetic variance follows a scaled inverted chi-square prior distribution, with *v* number of degree of freedom and *S* variance that is a scale parameter.

The BayesB, in turn, assumes that many loci have null genetic variance (${\sigma^2}_{gi} = 0$, with π probability) and a few have genetic variance ($\sigma_{gi}^2 > 0$, with 1- π probability), that is, assumes π as known and specified arbitrarily. When the genetic variance is different from zero, the prior distribution assumes the same of BayesA, a scaled inverted chi-square prior distribution, with *v* number of degree of freedom and *S* variance that is a scale parameter.

For BayesB models, we assumed two different values of π: 0.99 and 0.9912 that was estimated by $[1 - \frac{n_{animals}}{n_{markers}}]$, where $n_{animals}$ is the total number of animals and $n_{markers}$ is the total number of markers available for analysis.

For this analyses were used 41,000 iterations, where initially 1,000 iterations were discarded (burnin), and among 40,000 iterations, was get iteration by 10 to 10 (thin). Both Bayesian regression models were fitted by the GenSel software [15].

Estimation of accuracy using genomic selection

Models were initially selected based on convergence of marker genetic variance, residual variance, and heritability for models with fixed π based on Heidelberger & Welch convergence test[16]. After this initial filter of models, the genomic estimated breeding value (**GEBV**) was predicted for each animal by considering the model [equation 4.7].

$$GEBV = \sum_i^n W_i \hat{g}_i \text{ [4.7]}$$

Where *n* is the number of markers, $W_i$is the matrix with the genotypes (coding as 0, 1 and 2) for each marker *i* from each animal; $\hat{g}_i$ is the marker effect obtained by equation 4.6. The estimation of marker effects was made using training population that consisted of half of the genotyped and phenotyped animals. The remaining animals were used to evaluate the accuracy of the predictions (validation group). Evaluation based on the estimation of genomic breeding value using only genotype information and marker effects from animal contained in the training population.

For accuracy estimation, we used the repeated subsampling validation process, where the data was randomly divided in training and validation groups (20 replicates). The accuracy was considered the correlation of GEBV [equation 4.7] and the phenotype adjusted for the fixed effects [equation 4.4].

$$\overline{r_{GEBV}} = \sum_1^n \left(r_{GEBVs,Fen} / n \right) \text{ [4.8]}$$

Where $\overline{r_{GEBV}}$ is average accuracy of GEBV and $r_{GEBVs,Fen}$ is the Pearson correlation between the GEBV and the adjusted phenotype, and *n* is number of validation group (20 groups).

4.3 Results and discussion

Genomic Predictions

The models used to predict the GEBV for this small population presented low average accuracy, ranging from 0.033 (BayesB with π=0.9912) to 0.036 (BayesA) (Table 4.2). We observed that the accuracy do not have large differences across methods.

The Pearson's correlation among markers effects obtained by methods BayesA and BayesB with π=0.99 and π=0.9912 were equal to 0.569 (Figure 4.1a) and 0.587 (Figure 4.1b), respectively. Pearson's correlation between markers effects for BayesB method with different π (0.99 and 0.9912) was equal to 0.707 (Figure 4.1c). Additionally, Meuwissen et al. (2001) also observed similar accuracies among BayesA ($r_{x,y}$= 0.798) and BayesB ($r_{x,y}$= 0.848) methods[14].

Table 4.2 Average (standard error) accuracy ($r_{x,y}$), minimum and maximum.

Methods	BayesA	Bayes (π=0.99)	Bayes (π=0.9912)
Mean (SE)	0.036(0.0132)	0.034 (0.0132)	0.033 (0.0132)
Min	-0.094	-0.084	-0.075
Max	0.138	0.135	0.125
SE: standard error; Min: minimum; Max: maximum			

Encompassing for other Bayesian regression models, Habier et al. (2011)[17] found a similarity between GEBV accuracy for BayesA, BayesB, BayesCπ and BayesDπ for milk yield, fat yield, protein yield and somatic cell score. In plants, Resende et al. (2012)[18] also observed similar prediction ability of genomic selection for many traits among three methods (bayesian LASSO, BayesA and BayesCπ).

In the face of similarity among models used, enabling concluded that relationship between the number of SNPs per equation (iteration) and the number of animals are not necessary, in our case. Consequently, the model is able to estimate the marker effects with a number of marker per equation/iteration much higher than animals' number.

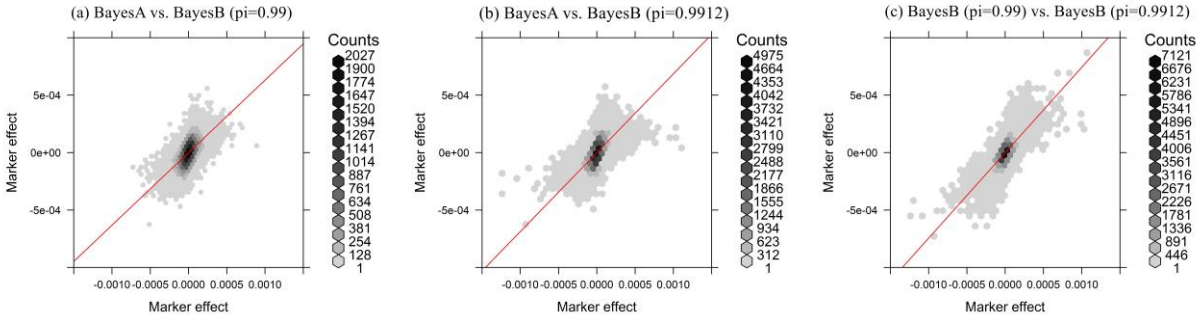

Figure 4.1 Correlation between the marker effects estimated by different models (pi is the π).

Negative accuracies values were found in five groups of validation. These negative values were observed in the same validation groups and for all methods (Table 4.3). One of the potential reasons for this is the genetic relationship between the animals in training

population and validation group. A high relationship between both groups is favorable for accuracy of prediction. However, we did not observe this behavior, founding average correlation among the accuracy and relationship between animals from validation and training group equal to -0.148.

As a part of quest for justification of negative and low accuracies, Hayes et al. (2009)[19] cited parameters that the accuracy of GEBV is dependent, such as level of linkage disequilibrium (**LD**) between the markers and the QTL; the number of animals with phenotypes and genotypes in the training population; and, additionally, the relationship between animals.

Accuracies of GEBV equal to 0.85 were achieved in the level of LD between adjacent markers greater than 0.2 (allelic frequency correlation, $r^2 \geq 0.2$)[14]. In this study using Santa Inês breed, and in other *Ovis aries* breeds, a low LD with average r^2 equal to 0.166 was found. Thus, an additional reason for the low average accuracy observed is the low average LD.

The third potential reason is the size of the training population that might influence the accuracy. Probably, the small size of the training group does not allow proper makers effects estimation. The definition of records number is depended on trait heritability; greater heritability, fewer records are required [19]. In this study, high genomic heritability for RFI was observed (0.75). For traits with heritability near 0.7, the number of phenotypic records required to achieve a desired accuracy of GEBV was approximately 2,000 [20]. Therefore, it is likely that the number of animals was not sufficient.

Table 4.3 Correlation between genomic estimated breeding value and adjusted phenotype by group training-validation between methods.

$G_{t\text{-}v}$	BayesA	BayesB (π=0.99)	BayesB (π=0.9912)	$r_{x,y}$
1	0.038	0.017	0.002	0.0018
2	0.061	0.075	0.084	-0.0020
3	0.040	0.043	0.028	0.0004
4	0.064	0.077	0.069	-0.0062
5	0.128	0.123	0.125	0.0025
6	0.087	0.076	0.078	-0.0011
7	0.071	0.060	0.058	-0.0012
8	-0.094	-0.084	-0.075	0.0033
9	-0.016	0.012	0.000	-0.0029
10	-0.052	-0.060	-0.060	-0.0012
11	-0.029	-0.029	-0.013	-0.0063
12	0.054	0.064	0.076	-0.0040
13	-0.002	0.012	0.016	-0.0012
14	0.065	0.048	0.045	0.0020
15	0.138	0.135	0.109	-0.0040
16	0.012	-0.005	0.011	0.0021
17	0.039	0.038	0.039	-0.0025
18	0.023	0.007	0.007	-0.0035
19	0.052	0.046	0.032	-0.0017

$G_{t\text{-}v}$: group training-validation; $r_{x,y}$: average of relationship of animals

The final hypothesis for negative and low average accuracies was the average of the relationship between all animals that was low, close to zero. The average genetic relationship between animals (standard deviation) was 0.0011 (0.06340), with minimum and maximum values equal to -0.1353 and 0.9341, respectively. This finding is also presented in Figure 4.2 as a heatmap of genetic relationship among animals. Hayes et al. (2009) [21] verified the GEBV accuracy using different training and validation groups. For instance, they used two dairy cattle breeds data (Jersey and Holstein). The marker effects were estimated by the BayesA with Holstein breed training data and were found accuracies of GEBV for milk production equal to 0.59 and 0.37 for Holstein and Jersey group-validation, respectively [21]. Using Jersey breed data training were observed accuracies for milk production equal to 0.59 and -0.02 for Jersey and Holstein group-validation, respectively [21].

Based on the results and computing time required for the analyses, the better model was the BayesA as it presented lower average (1,013 seconds) than BayesB with π=0.99 (2,232 seconds) and π=0.9912 (2,268 seconds).

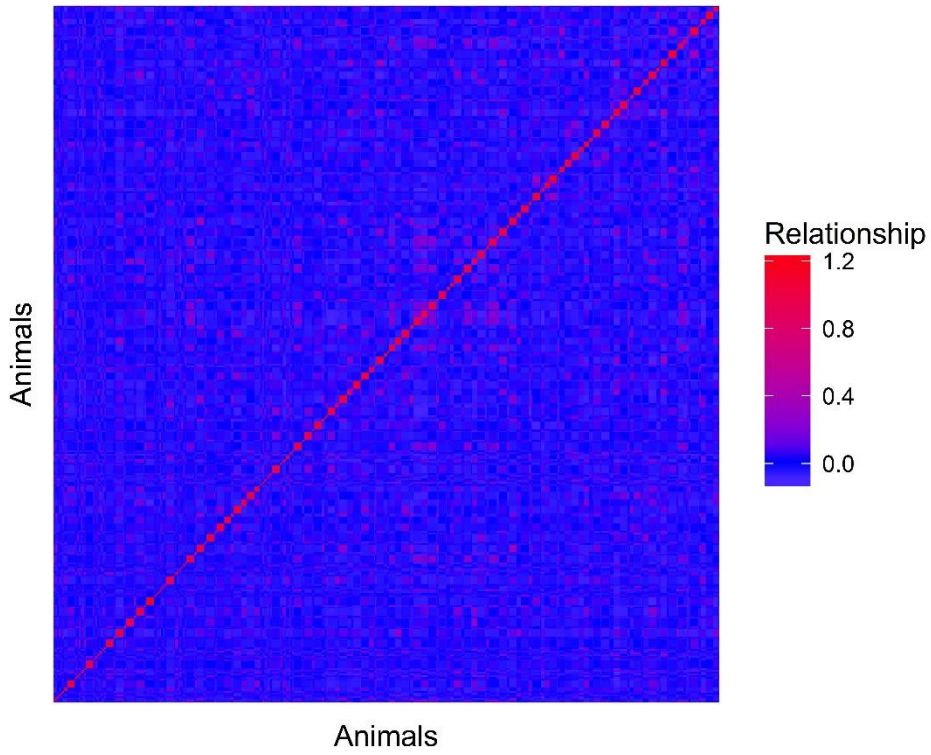

Figure 4.2 Heatmap of genetic relationship between animals.

4.4 Conclusion

The limiting factor for obtaining medium/high accuracy in GS was the low relationship between animals and the size of training population. Consequently, for the marker effects estimation is necessary to systematically training population chosen with a significant number of animals and minimum of the relationship between animals, or develop methods that optimize both challenges.

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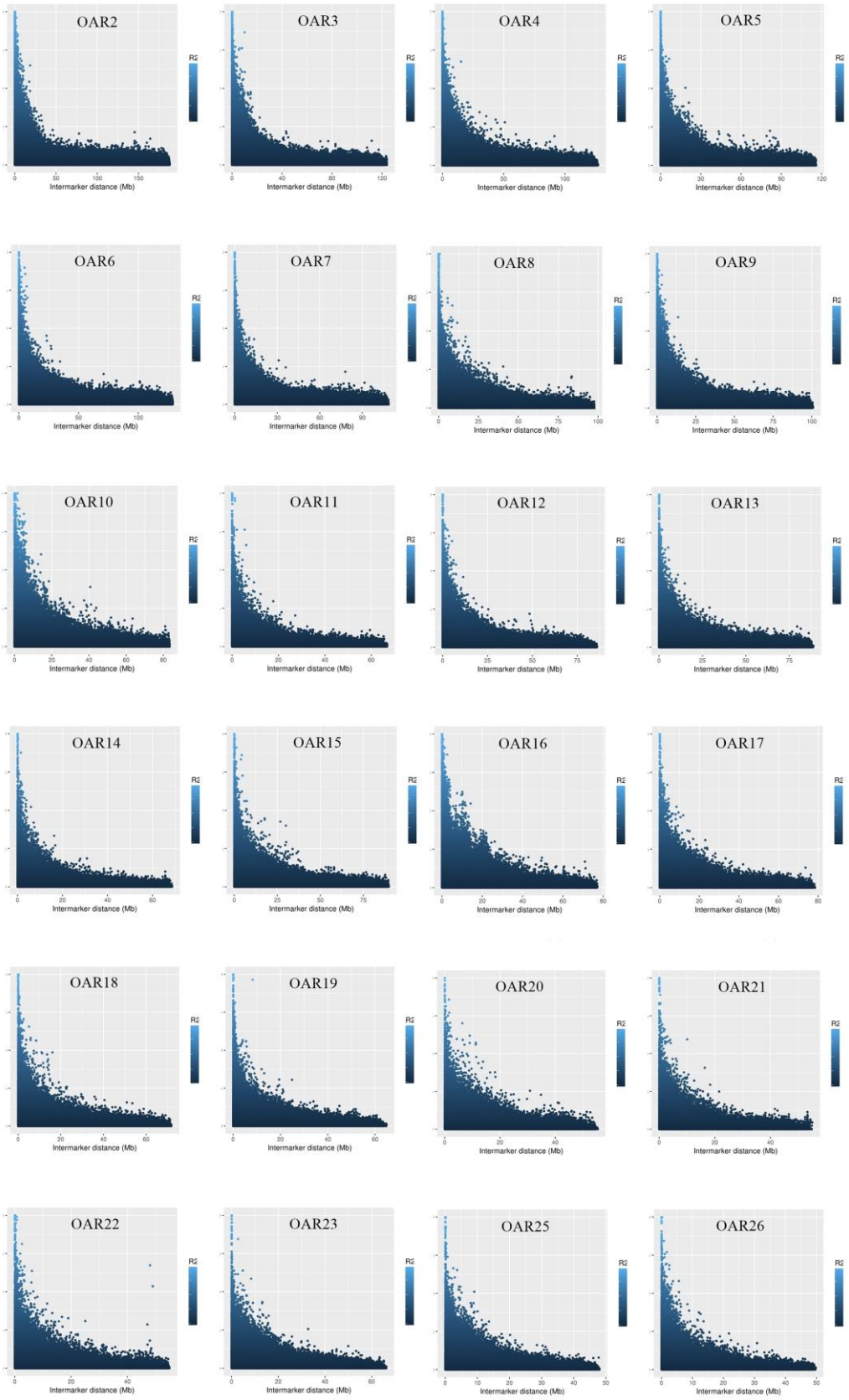
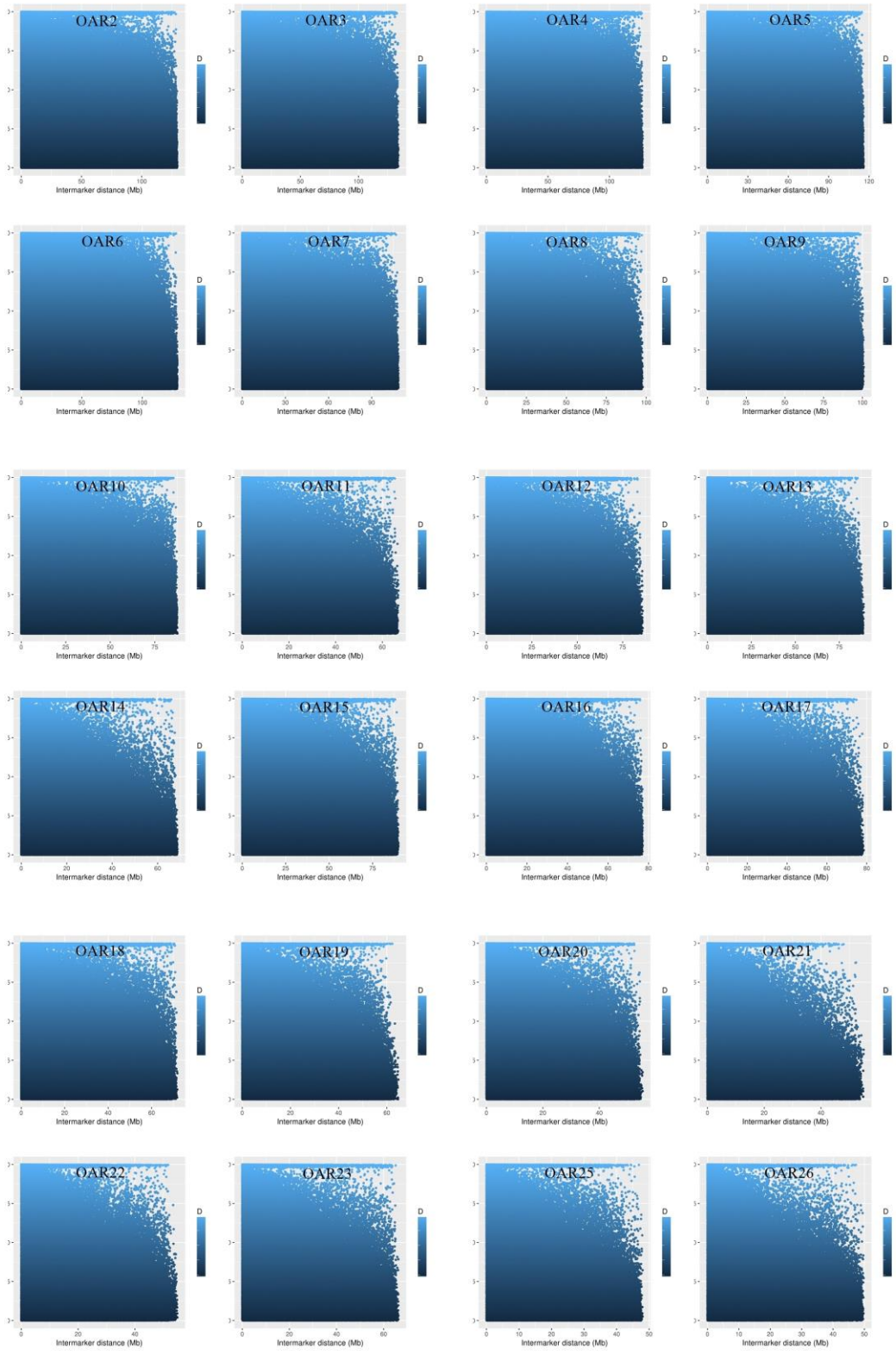
5. FINAL CONSIDERATION

The development and enhancement of reproductive and molecular technologies have allowed the acceleration of genetic progress through the genetic evaluation of young individuals to a certain accuracy, even in characteristics of low heritability, of difficult and onerous measurement or delayed measurement in the life of the animal. This study represents an initial step for genomic-quantitative studies and genomic selection for feed efficiency traits in domestic sheep. First, we study the genome structural by mapping of linkage disequilibrium, concluding that is necessary high density of markers panel for genomic approaches application in Santa Inês sheep. For the residual feed intake trait, we observed high genomic heritability, suggesting greater opportunity and results for animal breeding than other feed efficiency traits. For the comprehension of genomic selection used, it is still necessary to systematically collect phenotypic and genotypic information to include a larger number of animals in the training group, and with at least of animal's relationship for a possible improvement in accuracy; and finally, the analysis of costs and revenues associated with the characteristics of interest using genomic information.

SUPLEMENTARY FIGURES



Supplementary figure 2.1 LD measured by r^2 plotted as a function of intermarker distance (Mb) for chromosomes.



Supplementary figure 2.2 LD measured by D' plotted as a function of intermarker distance (Mb) for chromosomes.

SUPLEMENTARY TABLES

Supplementary File 3.1 SNP associated, respectively gene, chromosome, position, SNP effect and bayes factor.

Trait	SNP_novo	Ensemble (Gene)	Gene name	Position	Effect	% VarGen	alelo	BP
RFI	rs414735594	ENSOARG00000026794	uncaracterized protein	26:28702214	-0.01147	0.00118947	[A/G]	32.7189995
RFI	rs410141696			6:85018309	0.01558	0.00132226	[T/C]	27.7443349
RFI	rs430321012			7:98174186	0.01146	0.00099079	[T/C]	22.2047013
RFI	rs402761680	ENSOARG00000015069	MTFR2	8:61268758	-0.008097	0.00076862	[T/C]	17.9383416
RFI	rs411518548	ENSOARG00000003036	uncaracterized protein	15:49746734	0.00572	0.00065487	[A/G]	16.6947528
RFI	rs411784503			3:198973527	-0.005693	0.00061347	[T/C]	15.331909
RFI	rs428751386	ENSOARG00000015133	CEP95	11:48125112	-0.004115	0.00051909	[A/G]	12.3736078
RFI	rs408272283			23:38230104	-0.004408	0.00046255	[A/G]	10.9023091
RFI	rs409559449			5:310744	-0.003667	0.00044356	[T/C]	10.9023091
RFI	rs413247589			3:203798865	0.003736	0.00044369	[T/C]	10.5375083
RFI	rs408365913	ENSOARG00000012755	GDA	2:63837976	-0.004148	0.00042388	[A/G]	9.87495876
RFI	rs419463225	ENSOARG00000003379	EFCAB11	7:98396585	-0.005159	0.00047014	[T/C]	9.75535538
RFI	s56827.1			4:114893675	-0.003772	0.00042481	[A/G]	9.45749343
RFI	rs412816959			8:7457271	-0.002997	0.00037407	[A/G]	8.97251609
RFI	rs413057852			22:32429371	0.002953	0.00036108	[T/C]	8.51520417
RFI	rs417128974	ENSOARG00000006995	FCHSD2	15:50781754	-0.003329	0.000363	[T/C]	8.49185668
RFI	rs408441367			11:48556687	-0.002807	0.00035263	[T/C]	8.24731882
RFI	rs406251433			8:90234353	-0.00416	0.00040341	[T/C]	8.05017301
RFI	rs398957969			4:92688260	0.002717	0.00033697	[A/G]	7.85375067
RFI	rs419579453	ENSOARG00000014668	ZFYVE16	5:77888494	0.002772	0.00031847	[T/C]	7.17760618
RFI	OAR8_76092068.1			8:76092068	0.002335	0.0003207	[T/C]	7.0865838
RFI	rs411082902			2:76748382	-0.003911	0.00035523	[A/G]	6.99571734
RFI	rs414518818			23:22393678	0.002409	0.00029264	[T/C]	6.98436998
RFI	s23148.1			9:15350447	-0.002208	0.00030361	[T/C]	6.65635005
RFI	rs405076950			4:89310177	-0.002179	0.00027676	[A/C]	6.4088586
RFI	OAR20_46803189.1			20:46803189	-0.003276	0.00031889	[A/G]	6.17369595
RFI	rs415988359			4:9108090	0.003658	0.000323117	[A/G]	6.17369595
RFI	OAR5_79829554.1			5:79829554	-0.002146	0.000244831	[T/C]	6.0955414
RFI	rs400802261	ENSOARG00000005292	CPA5	4:94145887	-0.002033	0.000256574	[T/C]	6.00636402
RFI	rs401602228			5:17836037	-0.002204	0.00026885	[A/C]	5.76190476
RFI	rs403066959			11:51650294	-0.001809	0.000255154	[A/G]	5.69543147
RFI	OAR1_153662211.1			1:153662211	-0.002517	0.000257036	[A/G]	5.61798584

Trait	SNP_novo	Ensemble (Gene)	Gene name	Position	Effect	% VarGen	alelo	BP
RFI	rs400411648			4:108494061	-0.002111	0.000239742	[T/C]	5.58482992
RFI	rs414816492	ENSOARG00000003012	PLEKHG1	8:74555627	-0.001732	0.00022629	[A/C]	5.36432638
RFI	OAR15_40099623.1			15:40099623	-0.00208	0.00023788	[A/G]	5.23246999
RFI	rs425134950	ENSOARG00000020315	MAN1A2	1:94050486	-0.001498	0.000207985	[A/G]	4.9588365
RFI	OAR11_27752920.1			11:27752920	0.001819	0.000222699	[A/G]	4.9588365
RFI	OAR16_72265538.1			16:72265538	-0.002618	0.000248341	[T/C]	4.81711409
RFI	rs423570605	ENSOARG00000013828		1:60733952	0.001719	0.000200672	[T/C]	4.53482535
RFI	rs417223706	ENSOARG00000010362	MYL12A	23:37749930	0.001597	0.000191129	[T/C]	4.30794115
RFI	rs411012150			13:36682642	0.001572	0.000182584	[T/G]	4.2648378
RFI	rs160106606	ENSOARG00000009032	uncaracterized protein	5:60421059	-0.001674	0.000184751	[T/G]	4.2648378
RFI	rs425904136			8:7501857	0.00132	0.000180834	[T/C]	4.24329961
RFI	rs415141804			4:92807686	-0.001341	0.000176504	[T/C]	4.23253389
RFI	rs410904559			4:840726	0.002257	0.000222249	[A/G]	4.18949343
RFI	OAR9_68618966.1			9:68618966	0.001343	0.000185358	[T/C]	4.18949343
RFI	s69512.1			15:21926139	0.00135	0.000177444	[T/C]	4.16798666
RFI	rs398217340	ENSOARG00000013809	LURAP1L	2:80731070	0.001516	0.000194972	[A/C]	4.1035201
RFI	OAR5_77162689.1			5:77162689	0.0015	0.00016696	[A/C]	4.09278351
RFI	OAR8_69071490.1			8:69071490	-0.001309	0.000167117	[A/G]	4.01768991
RFI	rs402543445			19:58097089	-0.001929	0.000180793	[A/G]	3.91060291
RFI	rs426937018			8:72055247	0.001235	0.00016819	[A/G]	3.86783042
RFI	OAR15_52395182.1			15:52395182	-0.00141	0.000172353	[A/G]	3.82509348
RFI	OARX_16441119.1			27:16441119	0.001533	0.000169102	[A/G]	3.82509348
RFI	s03769.1			2:22928115	0.001148	0.000158432	[A/C]	3.7610546
RFI	rs421479854			2:63675988	-0.001421	0.000172028	[T/C]	3.75038921
RFI	rs419342821	ENSOARG00000014403	uncaracterized protein	9:35529619	-0.001169	0.000163295	[T/G]	3.73972603
RFI	rs406967914			4:94552733	0.001277	0.000162703	[T/C]	3.72906506
RFI	OAR26_32903035.1			26:32903035	-0.001263	0.000158684	[A/G]	3.6757934
RFI	rs424506880			10:52459290	0.001179	0.000155123	[T/C]	3.6651457
RFI	rs405615582	ENSOARG00000013544	RIC1	2:73333474	-0.001332	0.00015436	[T/C]	3.60130583
RFI	rs426940450	ENSOARG00000023774	uncaracterized protein	20:43161083	0.002047	0.000184428	[T/C]	3.60130583
RFI	rs415294045	ENSOARG00000013596	COBL	4:4713517	0.001137	0.000149133	[T/C]	3.60130583
RFI	s07134.1			15:5988222	0.001197	0.000160527	[T/G]	3.59067358
RFI	rs414207473	ENSOARG00000007560	ARHGAP42	15:7536215	-0.001291	0.000151754	[A/C]	3.59067358

Trait	SNP_novo	Ensemble (Gene)	Gene name	Position	Effect	% VarGen	alelo	BP
RFI	rs426186644	ENSOARG00000025287	uncaracterized protein	5:6895007	-0.001157	0.000153254	[T/C]	3.58004352
RFI	rs426186644	ENSOARG00000001029	uncaracterized protein	5:6895007	-0.001157	0.000153254	[T/C]	3.58004352
RFI	OAR12_8785706.1			12:8785706	-0.001219	0.000145048	[A/G]	3.56941567
RFI	rs420380888			2:9877191	0.001078	0.000164689	[A/G]	3.52692626
RFI	rs416863154	ENSOARG00000004438	uncaracterized protein	8:81812309	-0.001389	0.000160036	[A/G]	3.49508231
RFI	rs415843943	ENSOARG00000002063	SRRM4	17:55842659	0.001182	0.000150063	[A/C]	3.47386399
RFI	OAR26_32961768.1			26:32961768	-0.001201	0.000155195	[A/G]	3.47386399
RFI	rs413139541	ENSOARG00000025200		4:718077	-0.001841	0.000174847	[A/G]	3.47386399
RFI	rs399102597	ENSOARG00000011518	PAX5	2:51529698	0.001336	0.000155489	[T/C]	3.45265446
RFI	rs423217515			5:95970638	0.001232	0.00015919	[T/C]	3.44205298
RFI	s71989.1			2:23659780	-0.001461	0.000146422	[A/G]	3.4314537
RFI	rs411475019			1:205107119	-0.001419	0.00014595	[A/G]	3.36790404
RFI	rs423531461			1:70855490	0.001401	0.000157159	[T/C]	3.3573201
RFI	rs414345721			10:74465731	0.001004	0.000144667	[T/C]	3.18827415
RFI	rs416676032			5:95329244	0.001	0.000130589	[A/G]	3.18827415
RFI	OAR5_112405141.1			5:112405141	-0.001056	0.00013993	[T/C]	3.16718266
RFI	rs412019471	ENSOARG00000026185	uncaracterized protein	23:36854999	0.001368	0.000145189	[T/C]	3.16718266
RFI	rs407100968			12:45742771	-0.000996	0.000134846	[A/G]	3.13556175
RFI	rs415267900	ENSOARG00000016117	uncaracterized protein	1:68632937	0.001404	0.000146856	[T/C]	3.1039604
RFI	rs399135205			3:9958035	-0.001217	0.000140856	[A/G]	3.09343096
RFI	OAR4_41049923.1			4:41049923	-0.001643	0.000155002	[A/G]	3.06185567
RFI	rs403391311			23:22105490	0.0009791	0.000132935	[A/G]	3.06185567
RFI	rs401422903			7:59408108	-0.001127	0.000137557	[T/C]	3.05133491
RFI	rs411315526			10:64913571	-0.0009853	0.000130541	[T/C]	3.03029991

Trait	SNP_novo	Ensemble (Gene)	Gene name	Position	Effect	% VarGen	alelo	BP
FE	rs402836082	ENSOARG00000011009	uncaracterized protein	11:9074516	0.0003909	0.000142585	[A/G]	3.47386399
FE	rs408501145	ENSOARG00000005184	PIK3C3	23:14088280	0.0006795	0.00013288	[A/C]	3.29386237
FE	rs412052666			13:70886211	-0.0004808	0.000150472	[A/G]	3.72906506
FC	rs416210172			1:53710321	0.01949	0.002458726	[A/G]	8.76096658
FC	rs430136740	ENSOARG00000020436	SLCO1C1	3:193827830	-0.00874	0.000940729	[A/G]	5.07905803
FC	rs406111016	ENSOARG00000020821	uncaracterized protein	7:46878437	0.008686	0.001035656	[A/G]	4.751834
FC	rs425422822			20:46556750	0.006286	0.00075029	[T/C]	3.89990645
FC	rs417293333			23:44559717	-0.005761	0.000630313	[T/C]	3.64385692
FC	rs402836082	ENSOARG00000011009	uncaracterized protein	11:9074516	-0.005047	0.000540339	[A/G]	3.62257697
FC				19:1206663	0.005074	0.000544214	[T/G]	3.55879001
FC	rs422431427	ENSOARG00000008837	uncaracterized protein	16:31882270	0.007645	0.001079394	[T/C]	3.46325812
FC	rs428620176			2:236977003	0.005263	0.000610997	[T/C]	3.4314537
FC	rs406809081			3:24374242	-0.005074	0.000579704	[T/C]	3.41026172
FC	rs402039066			5:34773855	-0.004339	0.000442463	[T/C]	3.241041
FC	s36178.1			17:13746553	0.004149	0.000403458	[A/G]	3.17772732
FC	rs429351668	ENSOARG00000013534	MYOC	12:37251694	-0.004164	0.000434504	[A/G]	3.1039604
FC	rs410797913			2:140622573	-0.003967	0.000389464	[A/G]	3.1039604
FC	OARX_11655327.1			27:11655327	0.004325	0.000457875	[T/C]	3.04081633
FC	rs421641135	ENSOARG00000013390	GIPC2	1:53875325	0.004139	0.000420154	[A/G]	3.01978566
RFI	rs423726969			5:528815	0.001044	0.000137024	[T/C]	3.29386237
RFI	rs426828423			13:13066280	0.0011	0.000141806	[T/C]	3.262163
RFI	rs424765042			4:10206259	0.001668	0.000162124	[T/C]	3.21992772