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Genomic study for maternal related traits in Santa Inês sheep breed

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ABSTRACT

The aim of this study was to estimate variance components and to identify genomic regions and pathways associated with maternal related traits in Santa Inês sheep breed adapted to tropical climate. Phenotypic records for maternal efficiency (ME), metabolic maternal efficiency (MME), twin lambing (TL), adult weight (AW), metabolic adult weight (MAW), and body condition score (BCS) from 1333 ewes from Santa Inês breed were used. A total of 576 animals were genotyped with the Ovine SNP12k BeadChip (Illumina, Inc.), that contains 12,785 bialleleic SNP markers. The variance components were estimated using a single trait animal model by single step genomic BLUP procedure. For AW, MAW, BCS, ME, MME and TL the mean values were 50.30 (± 9.76), 19.2 (± 2.33), 2.76 (± 0.72), 34.6 (± 15.95), 91.8 (± 42.52), and 1.27 (± 0.44), respectively. The heritabilities estimated were moderate for AW (0.32) and MAW (0.33) and low for BCS (0.04), ME (0.07), MME (0.08), and TL (0.10). A total of 7, 8, 13, 16, 19, and 09 candidate regions for ME, MME, TL, AW, MAW and BCS traits were identified, respectively. AW and MAW had a total of 15 regions in common, while AW and BCS had a common region on chromosome 21. ME and MME had six candidate regions in common, and TL had no common regions with any other features. The maternal indicator traits have genetic variability to respond to selection in Santa Inês breed, and it would be expected higher genetic gain for ewe adult weight when compared to the others studied traits. Several candidate regions related to growth, reproduction, lactation and enviromental adaptability were identified in this study. These candidate regions would give support to identify and select animals with higher maternal efficiency and fitness, and consequently, increase the productivity of Santa Inês sheep. Moreover, the results of this study should help to understand the genetic and physiologic mechanism associated with maternal related traits in Santa Inês breed.

1. Introduction

Sheep farming is present in worldwide, and its diffusion is related to the environmental adaptation of the species. It is estimated that 56% of global population of domestic ruminants are small ruminants, approximately 1.1 billion are sheeps and 0.9 billion are goats, totaling 2.0 billions of animals (Statistical Yearbook of the Food And Agricultural Organization for the United Nations - FAOSTAT, 2013). Usually these animals are small and very efficient in terms of survival and adaptation in several ecosystems, unlike other ruminants that probably would have its performance compromised in unfavorable environmental conditions (Zygoyiannis, 2006). Farmers can positively influence the relationship between animal and environment using tools like selection to produce in different climates (Starling et al., 2002). Tropical semi-arid regions

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are characterized by high levels of solar radiation, high temperatures and rainfall indexes lower than 800 mm per year (Marino et al., 2016). Hair sheep breeds demonstrate robust production in critical enviroments, in northeast region of Brazil, Santa Inês sheep breed showed higher productive performance compared to other hair sheep breeds (Garcia et al., 2009). This breed was developed from successive crossbreedings between Bergamácia breed with Crioula and Morada Nova sheep breeds (Landim et al., 2011). Compared to other sheep breeds in Brazil, the Santa Inês breed has the higher number of registered herds (McManus et al., 2014), and there are several reasons that explain this fact, such as management requirements of this breed are simpler than those of wool breeds, because the animals do not need to be sheared, crutched, or docked. Also, their lack of wool eliminates several health concerns and drastically reduces the cost of production, and Santa Inês animals have shown superior adaptability to tropical climate conditions (Garcia et al., 2014; Seixas et al., 2017). Besides, Santa Inês owns interesting traits like prolificity, rusticity, maternal ability and parasite resistance (Cunha et al., 2008), for these reasons, more genomic studies with Santa Inês animals are important to the development of sheep farming in tropical environments.

The genetic improvement of reproductive traits has a larger economic impact than productive traits (Rosati et al., 2002). Therefore, traits like maternal efficiency and twin lambing can be used to evaluate the ewe productive efficiency, and consequently improve the productive performance of the herd. The selection for reproductive traits is complex due to its difficulty of measurement, low heritability estimates, and consequently low response to selection (Rosati et al., 2002). Traits like ewe adult weight are related to nutritional requirements of the herd and showed enough genetic variation to respond to selection (Safari et al., 2005). However, this kind of trait took a long time to be measured in animals life, which can be a problem for breeding programs since it increase the generation interval. In tropical conditions where scarcity of water and feed through the year is a challenge in production systems, Santa Inês breed represents a genetic resource to study biological mechanisms of adaptation in extreme environments.

Several GWAS studies identified genetic variants related to productive traits in sheep breeds (Al-Mamun et al., 2014; Demars et al., 2013; Zhang et al., 2013), but these studies were performed in wool breeds. Recently, Berton et al. (2017) identified several candidate genes and pathways related to parasite resistance of Santa Inês animals, like development and activation of immunological system, inflammatory response, lymphocyte regulation and proliferation. The identification of genomic regions that play a role in productive and reproductive traits would become an important tool for genetic improvement of Santa Inês breed and other breeds in tropical conditions. Therefore, the objective of this study was to estimate variance components and identify genomic regions and pathways associated to prolificity, maternal efficiency and ewe adult weight in Santa Inês breed in tropical conditions.

2. Material and methods

2.1. Animals and phenotypes

The phenotypic records were collected from 1333 ewes from Santa Inês breed belonging to four flocks located in Southeast and Northeast states of Brazil, in Minas Gerais, São Paulo and Sergipe. The relationship matrix was composed by 32,292 animals, and a total of 576 animals were genotyped. The following traits were evaluated: maternal efficiency (ME), metabolic maternal efficiency (MME), twin lambing (TL), ewe adult weight (AW), metabolic ewe adult weight (MAW), and body condition score (BCS).

The adult weight was admitted as the weight of the ewe weighed at 4 years old. Metabolic adult weight was defined as the weight of the ewe weighed at years old to the power of 0.75, as proposed by Van Soest (1994):

$$MAW = AW^{0.75}$$

The maternal efficiency was given by the relation between the lambs weight at 60 days old and the adult weight of the ewe, or as the conversion of available feed into kilograms of lamb weaned per kilogram of ewe joined at 60 days of age. Metabolic maternal efficiency was evaluated as the relation between the lambs metabolic weight at 60 days old, and the metabolic adult weight of the dam.

Body condition score and twin lambing were admitted as categorical traits. The BCS was visually evaluated, for each score were attributed grades from 1 to 5 according to fat deposition in the lower back. Animals with lower fat deposition received score 1, and animals with the highest fat deposition received score 5 (Schröder and Staufenbiel, 2006). For TL, ewes with twin lambing were admitted as score 2 for success, and the ewes with only one lamb at birth had score 1 for failure. The contemporary groups were formed by ewes borned in the same year and farm. The contemporary groups with less than three ewes were discarded. For ME, MME, AW and MAW the covariable (linear effect) BCS and the animal age at measurement were included in the model. For BCS and TL only the covariable (linear effect) animal age at measurement was included in the model

2.2. Genotyping of the animals

A total of 576 animals were genotyped using the Ovine SNP12k BeadChip (Illumina, Inc.), with 12,785 biallelic SNP markers. The quality control excluded markers with unknown genomic position, located at the sexual chromosomes, monomorphic, with minor allele frequency (MAF) lower than 0.05, call rate lower than 90%, and with excess heterozygosity. After quality control, there were 12,315 SNPs and 574 samples left for analyses. The overall mean linkage disequilibrium (r^2) obtained between pairs of markers for a distance lower than 50 kb was 0.16 (Berton et al., 2017).

2.3. Estimation of variance components

The variance components were estimated using a single animal trait model by the single step genomic BLUP (ssGBLUP) procedure. The ssGBLUP is a modified version of the animal model (BLUP), that consists of integrating additive relationship matrix (**A**) and genomic relationship matriz (**G**) into a single matrix (**H**) (Legarra et al., 2014). The genomic relationship matrix was obtained according to VanRaden (2008).

The repeatability linear model adopted for maternal efficiency and metabolic maternal efficiency was:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Za} + \mathbf{Wc} + \mathbf{e}$$

where, **y** is represented by the vector of dependent variables; **X** is the incidence matrix for fixed effects; $\boldsymbol{\beta}$ is the vector of fixed effects (contemporary groups and covariables); **Z** is the incidence matrix for additive genetic effect; **a** is the vector of additive genetic effects; ***c*** is the vector of permanent environmental effects; ***W*** is the incidence matrix for permanent environmental effect; and **e** is the random residual effects associated with the observations.

For TL analyses a threshold repeatability model was adopted, assuming that the underlying distribution (**U**) is determined by:

$$\mathbf{U} \sim \mathbf{N}(\mathbf{X}\beta + \mathbf{Za} + \mathbf{Wc} + \mathbf{I}\sigma_{\mathbf{e}}^2)$$

Priori distributions for additive genetic effect, permanent environmental effect and residual followed the multivariate normal distributions, as follows:

$$\mathbf{P}(\mathbf{a}|\sigma_{\mathbf{a}}^2) \sim \mathbf{N}(0,\ \sigma_{\mathbf{a}}^2)$$

$$\mathbf{P}(\mathbf{c}|\sigma_{\mathbf{c}}^2) \sim \mathbf{N}(0, \sigma_{\mathbf{c}}^2)$$

$$\mathbf{P}(\mathbf{e}|\sigma_{\mathbf{e}}^2) \sim \mathbf{N}(0,\ \sigma_{\mathbf{e}}^2)$$

Since the variable at underlying distribution was not observed, we adopted $\sigma_e^2 = 1$ obtain identifiability in the likelihood function (Sorensen and Gianola, 2002). After defining the model parameters, the link between categorical and continuous scales could be established based on the contribution of the probability of an observation that fit the first category, which is proportional to:

$$\mathbf{P}(\mathbf{y_r} = 0|\mathbf{t}, \theta) = \mathbf{P}(\mathbf{U_r} < \mathbf{t}|\mathbf{t}, \theta) = \varnothing((\mathbf{t} - \mathbf{W'_r}\theta)/\sigma_\mathbf{e})$$

where: y_r is the response variable for the rth observation, with values equal to 1 or 2. If the value belongs to first or second category (failure or success); t is the threshold value arbitrarily assigned as the true value is unobservable; U_r is the value of the underlying variable for the rth observation; ($\varnothing$) is the cumulative distribution function of a standard normal variable; W'_r is the scale of the incidence matrix that linked $\varnothing$ to the rth observation; $\varnothing = (b', a')$ is the vector of the parameters of s with b (systematic effects) and a (random effects).

For AW and MAW, a linear model was used as follows:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Za} + \mathbf{e}$$

y is the observations vector; $\boldsymbol{\beta}$ is the vector of fixed effects (contemporary group and covariable); $\mathbf{a}$ is the vector of additive genetic effects; $\mathbf{X}$ is the incidence matrix for fixed effects; $\mathbf{Z}$ is the incidence matrix for additive genetic effects; $\mathbf{e}$ is the residual effect vector.

The analyses for ME, MME, AW e MAW were performed using the AIREMLF90 software and for TL using the THRGIBBSF90 software (Misztal et al., 2015). The Bayesian analysis consisted of chain of 500,000, burn-in of 100,000 cycles, taking a sample at every 100 iterations. High-density regions were constructed for all variance components and genetic parameters estimated at 90% level of credibility. The convergence was tested using the Bayesian Output Analysis (BOA) implemented in R (2010) program.

2.4. Genome-Wide Association Study (GWAS)

The GWAS analyses were performed using the single-step method proposed by Wang et al. (2012b) considering the same linear animal model for continuous traits or threshold animal model described before to estimate the variance components. The animal effect was split into genotyped (a_g) and non-genotyped (a_n) animals as described by Wang et al. (2012b), with the animal effect of genotyped animal:

$$\boldsymbol{a_g} = \boldsymbol{Mu},$$

where $\boldsymbol{M}$ is a matrix that relates genotypes of each locus and $\boldsymbol{u}$ is a vector of marker effects. The variance for animal effect was assumed as:

$$\text{var}(a_g) = \text{var}(\boldsymbol{Mu}) = \boldsymbol{MDM'}\sigma_u^2 = \boldsymbol{G^*}\sigma_a^2,$$

where $\mathbf{D}$ is a diagonal matrix of weights for variances of markers ($\mathbf{D} = \boldsymbol{I}$ for GBLUP), σ_u^2 is the genetic additive variance captured by each SNP marker when no weights are present and $\mathbf{G}^*$ is the weighted genomic relationship matrix.

The markers effects were obtained as described by Wang et al. (2012b):

$$\widehat{\mathbf{u}} = \lambda \mathbf{D}M''\mathbf{G}^{*-1}\widehat{\mathbf{a}}_\mathbf{g} = \mathbf{D}M''[M\mathbf{D}M'']^{-1}\widehat{\mathbf{a}}_\mathbf{g}$$

where λ is a variance ratio defined by VanRaden et al. (2009):

$$\lambda = \frac{\sigma_u^2}{\sigma_a^2} = \frac{1}{\sum_{i=1}^{m} 2pi(1 - pi)},$$

where m was the number of SNPs, and p_i was the allele frequency of the second allele of the ith SNP.

The iterative process described by Wang et al. (2012b) was used to estimate the SNP effects updating the GEBV of all animals in three iterations. The proportion of additive genetic variance explained by the k-th region was obtained as proposed by Wang et al. (2012b):

$$\frac{Var\ (u_k)}{\sigma_a^2} \text{ x } 100\% \ = \ \frac{\text{Var}(\sum_{\text{j=k}}^{\text{n}} \text{Z}_\text{j}\ \hat{\text{u}}_\text{j})}{\sigma_\text{a}^2} \text{ x } 100\%$$

where u_k was the genetic value of the k-th region that consists of 10 continuous adjacent SNPs, σ_a^2 was the total genetic variance, Z_j is vector of SNP content of the jth SNP for all individuals, and $\hat{u}_j$ is the marker effect of the jth SNP within the kth region. The GWAS analyses were performed using the BLUPF90 family software (Misztal et al., 2002) modified to include genomic information (Aguilar et al., 2010).

2.5. Analysis of QTL regions for candidate gene identification

The chromosome regions that explained more than 1.0% of the additive genetic variance were selected to explore and determine possible quantitative trait loci (QTL). The windows were defined by 10 continous adjascent SNPs. Map Viewer tool of ovine (*Ovis aries*) genome was used for identification of the genes, available at "National Center for Biotechnology Information" (NCBI - http://www.ncbi.nlm.nih.gov) database using the bank references of HuRef assembly, CHM1 1.0, CRA TCAGchr7v2 and Ensembl Genome Browser (http://www.ensembl.org/index.html). The classification of genes regarding their biological function was performed by DAVID tool v6.8 (https://david.ncifcrf.gov/) and GeneCards (http://www.genecards.org/), using all annotated genes in the *Ovis aries* genome as background. Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathways were considered used for functional enrichment analysis considering a $P < 0.01$ threshold for significance.

3. Results

3.1. Genetic parameters estimates

The descriptive statistics and genetic parameter estimates for ME, MME, AW, MAW, BCS and TL were presented in Table 1. The maternal efficiency, corporal condition and twin lambing presented low heritability estimates, and adult ewe weights (AW and MAW) moderate. These estimates corroborate with the review by Safari et al. (2005)

Table 1
Descriptive statistics and genetic parameters for maternal efficiency (ME), metabolic maternal efficiency (MME), adult weight (AW), metabolic adult weight (MAW), body condition score (BCS) and twin lambing (TL) for Santa Inês sheep breed.

Trait	N	Mean ± SD	σ_a^2 ± SE	σ_c^2 ± SE	σ_e^2 ± SE	h^2 ± SE	c^2
ME[1]	1,395	34.6 ± 15.95	14.11 ± 6.08	10.70 ± 6.86	170.02 ± 5.81	0.07 ± 0.01	0.05 ± 0.01
MME[1]	1,395	91.8 ± 42.52	109.61 ± 43.36	11.36 ± 44.3	1222.8 ± 41.3	0.08 ± 0.01	0.01 ± 0.01
TL[2]	1,395	1.27 ± 0.44	–	–	–	0.10 (0.00–0.21)[2]	0.22 (0.10–0.34)[2]
AW	1,333	50.3 ± 9.76	14.77 ± 4.02	–	30.82 ± 3.42	0.32 ± 0.01	
MAW	1,333	19.2 ± 2.33	1.18 ± 0,30	–	2.31 ± 0.25	0.33 ± 0.01	
BCS	1,333	2.76 ± 0.72	0.02 ± 0.01	0.40 ± 0.02	0.39 ± 0.02	0.04 ± 0.03	

σ_a^2: Genetic additive variance; σ_c^2: Permanent environmental variance; σ_e^2: Residual variance; h^2: Heritability; c^2: permanent environmental effect. [1]The average of ME and MME are presented in percentage. [2]TL was considered in a threshold model, then are presented highest posterior density (HPD) for this trait.

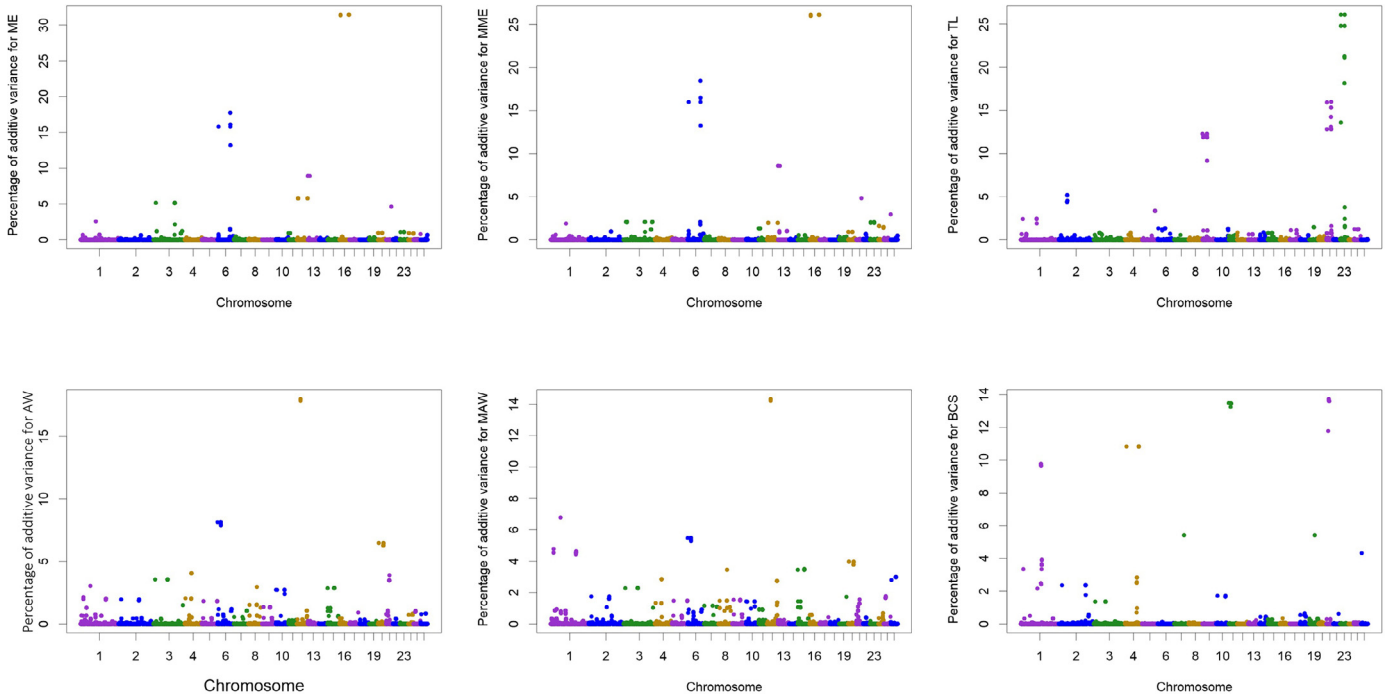

Fig. 1. Manhattan plots of the genome-wide association of the studies traits. The *X*-axis represents the chromosomes, and the *Y*-axis shows the proportion of genetic variance explained by windows of 10 adjacent SNPs.

which gathered 165 studies from 1992 to 2003 that estimated genetic parameters for growth, wool, meat and reproduction traits in sheep and reported heritability estimates for adult weight varying from 0.30 to 0.41. In addition, the estimates for AW and MAW obtained in this study were similar to those described by Sousa et al. (1999) also for Santa Inês sheep.

3.2. Genomic regions

The SNPs windows regions which accounted for more than 1% of the genetic additive variance were used to search for candidate genes (CG), which were described in Additional File 1. A total of 7, 8, 13, 16, 19, 9 candidate regions for ME, MME, TL, AW, MAW and BCS traits were identified, respectively. AW and MAW had a total of 15 regions in common on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 20, 21 and 25, showing a high number of genes associated with energy production and conversion. AW and BCS had a common region on chromosome 21. For BCS, genes linked to lipid binding, lipid catabolic process, fat cell differentiation and generation of percursos metabolites and energy were found. ME and MME displayed six candidate regions in common on chromosomes 3, 6, 12, 13, and 16. Genes related to reproductive functions, feeding behavior, lipid byosintesis were identified. TL had no common regions with any other features (Additional File 1), and it was possible to identify genes related to reproductive functions in the candidate regions studied.

3.3. Enrichment analisys

To assess the functional annotation of these significant regions, gene ontology analysis (GO) using the Database for Annotation, Visualization and Integrated Discovery (DAVID) functional annotation tool were performed (Huang et al., 2009a, 2009b). The analysis set comprised 829 genes ID which were used to perform the functional analysis. A total of 144 ovine gene IDs were identified by DAVID. The gene-annotation enrichment analysis by DAVID tool identified 30 biological processes, five molecular functions and six cellular components as significant ($P < 0.01$) (see Additional File 2).

In addition, DAVID identified 31 KEGG pathway enriched ($P < 0.01$) (Table 7). Several significant KEGG pathways identified are related to immunity and inflammatory host defenses, such as measles (oas05162), amoebiasis (oas05146), and salmonella infection (oas05132), intestinal immune network for IgA production (oas04672), TNF signaling pathway (oas04668), cytokine-cytokine receptor interaction (oas04060), apoptosis (oas04210), among others discussed below (Table 8). Also showed that several significant regions found in this study is associated with known-disease related genes, corroborating with the results found by Liu et al. (2013).

4. Discussion

4.1. Genetic parameters estimates

The low heritability estimated for body condition is related mainly to the nutritional status of the sheep, since the residual variance of this trait composes 96% of the phenotypic variance, indicating that the environmental effects modulate the phenotypic expression of this trait. Gonzalez et al. (1999) demonstrated that sheep and goats reared in tropical conditions with low body condition score presented longer postpartum anestrus, lower fertility rate and lower prolificacy index than females with higher BCS. In this regard, the increase in BCS seems to be more related to factors that are set than to the genetic ones.

Heritability estimated for ME, MME and TL indicated that these traits were highly influenced by environmental factors (Table 1). For TL, the direct permanent environment effect was higher than the heritability estimate, indicating that higher proportion of the phenotypic variance is due to the direct permanent environment effect, in other words, for this trait the permanent environment effects, such as management and nutritional decisions have more importance than the genetic background (Table 1). Genetic parameter estimates for these traits in Santa Inês sheep were not reported in the literature yet, since most of studies about genetic parameter estimates for maternal related traits were with wool breeds. Despite the heritability estimated in this study were low to moderate magnitude, these traits should be considered in breeding programs of Santa Inês breed due to the economic importance

of the maternal related traits for sheep production. The results of this study pointed out that there is genetic variability in selecting for maternal related traits in Santa Inês sheep breed. Thus, it is important to known whether there are more genes involved to better understand the genetic architecture of these traits, especially for reproductive traits that can reduce the production cycle and increace productivity in the system.

4.2. Maternal efficiency

For ME, the window that is responsible for the most part of additive genetic variance was located in chromosome 16 at the 81 Mb position that is responsible for 31.447% of the additive genetic variance (Fig. 1).

Genes involved in the energy and lipid metabolism may play an important role to keep body homeostasis against environmental changes. These genes can affect performance and may explain the trade-off between energy for growth and energy for homeostasis. In this sense, the *CARTPT* (Cocaine and Amphetamine-Regulated Transcript Prepropeptide) gene which is located at chromosome 16, plays a role in appetite, energy balance, maintenance of body weight, and stress response (Lau and Herzog, 2014). Results of other studies have suggested that this gene is related to susceptibility of obesity in humans (Yeo et al., 2012). Polymorphisms in *CARTPT* gene has been associated with growth traits in cattle (Zhang et al., 2008). The *PIK3R1* (Phosphoinositide-3-Kinase Regulatory Subunit 1) gene, also located at chromosome 16, encodes a regulatory subunit and plays a role in the metabolic actions of insulin, also Puig-Oliveras et al. (2016) reported that *PIK3R1* as a key regulator affecting intramuscular fatty acid content in porcine meat. *PIK3R1* is a catalytic subunit of the AMP-Activated Protein Kinase (*AMPK*) which is in charge of regulating lipid synthesis by phosphorylating lipid metabolic enzymes, it acts regulating key enzymes of fatty acid uptake, esterification, lipolysis and oxidation (O'neill et al., 2013). Several studies have shown the key roles of the *GHR* gene (Growth Hormone Receptor) in postnatal growth and carbohydrate metabolism in mice (Bartke and Brown-Borg, 2004; Coschigano et al., 2003; Liu et al., 2008; Rowland et al., 2005). Additionally, Valeh et al. (2009) reported a *GHR* gene polymorphism and its association with growth and meat production traits in sheep.

4.3. Metabolic maternal efficiency

For MME trait, the window responsible for the most part of additive genetic variance was also in chromosome 16, followed by chromosome 6 at 10 Mb, that is responsible for 18.46% of the additive genetic variance (Fig. 1). Genes like *MSX1* (Msh Homeobox 1), and *DRD5* (Dopamine Receptor D5) have important functions in utero embryonic development and reproductive behavior, respectively (Gerhard et al., 2004; Wang et al., 2012a). Tesfaye et al. (2010) identified that the suppression of *MSX1* gene affects oocyte maturation, embryo cleavage rate and the expression of other genes, suggesting its potential role in the development of bovine preimplantation embryos. Mann (2014) studying gene expression profiling during pregnancy in rats, noticed that the expression of *DRD5* gene was reduced at the end of pregnancy, resulting in changes in feeding behavior and energy metabolism in favor of the fetus (Ladyman et al., 2010, 2012).

Genes related to lipid biosynthesis process, catabolism, oxidation, transport, metabolism, and degradation were also identified (see Additional File 1). The functions described above corrobates with several studies of regulation of milk lipid biosynthesis in human mammary epithelial cells during secretory activation. However, milk lipid synthesis is a complex process and requires a orchestration of a wide variety of pathways and several genes may play a role (Mohammad and Haymond, 2013; Zou et al., 2013). In domestic sheep, a different pattern of adipose tissue was found. During the last third of pregnancy, the growth of the fetus and mammary gland place a considerable burden on the ewe, precipitating a switch to lipid mobilization which continues into lactation. Ewes with a better corporal condition and with a single lamb are unlikely to lose lipid during lactation, but if they have two lambs there is an extensive loss of lipid from adipose (Robinson, 1986). These results probably shown the strong influence of this group of genes on reproductive traits in Santa Inês sheep.

4.4. Twin lambing

For TL were identified 13 windows with 20 potential candidate genes (Fig. 1). The window responsible for the most part of additive genetic variance was identified on chromosome 23, that is responsible for more than 25% of the additive genetic variance.

During pregnancy, the placenta regulates the transfer of solutes, nutrients and water between maternal and fetal blood. The *SLCO4C1* (Solute Carrier Organic Anion Transporter Family Member 4C1) gene is a member of SLC family of transporters that play a role in this biological process. This gene is located on ovine chromosome 5, and is related to preeclampsia in humans (Morrison et al., 2010). Animals require molecular signals to determine when to divert resources from somatic functions to reproduction functions. Genes linked to embryonic development, progesterone, oocyte maturation, and oogenesis were found in this study, like *OOEP (*Oocyte Expressed Protein) *GATA6 (*GATA Binding Protein 6), *CUL4A* (Cullin 4A), and *ZFAND5* (Zinc Finger AN1-Type Containing 5)This cluster of genes plays an important role in physiology of reproduction, since they signal energy sufficiency for the neuroendocrine reproductive axis. The *OOEP* gene is linked to the subcortical maternal complex (SCMC) which is a multiprotein complex expressed in mammalian oocytes and early embryos, fundamental for zygote progression beyond the first embryonic cell divisions (Bebbere et al., 2016). Also, other studies performed by Moorthy et al. (2017), Tashiro et al. (2010), and Tran et al. (2015) described that *OEEP* is expressed in oocytes and in embryonic stem cells. Bebbere et al. (2014) identified the *OPEP* transcription in ovaries and testis, which expression of maternally derived *OOEP* is associated with oocyte developmental competence in ovine.

The *CUL4A* gene is essential for spermatogenesis and male fertility. Kopanja et al. (2012) and Li et al. (2002) described *CUL4A* gene also as critical in early embryonic development, while *GATA6* and *ZFAND5* genes are also related to utero embryonic development. Taniguchi et al. (2009) identified that *GATA6* gene contribute to the transcriptional regulation of steroidogenic gene expression and hence progesterone production in the bovine corpus luteum. Bai et al. (2014) studied the xpression of *GATA6* in ovine conceptuses and uterine endometria during the peri-implantation period and suggested that *GATA6* may also play a potentially novel role in the development of ovine trophectoderm, endoderm and/or uterine endometria following conceptus attachment to the uterine epithelium. PAGS (Pregnancy-associated glycoproteins) were first described as placental antigens present in the blood serum of the mother soon after implantation. Gonzalez et al. (1999) and Ledezma-Torres et al. (2006) reported rapid increase in PAG concentration during pregnancy in caprine and ovine species. Barbato et al. (2013) performed a purification of several PAGS in water buffalo placenta which was observed a rapid increase in PAG concentrations from day 30 to day 37 of pregnancy, further they were able to develop radio immune assay for early pregnancy diagnosis in buffalo species. Genes play multiple roles in metabolism and physiology, therefore, specific studies demonstrating the role of this group of genes in twin lambing in sheep are needed.

4.5. Adult weight

For ewe AW, a total of 16 putative candidates genes related to regulation of growth, skeletal muscle tissue development, growth factor activity, regulation of muscle contraction, multicellular organism growth, and muscle cell differentiation were identified (Fig. 1).

The *TNNT2* (Troponin T2, Cardiac Type) gene is essential for the

contraction of striated muscles because it has a central player in the calcium regulation of actin thin filament function (Gordon et al., 2000). The *HTRA3* (HtrA Serine Peptidase 3) gene is a member of the HtrA protease family highly expressed in the developing placenta, including the maternal decidual cells in mice. Li et al. (2017) investigated the importance of *HTRA3* gene in mouse placentation using a gene deletion strategy. These authors shown the importance of maternal environment like low protein diet, ion deficiency, or maternal undernutrition on fetal growth and growth trajectory of adulthood animals.

4.6. Metabolic adult weight

For ewe MAW, many regions in common with AW were found, but MAW have shown more candidates genes than AW, showing a high number of genes associated with energy production and conversion (Fig. 1). The *LDHA* (Lactate Dehydrogenase A) gene is the key regulator of glycolysis, and transcript expression and post-transcriptional modification of *LDHA* is regulated by several known genes and deacetylases (Jin et al., 2017; Wang et al., 2015), such as *MYC* (MYC Proto-Oncogene, BHLH Transcription Factor), that was also found in this study.

The *MDFIC* (MyoD Family Inhibitor Domain Containing) is known as a functionally important gene involved in growth and development. Zhang et al. (2014) related *MDFIC* with the improvement of piglet birth weight. The *MSTN (*Myostatin) gene is known as a negative regulator of muscle growth responsible for double muscling in cattle (Grobet et al., 1997). The genes presented here as candidates can possible influence in the increase of muscle mass and potential greater commercial value of the animals.

4.7. Body condition score

Genes linked to lipids, metabolites and energy were associated to BCS (Fig. 1). *AOX1* (Aldehyde Oxidase 1) gene is related oxidoreductase activity and electron carrier activity. Gan et al. (2015) used ELISA test to detect the key enzymes of fatty acid synthesis, and the key enzymes of fatty acid mobilization such as *AOX1* in mice adipocytes. The *LTBP1* (Latent Transforming Growth Factor Beta Binding Protein 1) gene is an insulin-like growth factor binding protein, and known to be calcium binding, this interaction is thought to induce structural changes and protect the protein from proteolysis (Colosetti et al., 1993). Fu et al. (2017) studied mitochondrial energy metabolism in skeletal muscle tissue, and they reported that is negatively correlated with feed efficiency in pigs. Ijuin and Takenawa (2012) studied the skeletal muscle and kidney-enriched inositol polyphosphate phosphatase (*SKIP*), which has a previously been implicated in the regulation of insulin signaling in skeletal muscle. The *PAK1* (P21 (RAC1) Activated Kinase 1) gene is responsible for cellular response to insulin stimulus, and it was reported in their research. *PAK1* has also been implicated as a positive effector of mechanisms in skeletal myotubes that is crucial to maintaining glucose homeostasis *in vivo* (Chiu et al., 2011). The *THRSP* (Thyroid Hormone Responsive) gene is regulated by hormonal and dietary intervention and is involved in lipogenic processes in rodents. It is expressed in mammary gland, liver, white and brown adipose tissue of rats (Seelig et al., 1981). Studies in cattle suggests that *THRSP* is a potential molecular marker for intramuscular fat deposition (Schering et al., 2017).

4.8. Enrichment analysis

The gene ontology terms anotation analysis enriched ($P < 0.01$) and main KEGG pathways significantly enriched ($P < 0.01$) from the set of genes previously identified (Additional File 2) are discussed ahead.

The mammary gland involution (GO:0060056) biological process is defined as the tissue remodeling that removes differentiated mammary epithelia during weaning (Ashburner et al., 2011). Mammary gland involution is a highly complex multi-step process in which the lactating gland returns to a morphologically near pre-pregnant state, with is characterized by a high degree of epithelial cell death redevelopment of the mammary adipose tissue and tissue remodeling (Stein et al., 2007). This process is directly related to lactation process, which was another significant term obtained on the enrichment analyses. The lactation (GO:0007595) biological process is defined as the regulated release of milk from the mammary glands and the period of time that a mother lactates to feed her progeny (Ashburner et al., 2011), thus, a term of great importance for the study in question since its traits are directly linked to the lactation process.

A fact that can influence the sheep behavior, as well their production, is the mechanisms that regulate the body temperature. The climate of a particular region, especially air temperature and relative humidity, directly influences the animal's production potential (McManus et al., 2009). Heat can significantly affect pregnant females as demonstrated in the study of Quesada et al. (2001). In this sense, two GO terms related to heat tolerance was enriched in this study: regulation of blood pressure (GO:0008217) and hair follicle morphogenesis (GO:0031069)The term hair follicle morphogenesis was reported by Lemos et al. (2017) to be significant in a copy number variation characterization study with Nelore cattle and associated with the heat tolerance of these animals. The authors emphasized the fact of the Nelore breed have thin, short, smooth and shiny hair coats, which facilitates the spread of heat.

The Jak-STAT signaling pathway (oas04630) is one of pleiotropic cascades used to transduce signals from cell-membrane receptors to the nucleus for mediates the action of several hormones, cytokines and growth factors that are critical to a variety of physiological processes including development, hematopoiesis, lactation and inflammation (Liongue et al., 2012; Seif et al., 2017). Modulation of the Jak–STAT pathway regulates adipocyte development and function (oas04920: adipocytokine signaling pathway). Adipocytes produce and secrete substances that modulate appetite, lipid and glucose homeostasis, insulin sensitivity, inflammation, and homeostasis (Athyros et al., 2010; Richard and Stephens, 2014). Additional functions of Jak-STAT signaling in adipocytes include the transcriptional regulation of genes related to insulin action and lipid and glucose metabolism (Richard and Stephens, 2011). In this context, we also identified the insulin signaling (oas04910), insulin resistance (oas04931), and glucagon signaling (oas04922) pathways enriched ($P < 0.01$) in this study.

Insulin signaling (oas04910) pathway also plays an important function of female reproduction, since that hyperinsulinemic and hypoinsulinemic condition are related with certain types of ovarian dysfunction, such as altered steroidogenesis and infertility. Insulin signaling acts cooperatively with gonadotropins in mammals to mediate several aspects of ovarian development (Das and Arur, 2017). Glucagon signaling (oas04922) pathway also has been associated to important disorders of female reproduction including hypoglycemic pregnancies, altered placentation, poor fetal growth and increased fetal–neonatal demise (Charron and Vuguin, 2015). Insulin signaling (oas04910) pathway has being associated with important economically traits such as carcass composition, milk yield, adipose tissue development, lean tissue accretion rates, among others (Baumgard et al., 2015).

The FoxO signaling pathway (oas04068) integrate insulin signaling with glucose and lipid metabolism. They mediate the inhibitory action of insulin or insulin-like growth factor on key functions involved in cell cellular physiological events including apoptosis and glucose metabolism (Lee and Dong, 2017). FoxO transcription factors also play an important role in adipose tissue by regulating adipogenesis and in skeletal muscle acting on development and atrophy (Gross et al., 2008).

The AMPK signaling pathway (oas04152), also identified in this study, also acts as a sensor of cellular energy status promoting a concomitant inhibition of energy-consuming biosynthetic pathways, such as fatty acid, protein and glycogen synthesis.The transforming growth factor-beta (TGF-beta) signaling pathway (oas04350) family members comprises growth and differentiation factors that include TGF-βs,

activins, inhibins, and bone morphogenetic proteins. It has been well established that TGF-β superfamily members are key regulators of follicle development in mammals, because many of them are expressed by ovarian somatic cells and oocytes in a developmental, stage-related manner and function as intraovarian regulators of folliculogenesis (Knight and Glister, 2006). Differential expression analyses in cattle identified this pathway overrepresented in studies about heat stress (Mehla et al., 2014) and fatty acid composition of intramuscular muscle (Lemos et al., 2016).

5. Conclusions

The ewe maternal indicator traits have genetic variability to respond to selection in Santa Inês breed, and it would be expected higher genetic gain for adult ewe weight when compared to the others studied traits. Despite the low density SNP chip used, the level of LD estimated for markers separated by less than 50 kb indicates that the Ovine SNP12k BeadChip will likely be a suitable tool for identifying genomic regions associated with those traits related to maternal related traits.

Several candidate regions related to growth, reproduction, lactation and enviromental adaptability were identified in this study. These candidate regions would give support to identify and select animals with higher maternal efficiency and fitness under tropical conditions, and consequently, increase the productivity of Santa Inês sheep breed.

The results of this study should help to better understanding the genetic and physiologic mechanism associated with maternal efficiency related traits in Santa Inês breed. In addition, the results obtained would support to customized low density SNPs arrays through selecting genetic markers to use for genomic selection for maternal efficiency related traits in Santa Inês breed.

Declarations

Ethics statement: All experimental procedures involving animals were approved by the Ethical Committee of 625 FZEA/USP CEUA no 7718021216.

Consent for publication

Not applicable.

Availiability of data and material

The phenotypic and genomic information used in this study belongs to a private sheep breeding program company, so we do not have authorization to share the data.

Competing interests

The authors declare that they have no competing interests.

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Authors' contribution

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Supplementary materials

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