animal

Single nucleotide polymorphisms in the growth hormone and IGF type-1 (IGF1) genes associated with carcass traits in Santa Ines sheep

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*Polymorphisms in the growth hormone (*GH*) and IGF type-1 (*IGF1*) genes have been associated with the economic traits in farm animals, including BW of some sheep breeds. However, it remains unknown if these polymorphisms also affect carcass traits in sheep. Thus, we aimed to identify polymorphisms in the* GH *and* IGF1 *genes in Santa Ines sheep in order to describe their allelic and genotypic frequencies as well as to test the hypotheses that they are associated with the carcass traits. Fragments of 4550 bp (*IGF1*) and 1194 bp (*GH*) were sequenced in up to 191 lambs. In all, 18 polymorphisms were identified in the* IGF1 *and 21 in the* GH *gene. The* IGF1 *polymorphisms rs430457475, rs412470350, rs409110739 and rs400113576 showed an additive effect on the internal carcass length (−0.9265 ± 0.4223), rump girth (−2.9285 ± 1.1473), rib yield (−1.0003 ± 0.4588) and neck weight (−0.0567 ± 0.0278), respectively. In addition, the polymorphisms rs58957314 in the* GH *affected the rib weight (−0.4380 ± 0.1272) and rib yield (−2.2680 ± 0.6970), loin weight (−0.1893 ± 0.0516) and loin yield (−0.9423 ± 0.3259), palette weight (−0.2265 ± 0.0779) and palette yield (−0.9424 ± 0.4184), leg weight (−0.3960 ± 0.1375), neck weight (−0.0851 ± 0.0394) and carcass finishing score (−0.1700 ± 0.0839). These results allow us to conclude that there are polymorphisms in the* IGF1 *and* GH *genes associated with carcass traits in Santa Ines sheep, which can provide important information for marker-assisted selection.*

Keywords: genomic, lamb, ovine, selection, sequencing

Implication

The carcass attributes are difficult to measure on a large scale. Consequently, these variables are rarely included in sheep breeding schemes. However, molecular markers can provide useful information for marker-assisted selection in order to improve them. Our results indicated the existence of mutations associated with the carcass traits in Santa Ines sheep. This represents an important advance in knowledge of the genetic control of these variables. In addition, our results can impact the production system of this breed, as the breeders have a new source of information to improve carcass quality.

Introduction

The Santa Ines is a hair sheep breed with two important characteristics for use in the tropical areas: it is relatively

resistant to heat (McManus *et al.*, 2009) and endoparasites (Mexia *et al.*, 2011) compared with wool sheep breeds; its performance in a pasture system (Jucá *et al.*, 2014) and its meat and carcass traits (Jucá *et al.*, 2016) have been extensively studied, confirming that this breed requires selection to improve its carcass traits. However, classical selection methods often fail to improve the carcass traits because they are difficult to evaluate *in vivo*. In this context, molecular markers in candidate genes may provide new information for selection.

The growth hormone (*GH*) and IGF type-1 (*IGF1*) affect several tissues and organs and play key roles in the longitudinal growth of an organism (Devesa *et al.*, 2016). The *GH* and *IGF1* are closely associated because the *GH* induces liver *IGF1* transcription which in turn negatively controls the pituitary *GH* secretion. Then, polymorphisms in *GH* and *IGF1* may contain useful information for the selection of traits correlated with body growth, such as carcass traits.

Some polymorphisms in *GH* in sheep were tested for their association with BW and morphometric traits (Nasution *et al.*,

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2017; Abdelmoneim *et al.*, 2017), milk production (Dettori *et al.*, 2015), wool production (Farag *et al.*, 2016) and carcass traits (Gorlov *et al.*, 2017). Although previous studies have already mentioned about association between polymorphisms in *GH* gene and the carcass traits in sheep, several phenotypes remain to be evaluated.

Polymorphisms in *IGF1* have been evaluated in association studies for lactation persistence in Gentile di Puglia, Altamurana and Sarda sheep breeds (Scatà *et al.*, 2010), prolificity in Small Tail Han sheep (He *et al.*, 2012) and growth traits in Soviet Merino breed (Trukhachev *et al.*, 2016) and in Iranian Zandi sheep (Nazari *et al.*, 2016). Although there are no reports about polymorphisms in association with carcass traits in sheep, the *IGF1* expression levels have been associated with carcass traits in ovine (Su *et al.*, 2014; Sun *et al.*, 2014). In addition, Islam *et al.* (2009) reported about the effects of polymorphisms in *IGF1* on the carcass traits in beef cattle. Therefore, *IGF1* is also a potential candidate gene for carcass traits in sheep, requiring further exploration in association tests. Thus, the objective of this study was to identify polymorphisms in *GH* and *IGF1* in Santa Ines sheep in order to describe their allelic and genotypic frequencies as well as to test whether they are associated with carcass traits.

Material and methods

Animals and phenotypes

We evaluated 191 Santa Ines lambs at ∼ 240 days of age. Of these sheep, 105 were born between 2010 and 2012 on the experimental farm of the EMBRAPA Tabuleiros Costeiros; whereas, 86 were born in 2014 and raised on the experimental farm of the Federal University of Bahia. In all animals, BW at slaughter was measured after a fasting period of 16 h. After slaughter, the blood, head, feet, reproductive system and hot carcass weights (HCW) were measured (Table 1). In addition, the hot carcass yield (HCY = HCW/BW × 100) was recorded and expressed in percent. The carcasses were cooled in a cold chamber for 24 h at 2°C and weighed to obtain the cold carcass weight (CCW) and the cold carcass yield (CCY = CCW/BW × 100).

The carcass morphometric traits (Table 1) were obtained using a tape measure and compass. The carcass internal length (maximum distance between the cranial border of the pubis and the cranial border of the first rib at its midpoint), carcass external length (maximum distance between the cervical–thoracic joint and the first intercoccygeal joint), leg length (maximum distance between the major trochanter of the femur and the lateral border of the tarsus–metatarsal joint), carcass width at the thoracic region (maximum width of carcass measured at the rib level), thoracic depth (maximum distance between the sternum and the withers), carcass width at the rump region (maximum width of the carcass between the trochanters of the femurs) and rump girth (perimeter based on the trochanters of the femurs) were measured.

Table 1 *Sample size (*n*), mean and SD of the carcass traits in Santa Ines sheep*

Traits	*n*	Mean	SD
Hot carcass weight (kg)	185	16.17	3.57
Cold carcass weight (kg)	185	15.98	3.52
Hot carcass yield (%)	185	44.70	7.25
Cold carcass yield (%)	185	45.07	7.19
Carcass conformation score	185	2.22	0.35
Carcass finishing score	185	2.06	0.56
Carcass internal length (cm)	185	60.47	5.78
Carcass external length (cm)	185	64.20	7.34
Leg length (cm)	185	40.21	6.66
Rump girth (cm)	185	61.93	7.49
Carcass width at rump region (cm)	185	22.68	3.75
Carcass width at thoracic region (cm)	178	20.62	2.15
Thoracic depth (cm)	185	25.31	3.57
Palette weight (kg)	185	2.20	0.74
Palette yield (%)	185	18.80	2.96
Neck weight (kg)	185	1.12	0.39
Neck yield (%)	185	9.50	1.49
Rib weight (kg)	184	3.26	1.17
Rib yield (%)	184	28.44	8.33
Loin weight (kg)	185	1.14	0.46
Loin yield (%)	185	9.73	2.37
Leg weight (kg)	176	3.90	1.63
Leg yield (%)	176	31.99	6.34
Rib eye area (cm^2)	184	11.33	3.53

Visual scores from 1 to 5 were used to classify the carcasses for conformation and finishing (Table 1). Conformation visual scores were assigned as follows, according to the carcass musculature profile: 1 (concave profile – poor conformation), 2 (rectilinear profile – reasonable conformation), 3 (subconvex profile – good conformation), 4 (convex profile – very good conformation) and 5 (hyperconvex profile – excellent conformation). Carcass finishing visual scores were assigned according to the amount of fat on the carcass: 1 (very low quantity – very lean carcass), 2 (low amount – lean body), 3 (mean amount – mean carcass), 4 (high quantity – fatty carcass) and 5 (very high quantity – extremely fatty carcass).

The carcasses were sectioned in the middle; in the left half portion, a transversal cut was made between the 12th and 13th rib for the measurement of the rib eye area (Table 1). The contour of the cranial portion of the longissimus muscle was drawn on a transparent sheet with a pen to calculate the area after the scanning of the images in computational software.

The left half portion of the carcass was subdivided into five sections: palette (bone base: scapula, humerus, radius and ulna), neck (distance between the first and seventh cervical vertebrae), rib (distance between the first and 13th thoracic vertebra), leg (section between the last lumbar and the first sacral vertebras) and loin (lumbar vertebrae and muscles of this region). The yields (Table 1) of commercial cuts were calculated as a function of live weight at slaughter.

Extraction of genomic DNA and polymerase chain reaction
A 5-ml sample of blood was collected in ethylenediaminete-traacetic acid-containing vacutainer tubes and refrigerated at 4°C. Leukocytes were extracted following the protocol described by Oliveira *et al.* (2007) and sent to the São Paulo University for extraction of the genomic DNA. The DNA extraction was performed using the salt precipitation method and proteinase K digestion, according to Oliveira *et al.* (2007).

The primer design for amplification of the genes was made by observing the available sequence in the National Center for Biotechnology Information (NCBI) database NC_019460.2 (*IGF1*) and NC_019468.2 (*GH*), of the sheep genome (*Ovisaries*, version Oar_v4.0). The oligonucleotides were designed using the software package Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/). Net Primer was used to test the quality of the sequences using the rating parameters of >90%, the melting temperature (T_m) of the sense and anti-sense primers varied only by ±1°C and when the primers did not have dimer or cross dimer (http://www.premierbiosoft.com). After selecting the forward and reverse primers, basic local alignment search tool was used for sequence alignment in NCBI (http://blast.ncbi.nlm. Nih.gov/Blast.cgi) and to confirm the similarity with *Ovisaries*. The forward (GTGCTGCTTTTGTGATTTCTTG) and reverse (GATAGAAGAGATGCGAGGAGGA) primers were used for the amplification of 4.550 bp of *IGF1* between the regions of exon 1 to 2. For the *GH* gene, the forward (GCTGCTGA-CACCTTCAAAGA) and reverse (TGACCCTCAGGTACGTCTCC) primers were used for the amplification of 1194 bp, amplifying the region between exons 1 and 5.

For the PCR, we used 15 µl of the reaction mixture, containing 0.3 mM of each primer, Taq polymerase and 100 ng of the template DNA. The amplified products were evaluated for the presence of the desired bands using a pool of Santa Ines sheep DNA as a positive control. For the *IGF1*, a touch-down PCR was performed (initial denaturation: 98°C/5 min, followed by 10 denaturation cycles at 98°C/10 s, annealing at 61°C to 56°C, reducing −0.5°C at each cycle, for 30 s, and extension at 72°C/4 min. Following another 30 cycles with denaturation temperature at 98°C/10 s, annealing at 56°C/30 s and extension at 72°C/4 min, ending with an extension at 72°C/5 min). For *GH* amplification, a touch-down PCR was performed (initial denaturation: 98°C/5 min, followed by 20 denaturation cycles at 98°C/10 s, annealing at 63°C to 53°C, reducing −0.5°C at each cycle, for 30 s, and extension at 72°C/1 min. Following another 20 cycles with denaturation temperature at 98°C/10 s, annealing at 53°C/30 s and extension at 72°C/1 min, ending with an extension at 72°C/5 min).

Purification of amplicons and sequencing of libraries
The amplicons were purified with magnetic beads, and the recommended volume of AgencourtAMPure XP (Beckman Coulter, Brea, CA, USA) was used to homogenize the beads so as to bind to the amplified products. Immediately after this step, the samples were purified with 70% ethanol to remove the contaminants. The pellets were then diluted, and the beads were removed. The samples were diluted to 2 nM, considering the base pair size of the amplified products. The

samples were quantified with Qubit® fluorometer (Life Technologies, Carlsbad, CA, USA) and diluted to 0.2 ng/µl for library preparation.

The Nextera® XT DNA sample preparation and the Nextera® XT index (Illumina, San Diego, CA, USA) were used to prepare the library; all steps were performed based on the Nextera XT protocol. Sequencing was performed on the MiSeq platform (Illumina) by using the MiSeq Reagent Kit v2 (500 cycles).

Cleaning and aligning the reads
The qualities of the reads were verified by the FastQC software (www.bioinformatics.bbsrc.ac.uk/projects/). For the first data filtering, the SeqyClean software version 1.3.12 (Zhbannikov *et al.*, 2013) was used, adopting a quality parameter of 24 (Phred score) for each base and a minimum length of 50 bp. Subsequently, the reads were aligned against the reference sheep genome deposited in the NCBI (version Oar_v4.0) by using the Bowtie2 program (Langmead and Salzberg, 2012).

Polymorphism detection and structural annotation
The detection of polymorphisms was performed based on the position in the reference sheep genome (version Oar_v4.0): *IGF1* (171 037 883–171 113 228 bp) and *GH* (47 485 651 to 47 487 536 bp). In this step, the SAM tools software (version 1.4; Li and Durbin, 2009) was used. The PCR duplicates were then removed, the sequences were sorted and the index of the ordered file was constructed. The variant call was performed using the mpileup option of the SAM tools, covering a value set for the quality of the mapping through the genome reference (− q20) and a filter quality ⩾40 bases in the Phred (− q40) scale. We used the bcf tools command to convert the file form *.bcf to *.vcf. In this case, we performed more than 99 999 reads for this variant. Finally, the functional annotation of the single nucleotide polymorphisms (SNPs) and INDELs was performed using the variant effect predictor for the online annotation of Ensembl in order to identify the locations of the mutations across different regions of the genome and the possible functional effects of the variants.

Genotypic and allelic frequencies and association analysis
The allelic and genotypic frequencies were estimated for each locus by counting the alleles and genotypes, respectively, using the Statistical Analysis System (Statistical Analysis System, 2004). The Hardy–Weinberg equilibrium (HWE) was tested by comparing the predicted × observed heterozygosity. The predicted heterozygosity was obtained using the formula: $PH = 2(1 - MAF)(MAF)$, where, MAF is the minor allele frequency.

The association test was conducted using the Qxpak 5 program (Pérez-Enciso and Misztal, 2011), which performs a likelihood ratio test. The general model can be described as,

$y = \beta X + \sum_{k=1}^{n} Z\delta_k + \epsilon$, where y is the vector containing the

recorded traits, β is the vector of estimated solutions for the

fixed effects, δ_k is the solutions vector for the genetic effects for any of the n quantitative trait loci affecting the trait, X and Z are the incidence matrices that associate observations in y to the solutions β and δ_k vectors and ε is the residuals' vector. The fixed effects included in the model were farm (2 levels), year (4 levels), month of birth (10 levels) and the covariates BW and animal's age at the time of slaughter. For the yield traits, the covariate BW was not included in the model. The genetic effects included the additive and dominance effects. The additive effect is the contrast between homozygous genotypes, and when its value is positive, the mutant allele is associated with higher mean value; in another case, no-mutant homozygous showed higher mean values. The dominance effect indicates deviation in the heterozygous mean value from the homozygote mean value. When the dominance effect is positive, the mean value of the heterozygous is closer to the mean value of mutant homozygous; in another case, it is closer to the no-mutant homozygous. Bonferroni correction was used to establish the level of significance, and $P < 0.05$ was considered as suggestive associations.

Results

Polymorphisms

For *IGF1*, 20 samples did not amplify, which may be due to some mutation in the primer alignment region in these animals. Therefore, 171 animals were sequenced for *IGF1* and 191 animals for *GH*. In addition, 18 polymorphisms were found in the *IGF1* (Table 2) and 21 in the *GH* (Table 3).

All markers in the *IGF1* were present in intron-1, and the SNPs *rs1135847304* and *rs1135847305* are novel polymorphisms. The SNP *rs1135847304* is in HWE and showed genotypic frequencies of 77.19% (CC) and 22.81% (CA), whereas the *rs1135847305* showed frequencies of 98.25% (AA), 1.17% (AG) and 0.58% (GG), but not in HWE.

For the *GH*, 18 polymorphisms were present in intron and three in exon (Table 3). The SNPs *rs1135847308*, *rs1135847309*, *rs1135847310* and *rs1135847311* are novel polymorphisms that showed mutant allele frequencies of 1.30%, 4.95%, 1.04% and 14.84%, respectively. Among polymorphisms in *GH* gene, only the *rs1135847311* is not present in HWE.

The SIFT score predicts whether an amino acid change affects the function of the protein. The SNP with SIFT >0.05 is considered tolerable because its exchange cannot alter the function of the protein, but mutations with SIFT $\leqslant 0.05$ are considered non-tolerable, as the amino acid change alters the function of the protein, even inactivating its function (Ng and Henikoff, 2003). Among the polymorphisms detected in the exon region of *GH*, the SNP *rs1135847308* is a silent mutation located in exon-5, as the base exchange does not alter the amino acid threonine (amino acid 168 in the protein), whereas the other two mutations in exon are non-synonymous. The SNP *rs397514102* in exon-4 is a tolerable mutation, as its SIFT score was 0.22, which indicates that the exchange of valine by isoleucine (amino acid 123) does not

affect the function of the protein. The SNP *rs397514070* in exon-3 is not tolerable because its SIFT score was 0.01, which indicates that the exchange of the amino acid arginine for histidine (amino acid 60) can affect the function of the protein.

Association analysis

The *IGF1* polymorphisms *rs430457475*, *rs412470350*, *rs409110739* and *rs400113576* had additive effect on the internal carcass length (-0.9265 ± 0.4223), rump girth (-2.9285 ± 1.1473), rib yield (-1.0003 ± 0.4588) and neck weight (-0.0567 ± 0.0278), respectively. In addition, the SNP *rs430457475* showed dominance effects on the cold and HCYs, CCW and palette weight (Table 4).

The polymorphisms *rs58957314* in the *GH* had an additive effect on the rib weight (-0.4380 ± 0.1272) and rib yield (-2.2680 ± 0.6970), loin weight (-0.1893 ± 0.0516) and loin yield (-0.9423 ± 0.3259), palette weight (-0.2265 ± 0.0779) and palette yield (-0.9424 ± 0.4184), leg weight (-0.3960 ± 0.1375), neck weight (-0.0851 ± 0.0394) and the carcass finishing score (-0.1700 ± 0.0839).

Discussion

Polymorphisms

The present study revealed two novel polymorphisms in *IGF1* and four in the *GH* gene sequences of sheep. However, these polymorphisms showed small frequencies (Tables 2 and 3) for the mutant allele and several of them showed no-mutant homozygous animals. This made it difficult to use these novel polymorphisms to estimate the additive and dominance effects. We observed the well-conserved exon regions of these genes in Santa Ines. As the Santa Ines is a breed selected for growth, possible mutations in exon that positively affect the growth may have been fixed through selection. Similar results have been observed in previous studies with sheep. For example, Abdelmoneim *et al.* (2017) sequenced 2117 bp of *GH* in Harri sheep and found only one SNP at exon-4. Trukhachev *et al.* (2016) found 18 SNPs in *IGF1* in Russian Soviet Merino sheep, but only one at the exon region.

The three SNPs in the exons of *GH* showed mutant alleles with low frequencies. No homozygous mutant was observed for the SNPs *rs397514102* and *rs397514070*, whereas only one lamb was found to be homozygous for the mutant allele of *rs1135847308*. Previous studies with sheep indicated that mutations in the exons of *GH* may be deleterious. The variation A781G in the exon-2 of *GH* was studied by Seevagan *et al.* (2015) using Vembur sheep and Kumari *et al.* (2014) with nine other sheep breeds. This variation caused an amino acid change from serine to glycine, but the homozygous BB genotype was absent in both the studies. Thus, Seevagan *et al.* (2015) considered this variation as a possible embryonic deleterious effect. Thus, the missense mutation (*rs397514070*) found in exon-3 of *GH* in Santa Ines sheep may also be a deleterious mutation. However, a study with

Table 2 *Sample size (n), genotype (GF) and allelic (AF) frequencies, and Hardy–Weinberg equilibrium (HWE) for the single nucleotide polymorphisms (SNP) in IGF type-1 gene of Santa Ines sheep*

Polymorphisms*	Genotype	n	GF	Allele	AF	HWE P-value	Reference SNP
g.171108499T > G	TT	92	53.80	T	73.39		
	TG	67	39.18	G	26.61	1.00	rs430457475
	GG	12	7.02				
g.171108609C > A	CC	132	77.19	C	88.60		
	CA	39	22.81	A	11.40	0.17	rs1135847304
	AA	0	0.00				
g.171109001delA	GAGA	167	97.66	GA	98.83		
	GAA	4	2.34	A	1.17	1.00	rs595347398
	AA	0	0.00				
g.171109002A > G	AA	168	98.25	A	98.83		
	AG	2	1.17	G	1.17	0.04	rs1135847305
	GG	1	0.58				
g.171109151T > C	AA	9	5.26	A	5.56		
	AC	1	0.58	C	94.44	< 0.01	rs410261231
	CC	161	94.15				
g.171109262T > C	TT	110	64.33	T	77.49		
	TC	45	26.32	C	22.51	< 0.01	rs421621914
	CC	16	9.36				
g.171110364C > G	CC	161	94.15	C	96.49		
	CG	8	4.68	G	3.51	0.02	rs401398263
	GG	2	1.17				
g.171110428C > T	CC	40	23.39	C	48.25		
	CT	85	49.71	T	51.75	1.00	rs412470350
	TT	46	26.90				
g.171110492C > T	CC	151	88.30	C	94.15		
	CT	20	11.70	T	5.85	1.00	rs402300271
	TT	0	0.00				
g.171110600G > A	GG	125	73.10	G	85.96		
	GA	44	25.73	A	14.04	0.64	rs418030625
	AA	2	1.17				
g.171110688C > T	CC	123	71.93	C	85.38		
	CT	46	26.90	T	14.62	0.52	rs425204511
	TT	2	1.17				
g.171110860G > T	GG	136	79.53	G	89.18		
	GT	33	19.30	T	10.82	1.00	rs403521045
	TT	2	1.17				
g.171111015T > G	TT	11	6.43	T	6.43		
	TG	0	0.00	G	93.57	< 0.01	rs414846691
	GG	160	93.57				
g.171111287C > T	CC	90	52.63	C	72.51		
	CT	68	39.77	T	27.49	1.00	rs430449367
	TT	13	7.60				
g.171111426G > A	GG	25	14.62	G	32.16		
	GA	60	35.09	A	67.84	0.02	rs409110739
	AA	86	50.29				
g.171112440C > T	CC	159	92.98	C	96.20		
	CT	11	6.43	T	3.80	0.42	rs421570650
	TT	1	0.58				
g.171112488delCA	CCACCA	165	96.49	CCA	98.25		
	CCAA	6	3.51	A	1.75	1.00	rs600588782
	AA	0	0.00				
g.171112496C > T	CC	105	61.40	C	76.02		
	CT	50	29.24	T	23.98	0.02	rs400113576
	TT	16	9.36				

*All polymorphisms are in the intron-1.

Table 3 *Sample size (*n*), genotype (GF) and allelic (AF) frequencies, and Hardy–Weinberg equilibrium (HWE) for the single nucleotide polymorphisms (SNP) in growth hormone gene of Santa Ines sheep*

Polymorphisms	Genotype	*n*	GF	Allele	AF	HWE P-value	Region	Reference SNP
g.47485910G > C	GG	188	97,92	G	98,70			
	GC	3	1,56	C	1,30	0.05	exon-5	rs1135847308
	CC	1	0,52					
g.47485969G > A	GG	185	96,35	G	98,18			
	GA	7	3,65	A	1,82	1.00	Intron-4	rs397514078
	AA	0	0,00					
g.47486059G > A	GG	182	94,79	G	97,40			
	GA	10	5,21	A	2,60	1.00	Intron-4	rs397514077
	AA	0	0,00					
g.47486149A > C	AA	149	77,60	A	88,80			
	AC	43	22,40	C	11,20	0.14	Intron-4	rs397514076
	CC	0	0,00					
	TT	7	3,65	T	49,22			
g.47486221T > C	TC	175	91,15	C	50,78	< 0.01	Intron-4	rs397514074
	CC	10	5,21					
g.47486322C > T	CC	172	89,58	C	94,79			
	CT	20	10,42	T	5,21	1.00	exon-4	rs397514102
	TT	0	0,00					
	CC	39	20,31	C	57,55			
g.47486418C > G	CG	143	74,48	G	42,45	< 0.01	Intron-3	rs397514073
	GG	10	5,21					
	TT	5	2,60	T	48,70			
g.47486424T > C	TC	177	92,19	C	51,30	< 0.01	Intron-3	rs397514072
	CC	10	5,21					
	TT	2	1,04	T	9,64			
g.47486448T > G	TG	33	17,19	G	90,36	1.00	Intron-3	rs1092944696
	GG	157	81,77					
	CC	159	82,81	C	91,41			
g.47486453C > A	CA	33	17,19	A	8,59	0.44	Intron-3	rs1092437056
	AA	0	0,00					
	GG	173	90,10	G	95,05			
g.47486604G > A	GA	19	9,90	A	4,95	1.00	Intron-3	rs1135847309
	AA	0	0,00					
	CC	169	88,02	C	94,01			
g.47486736C > T	CT	23	11,98	T	5,99	0.99	exon-3	rs397514070
	TT	0	0,00					
	GG	174	90,63	G	95,31			
g.47486781G > T	GT	18	9,38	T	4,69	1.00	Intron-2	rs397514069
	TT	0	0,00					
	CC	130	67,71	C	82,55			
g.47486819C > A	CA	57	29,69	A	17,45	0.92	Intron-2	rs589527314
	AA	5	2,60					
	TT	171	89,06	T	94,53			
g.47486832T > C	TC	21	10,94	C	5,47	1.00	Intron-2	rs1087440770
	CC	0	0,00					
	CC	9	4,69	C	7,55			
g.47486836C > T	CT	11	5,73	T	92,45	< 0.01	Intron-2	rs397514068
	TT	172	89,58					
	AA	188	97,92	A	98,96			
g.47486837A > T	AT	4	2,08	T	1,04	1.00	Intron-2	rs1135847310
	TT	0	0,00					
	CC	182	94,79	C	97,40			
g.47486853C > T	CT	10	5,21	T	2,60	1.00	Intron-2	rs397514066
	TT	0	0,00					
	AA	162	84,38	A	92,19			
g.47486896A > G	AG	30	15,63	G	7,81	0.59	Intron-2	rs397514065

Table 3 (*Continued*)

Polymorphisms	Genotype	*n*	GF	Allele	AF	HWE *P*-value	Region	Reference SNP
	GG	0	0,00					
	GG	135	70,31	G	85,16			
g.47486910 G > T	GT	57	29,69	T	14,84	0.02	Intron-2	rs1135847311
	TT	0	0,00					
	GG	162	84,38	G	92,19			
g.47486914G > A	GA	30	15,63	A	7,81	0.59	Intron-2	rs397514064
	AA	0	0,00					

Table 4 *Additive (a) and dominance (d) effects with its respective SE, in the association tests of polymorphisms in Santa Ines sheep*

Traits	a(SE)	d(SE)	LRT	*P*-value
IGF type-1 (*IGF1*)				
g.171108499T > G				
Internal carcass length	− 0.9265 (0.4223)	–	4.74	0.0294
Cold carcass yield	–	3.4543 (0.9347)	13.12	0.0003*
Hot carcass yield	–	3.3643 (0.9153)	12.98	0.0003*
Cold carcass weight	–	1.4762 (0.4084)	12.57	0.0004*
Palette weight	–	0.1813 (0.0742)	5.86	0.0154
g.171110428C > T				
Rump girth	− 2.9285 (1.1473)	–	6.39	0.0115
g.171111426G > A				
Rib yield	1.0003 (0.4588)	–	4.69	0.0304
g.171112496C > T				
Neck weight	0.0567 (0.0278)	–	4.10	0.0429
Growth hormone (*GH*)				
g.47486819C > A				
Rib weight	− 0.4380 (0.1272)	–	11.49	0.0007*
Rib yield	− 2.2680 (0.6970)	–	10.29	0.0013*
Loin weight	− 0.1893(0.0516)	–	13.01	0.0003*
Loin yield	− 0.9423 (0.3259)	–	8.17	0.0043*
Palette weight	− 0.2265 (0.0779)	–	8.27	0.0040*
Palette yield	− 0.9424 (0.4184)	–	5.01	0.0253
Leg weight	− 0.3960 (0.1375)	–	8.10	0.0044*
Neck weight	− 0.0851 (0.0394)	–	4.61	0.0318
Carcass finishing score	− 0.1700 (0.0839)	–	4.06	0.0439

LRT = likelihood ratio test.
*Significant association at Bonferroni level (*P* < 0.0055).

more animals and other sheep breeds is required to confirm this hypothesis.

Insulin-like growth factor type-I association tests
Our results indicated that polymorphisms in *IGF1* can be used for improving some carcass traits, because the additive effect were observed for the internal carcass length, rump girth, rib yield and neck weight (Table 2). The differences between homozygous genotypes were 1.85 cm (internal carcass length), 5.86 cm (rump girth), 2.00% (rib yield) and 0.1134 kg (neck weight), which can result in economic impacts on large-scale activity. The no-mutant alleles of SNPs *rs43057475* and *rs412470350* were associated with the largest values of the internal carcass length and rump girth, respectively, whereas the mutant alleles of *rs409110739 and rs400113576* were associated with higher means of rib yield

and neck weight, respectively. The frequencies of alleles associated with the largest mean values were 73.39% (*rs430457475*), 48.25% (*rs412470350*), 67.84% (*rs409110739*) and 23.98% (*rs400113576*). Therefore, there are wide frequencies of these SNPs for the selection purpose.

According to the sequences deposited in the NCBI, *IGF1* have 75 346 bp, but due to financial reasons, we sequence 6% (4550 bp) of *IGF1*, including exons 1 to 2. We chose this region around the intron-1, as a previous study about sheep already reported effects on the growth traits. Trukhachev *et al.* (2016) evaluated Russian Soviet Merino sheep and found significant effect of the SNPs *c.-5363C > T, c.-5188G > C, c.-5186G > A* and *c.-4088G > A*, all located in the intron-1, associated with BW. In addition, the SNP *c-91A > C* influenced the BW, croup and withers heights as well as the width and length of rump.

This previous result already indicated the possibility of having effects of polymorphisms at intron-1 on the carcass traits. However, to the best of our knowledge, the present study is the first to report SNP in *IGF1*, affecting carcass traits in sheep. The results of the present study confirmed some other previous findings. Su *et al.* (2014) reported that *IGF1* expression in the longissimus dorsi muscle was positively and significantly correlated with the diameter of the muscle fibers, indicating that polymorphisms in this gene may affect meat production in sheep. In addition, Sun *et al.* (2014) reported that the level of *IGF1* expression was positively correlated ($P < 0.01$) with BW and carcass weight ($P < 0.05$) in Hu sheep.

Growth hormone association tests
We found association of SNP *rs589527314* with the weights and yields of several carcass cuts and the carcass finishing score (Table 4). The differences between the homozygous (*CC* and *AA*) were 0.876 kg and 4.54% (rib), 0.379 kg and 1.89% (loin), 0.453 kg and 1.89% (palette), 0.792 kg of leg, 0.170 kg of neck and 0.34 scores of carcass finishing. The no-mutant allele (*C*) was associated with higher mean values of these traits and it had high frequency (82.55%) in this population. However, the *CC* frequency (67.7%) is not very high and may be increased to improve the carcass quality of Santa Ines sheep.

According to the sequences deposited in NCBI, *GH* has 1886 bp, but for financial reasons, we could sequence 63.3% (1194 bp) of this sequence, including exons 1 to 5. Several previous studies evaluated polymorphisms in this fragment of sheep *GH* and reported associations with growth traits. Using PCR–SSCP, Moradian *et al.* (2013) reported an effect of polymorphism on exon-4, which affected BW in Makooei sheep, whereas Jia *et al.* (2014) reported polymorphisms in exon-4, which affected the BW and morphometric traits in the Tibetan and Poll Dorset breeds. Using PCR–RFLP and restriction enzyme, Malewa *et al.* (2014) identified polymorphisms associated with the weaning weight and growth rate in Donggala and East Java breeds, whereas Nasution *et al.* (2017) reported an association with growth and morphometric traits in sheep of the Jambi Province, Indonesia. Cauveri *et al.* (2016) found an association ($P < 0.05$) between the SNP *G480A* in the intron-1 and weaning weight and preweaning weight gain in Nilagiri sheep. Han *et al.* (2016) reported the effects of SNPs *g.616 G > A* (intron-2) and *g.498 G > C* (exon-2) on BW and morphometric traits of Chinese sheep breeds, whereas Abdelmoneim *et al.* (2017) reported SNPs in the intron-2 (*G871A*), exon-4 (*G1383A*) and intron-4 (*A1509G*), associated with BW and weight gain in Harri sheep in Saudi Arabia. Therefore, it is expected that there are also effects on carcass traits, since they are, at some level, correlated with the BW. However, only few carcass traits were explored in association tests with polymorphisms in *GH* in sheep. Gorlov *et al.* (2017) were the only ones to report a *GH*/HaeIII gene polymorphism associated with carcass traits such as carcass weight and yield of carcass as well as the heart and kidney weight in Russian Salsk sheep. Therefore, the present study reinforced the hypothesis that polymorphisms in *GH* influenced the carcass traits in sheep, but we identified potential SNPs that explained these effects.

In conclusion, we identified several mutations in *IGF1* and *GH* genes and some polymorphisms in association with carcass traits in Santa Ines sheep, including weights and yields of carcass and commercial cuts. Thus, this study showed that polymorphism in *IGF1* and *GH* may provide important information for marker-assisted selection schemes in Santa Ines sheep. However, we did not find causal mutation, and the main hypothesis is the linkage disequilibrium between intronic mutations associated with carcass traits and the causal mutation into these genes or nearby genes. Therefore, future investigations are necessary to extend our findings and to identify the causal mutation.

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