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Summary

The objective of this study was to describe the VNTR polymorphism of the mucin 1 gene (MUC1) in three Nelore lines selected for yearling weight to determine whether allele and genotype frequencies of this polymorphism were affected by selection for growth. In addition, the effects of the polymorphism on growth and carcass traits were evaluated. Birth, weaning and yearling weights, rump height, Longissimus muscle area, backfat thickness, and rump fat thickness, were analyzed. A total of 295 Nelore heifers from the Beef Cattle Research Center, Instituto de Zootecnia de Sertãozinho, were used, including 41 of the control line, 102 of the selection line and 152 of the traditional. The selection and traditional lines comprise animals selected for higher yearling weight, whereas control line animals are selected for yearling weight close to the average. Five alleles were identified, with allele 1 being the most frequent in the three lines, especially in the lines selected for higher means for yearling weight. Heterozygosity was significantly higher in the control line. Association analyses showed significant effects of allele 1 on birth weight and weaning weight while the allele 3 exert significant effects on yearling weight and back fat thickness. Despite these findings, application of this marker to marker-assisted selection requires more consistent results based on the genotyping of a larger number of animals in order to increase the accuracy of the statistical analyses.

Keywords: beef cattle, QTL, VNTR, marker assisted selection, zebu

Introduction

In beef cattle, growth and carcass traits are important economical traits. With improved selection schemes by marker assisted selection in mind, the approach of candidate gene has been applied by molecular biologists and animal breeders for identification of molecular markers in livestock. Several genes with significant associations for these traits has been identified including AMPD1 and *PPARγ* for carcass traits, and NUCB2, GAD1 and SDC1 for growth traits [1,2,3,4,5].

Epithelial mucins are heavily O-glycosylated proteins found in the mucus layer or at the cell surface of many epitheliums. They are responsible for the physical properties of mucus gels and are involved in epithelial cell protection. Thus, the central role played by mucins lies in accommodating the resident commensal flora and limiting infectious disease of mucosal tissues that presents an enormous surface area to the exterior environment [6].

Like other mucins, mucin 1 (MUC1), whose main function is the protection and lubrication of epithelial surfaces, is a transmembrane glycoprotein that is co-dominantly expressed on the apical surface of secretory epithelial cells of the respiratory, digestive, urinary and reproductive tracts of mammals [7, 8, 9]. Additionally, MUC1 has been reported to play a role in cell growth, fetal development, epithelial regeneration and differentiation, epithelial integrity, tumor genesis, and metastasis [10].

Bovine MUC1 is well characterized and is also known as PAS due to its affinity for periodic acid Schiff reagent. In Danish Holstein cattle, the complete molecule consists of 580 amino acids, with the 22-amino acid N-terminal sequence comprising the signal peptide. After cleavage of the signal peptide, the sequence can be divided into three domains: a phosphorylated intracellular domain consisting of 70 amino acids, a hydrophobic transmembrane domain, and a long extracellular domain. The SEA domain, comprising 120 amino acids, and the polymorphic VNTR, consisting of tandem repeats of 20 amino acids, are found in the extracellular domain [11]. The VNTR is an important region of the molecule due to its dense O-glycosylation and its relationship with the adhesive or anti-adhesive role of MUC1 in the organism.

In domestic ruminants, due to the abundant expression of MUC1 in the mammary glands [12], the VNTR polymorphism of the gene has been characterized mainly in species reared for milk production such as cattle (Bos primigenius taurus), goats (Capra hircus) and sheep (Ovies aries) [13-16]. In Holstein cattle, associations of the polymorphism with milk production and composition traits, as well as with

traits related to fertility and disease resistance, have been reported [17]. The polymorphism was also characterized in Zebu beef cattle (Bos primigenius indicus) and its effect on growth, fertility and carcass traits was investigated, but no evidence of an association was found [18-21].

In view of the scarcity of association studies between the MUC1 gene polymorphism and traits of economic interest in beef cattle, the objective of the present investigation was to analyze the VNTR polymorphism of the MUC1 gene in three Nelore lines selected for yearling weight in order to determine whether allele and genotype frequencies were affected by selection for growth. In addition, the effects of the polymorphism on growth and carcass traits of the animals were evaluated.

Methods

Animals

A total of 295 Nelore heifers born between 2003 and 2005, which belonged to three lines selected for growth at the Estação Experimental de Zootecnia de Sertãozinho (EEZS), a research facility of the Instituto de Zootecnia, located in the northern region of the State of São Paulo, Brazil (21°10′ south latitude and 48°5′ west longitude), were used. This region is characterized by a wet tropical climate, with average annual temperature and rainfall of 24°C and 1,312 mm, respectively. Pastures mainly consist of Panicum maximum and Brachiaria brizantha, which are common tropical grasses in Brazil.

The animals are part of the Zebu Selection Program started in 1978 and have been allocated to the following three selection lines since 1980: Nelore selection line (NeS), traditional Nelore line (NeT), and control Nelore line (NeC). In the NeS and NeT lines, males are selected for increasing weight adjusted to 378 days of age (W_{378}) after performance testing in confinement. Females are selected for weight adjusted to 550 days of age (W_{550}) on pasture. In the NeC line, the animals are selected based on W_{378} and W_{550} close to the average (selection differential of zero). The NeC and NeS lines are closed, whereas the NeT line differs from the NeS line because it eventually received sires from other herds, including commercial ones. The mean breeding values for yearling weight of animals born in the last four years (2004 to 2007), corresponding to 5.5 generations selected for growth, are 0.2, 49.5 and 55.3 kg for the NeC, NeS and NeT lines, respectively [22]. Forty-one heifers of the NeC line, 102 of the NeS line, and 152 of the NeT line were genotyped.

Traits studied

Birthweight (BW), weaning weight (adjusted to 210 days of age, W_{210}), and yearling weight (adjusted to 550 days of age, W_{550}) are obtained as part of the selection program. Yearling height (YH_{550}) is obtained at the time of weight recording at 550 days.

Longissimus muscle area (LMA), subcutaneous backfat thickness (BF), and rump fat thickness (RF) were measured by ultrasound at an average age of 22 ± 2.5 months. BF and LMA were measured on transverse ultrasound images taken between the 12th and 13th ribs over the Longissimus dorsi muscle. RF was measures at the intersection between the Gluteus medius and Biceps femoris muscles located between the hook and pin bone. Two ultrasound devices were used depending on the occasion of measurement: Aloka 500V (Corometrics Medical Systems, Inc., Wallingford, CT, USA) equipped with a linear 3.5-MHz probe (17.5 cm) (transducer: Aloka Co. Ltd., Tokyo, Japan), and Pie Medical 401347 – Aquila (Esaote Europe B.V., The Netherlands) equipped with a linear 3.5-MHz probe (18 cm). Vegetable oil and a standoff were used for capture of the images to guarantee acoustic contact between the linear probe and body of the animal. The images were saved and subsequently interpreted using the Echo Image Viewer 1.0 software (Pie Medical Equipment B.V., 1996).

Genotyping

Blood (5 mL) was collected from each animal by puncture of the jugular vein into Vacutainer tubes containing 7.5 mg EDTA. Genomic DNA was extracted according to the saline standard protocol.

Genotyping was performed by the polymerase chain reaction (PCR) using the Qiagen Hot Start Taq enzyme (Qiagen GmbH, Hilden, Germany). The reaction mixture contained 100 ng genomic DNA, 1X buffer, 1X solution Q, 1.5 mM $MgCl_2$, 1 mM dNTPs, 0.5 µM of each primer, and 2.5 units of the enzyme in a volume of 50 µL. The P4 - P5 primer pair described by Rasero [13] were used. PCR was carried out in a GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA, USA) under the following conditions: denaturation at 95º C for 15 min, followed by 37 cycles of denaturation at 94º C for 30 s, primer annealing at 60º C for 30 s, and extension at 72º C for 1 min and 30 s. A final extension was carried out at 72º C for 10 min.

The amplified fragments were separated by electrophoresis on 1.5% agarose gel. The length of the alleles was estimated using the GeneRuler^{TM} 100-bp DNA Ladder Plus molecular weight marker (Fermentas International, Inc., Burlington, Ontario, Canada).

Statistical analysis

Allele and genotype frequencies were compared between the three lines by Fisher's exact test using the population differentiation module of the GENEPOP program, version 3.4 [23] (http://genepop.curtin.edu.au/). A P value ≤ 0.05 was considered to be significant.

The association analyses were based on the methods described by Byun, Forrest, Lin, and Hickford [24-27].

Association analysis between MUC1 genotypes and the traits studied was performed using the PROC MIXED procedure of the SAS/STAT 9.1.3 program (SAS Institute, Inc., Cary, NC,USA). The analysis model included marker genotype (1, ..., 10), contemporary group (selection line and year of birth, 1, ..., 9) and month of birth (September, October, November) as fixed effects, age of dam and age at recording (only for YH_{550} and the carcass traits LMA, BF and RF) as linear covariates, and the random effect of sire (1, ..., 41). All genotypes were included in the analysis.

A second set of analyses was performed in order to evaluate the effect of the presence/absence of the MUC1 alleles on each trait. The allele presence or absence (coded as 1 or 0, respectively) was considered in place of the marker genotype. Initially, a "single-allele" model was performed testing the presence/absence of each allele in a trait. Subsequently, for the alleles that showed $P<0.20$ in the "single allele" model, a "multi-allele" model was performed which included all the alleles.

Finally, to analyze the effect of the number of copies of each allele on each trait the number of copies (0, 1, and 2 copies) of each allele was considered. Only alleles that had homozygous forms > 1% of all genotypes were included. Using this approach, each allele was tested separately and, subsequently, alleles with $P<0.20$ were included in a "multi-allele" model with all alleles. To ascertain whether additive, dominant or recessive effects were present, the significant effects were followed by multiple pair-wise comparisons (Least Significant Difference tests).

For the association analyses all P-values were considered statistically significant when $P<0.05$ and trends were considered when $0.05<P<0.10$.

Results

The means and standard deviations obtained for each trait according to selection line are shown in Online resource 1. In general, the NeS and NeT lines presented higher means for all traits, except for BF and RF.

Five alleles of different lengths were amplified and named allele 1, allele 2, allele 3, allele 4, and allele 5. Allele 1 was the predominant allele in the three selection lines, followed by allele 4 with a frequency of 0.25 in the NeC line, 0.08 in the NeS line, and 0.07 in the NeT line. In general, alleles 2 and 5 were the least frequent, with allele 2 not being detected in the NeC line. Compared to the selection lines NeS and NeT, the NeC line was characterized by a high frequency of alleles 4 and 5. Genotype 1/1 was the most frequent in all selection lines, followed by genotype 1/4 (Table 1). Although the NeC line presented only five genotype classes, heterozygosity (0.46) was higher than in the NeS line with seven genotype classes (0.16) and in the NeT that presented nine genotype classes (0.25).

Fisher's exact test showed a significant difference in allele and genotype frequencies between the lines selected for higher growth (NeT and NeS) and the control line. Regarding allele frequency, P values of less than 0.001 were observed for comparison between the NeC line and the NeS and NeT lines, whereas no significant difference (P = 0.38) was found between the two lines selected for higher growth (NeS and NeT). With respect to genotype frequency, the P values for comparison between the NeC line and the NeS and NeT lines were less than 0.001, whereas no significant difference (P = 0.46) was observed between the two lines selected for higher growth (NeS and NeT).

The statistical analysis most frequently applied in association studies in animal breeding revealed no evidence of a significant association between the genotypes and the traits analyzed (Online Resource 2). The statistical analysis considering the presence/absence of the MUC1 alleles revealed that the alleles 1 and 3 were associated with some economic traits in Nelore cattle (Table 2). Using the single-allele model, the absence of allele 3 was significantly associated with higher mean for W_{550} ($P<0.05$). A trend was also observed between the absence of allele 3 and higher mean for BF ($P<0.10$). Considering the multi-allele model, which included the alleles with $P<0.20$ in the single allele model, a significant effect ($P<0.05$) and a trend ($P<0.10$) for the allele 1 were found for higher means on BW and W_{210}, respectively.

For the allele 3, changing from the single-allele to the multi-allele model, its effect in W_{550} became non-significant whereas the trend between higher means for BF remained.

In the third set of analyses the effects of the number of copies of each allele in each trait was investigated (Table 3). The analysis was performed for those alleles with sufficiently common homozygous forms (>1% of all genotypes), so only the alleles 1, 3 and 4 were selected. In the single allele-model the significant effects of allele 1 on BW and of allele 3 on W_{550} were observed ($P<0.05$). Considering BW, animals with just one copy of allele 1 presented means significantly higher than animals with any copy or two copies of this allele. For W_{550} was observed that animals with no or one copy of allele 3 presented means significantly higher than animals with two copies of this allele. In the multi-allele model, which included the alleles with $P<0.20$ in the single allele model, just the effect of allele 3 on W_{550} persisted ($P\leq0.05$).

Discussion

In humans, MUC1 protein have several isoforms originating from alternative splicing, including MUC1/transmembrane, MUC1/secreted and MUC1/Y. MUC1/secreted lacks the hydrophobic transmembrane and cytoplasmic domains required for anchorage to the cell membrane, and MUC1/Y lacks the tandem repeat domains. The MUC1/transmembrane is the complete peptide expressed as a continuous polypeptide, however, the molecule is cleaved between the transmembrane and VNTR domains during its processing in complex Golgi complex although the two parts of the molecule remain associated via noncovalent forces [6].

Like all mucins, the best-described functions of MUC1/transmembrane are those of mucus layers: hydration, lubrication, protection from proteases, and defense against pathogens. The glycosilation pattern provides a hydrophilic environment ideal for hydration and lubrication of epithelia. In addition, the large protein core and dense sugar chains prevent access to the epithelium below. Thus, MUC1 extend considerably further from the cell surface than many cellular receptors, providing a mechanism that can disrupt the adhesion of cells and pathogens [28]

The gene MUC1 bovine lies in BTA3 and like in humans is formed by seven exons and six introns. In humans, the promoter of MUC1 gene contains numerous potential binding sites for transcriptional regulators, among them Sp1, AP1-4, NF-1, NF-·B, an E-box, GC boxes, and estrogen and

progesterone receptor sites. The VNTR in exon 2 of MUC1 gene is an important region because of the abundant presence of codons coding for serine and threonine residues in all mammalian species studied to date. These are the main amino acids that undergo dense O-glycosylation during processing of the protein in the Golgi complex, permitting steric hindrance that confers a broad functional role to the molecule in the organism of mammals [6].

The VNTR polymorphism of the MUC1 gene has been extensively studied in humans and domestic animals. In humans, the number of repeats at the VNTR locus ranges from 25 to 125 and this polymorphism has been associated with severe acne [29], conditions that precede gastric carcinoma [30], lung adenocarcinoma [31], and infertility due to failure in embryo implantation [32, 33]. In ruminants, the number of repeats ranges from 10 to 27 in cattle [13, 18], from 30 to 50 in goats [14], and from 19 to 22 bp in sheep [15]. An association between the polymorphism and milk production, health and fertility has been reported for the Holstein dairy breed (Bos primigenius taurus) [17]. The present study described the pattern of this polymorphism in three lines selected for yearling weight and its association with growth and carcass traits in Nelore beef heifers (Bos primigenius indicus).

As reported in previous studies, Nelore is characterized by 5 alleles of the MUC1 gene VNTR [18, 20, 21]. The allele 1 was the predominant allele in the Nelore breed in both the sample as a whole and in each selection line, especially in the NeS and NeT lines. However, in contrast to the results reported so far for Nelore animals, the second most frequent allele in all selection lines was allele 4, especially in the NeC line. The frequencies of allele 2 and allele 3 were lower than the frequencies reported in previous studies and these alleles showed a lower frequency than allele 5 in the NeC line. Alleles 4 and 5 have so far been considered the rarest alleles in the Nelore breed [18,20,21].

This is the first study reporting significant associations between the MUC1 gene polymorphism and economic traits in beef cattle. In previous studies using samples from a commercial Nelore herd [20,21], this polymorphism was characterized in this breed and its effects on early puberty and expected progeny differences for growth, carcass and fertility traits were investigated. No evidence of an association was found.

In the present study, the statistical methods commonly used in association studies between genetic polymorphisms and economic traits showed no significant associations. Therefore, the approach reported by Hickford [27] and Forrest [25] was used as an alternative for a more complete analysis.

The presence of allele 1 seems to be favorable for BW and W_{210} since it showed significant effects and trends on the multi allele model, respectively. For BW, the analysis of the number of copies also showed significant effects of this allele, being the heterozygous animals the heavier animals. Weight at different ages in Nelore and Japanese Black cattle have positive correlation that decreases with the increasing of the interval between the ages [34,35,36]. This allele showed higher frequencies in the two lines selected for yearling weight (NeS and NeT - 378 days for sires and 550 days for females), suggesting that this selection scheme is favoring the allele 1 in this lines and indirectly increasing the means for BW and W_{210} in the animals.

The allele 3 seems unfavorable for W_{550} and BF since its presence was associated with low means for these traits in the single allele model. In the multi allele model just the trend for BF remained for this allele. Smith [37] related low correlation (0.10) between slaughter weight and BF in Brahman cattle. Similarly, Yokoo [38], described a correlation close to zero (0.06) between W_{550} and BF in Nelore cattle, thus, these effects on W_{550} and BF cannot be attributed to genetic correlation. Considering the analysis of the number of copies, the allele 3 is significative in both analysis (single and multi allele models) for W_{550}, being animals with any or one copy heavier than animals with two copies. These results suggests that this allele have recessive effects for W_{550}, although caution is needed in drawing this conclusion, as the number of heifers carrying allele 3, especially in a homozygous genotype, is low. Although the frequency of the allele 3 in the line NeC is low its effect on W_{550} may explain the lower frequencies of the allele 3 in the lines NeS and NeT, which the females are selected considering higher means for W_{550}, but it is also necessary to consider the low frequency of this allele in other studies with Nelore cattle and the probability of its low frequency be a characteristic of the herd that initiated this three selection lines in the EEZS at 1978 [18, 20, 21].

The main explanation between these effects and MUC1 variation could lie in the protection and lubrication of epithelial tissues. The ability of bacteria to colonise the intestinal tract and initiate pathogenesis is intimately associated with their ability to bind receptors displayed on the surface of digestive epithelial cells [39]. The variations of MUC1 may influence the health of digestive tract of calves and mature animals permitting weight gain and to deposit fat. Thus, MUC1 acts as a barrier against opportunistic infections by some bacterial strains [9,16,40]. Another explanation is that the alleles 1 and 3 are in linkage disequilibrium with another DNA variation that influences directly the traits under study.

Indeed, there is evidence suggesting the existence of five QTLs in Bos primigenius taurus X Bos primigenius indicus crosses with additive effects on BW on chromosome 3, where the MUC1 gene is located [41]. Beever [42] also reported the existence of a QTL for weaning weight on this chromosome in nine half-sib families of sires originating from the European breeds Simmental, Gelbvieh, or South Devon. In addition to the QTL for BW, Casas [43] also found QTLs for subcutaneous fat thickness and for marbling on chromosome 3.

In conclusion, the results showed a high frequency of allele 1 in three selection lines, especially in the lines NeS and NeT selected for higher yearling weight. The allele 1 was associated with higher means for BW and W_{210}, while the allele 3 exert significant unfavorable on W_{550} and BF. However, application of this marker to marker-assisted selection requires more consistent results based on the genotyping of a larger number of animals in order to increase the number of representatives in each genotype class and, consequently, the accuracy of the statistical analyses.

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Table

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Table 1. Allele and genotype frequencies of the MUC1 polymorphism in the total sample and each Nelore selection line.

Total				NeC[1] (N = 41)				NeS[1] (N = 102)				NeT[1] (N = 152)			
Allele frequency		Genotype frequency		Allele frequency		Genotype frequency		Allele frequency		Genotype frequency		Allele frequency		Genotype frequency	
1	0.82	1/1	0.71	1	0.68	1/1	0.48	1	0.87	1/1	0.80	1	0.83	1/1	0.71
2	0.02	1/2	0.02	2	0	1/3	0.05	2	0.01	2/2	0.01	2	0.03	1/2	0.04
3	0.05	2/2	0.01	3	0.03	1/4	0.34	3	0.03	1/3	0.05	3	0.06	2/2	0.01
4	0.10	1/3	0.06	4	0.25	4/4	0.06	4	0.08	3/3	0.01	4	0.07	1/3	0.09
5	0.01	2/3	0.01	5	0.04	4/5	0.07	5	0.01	1/4	0.10	5	0.01	2/3	0.01
		3/3	0.02							4/4	0.02			3/3	0.01
		1/4	0.12							4/5	0.01			1/4	0.09
		4/4	0.03											4/4	0.02
		1/5	0.02											1/5	0.02
		4/5	0.01												

[1]NeC: control line; NeS: selection line; NeT: traditional line.

Table 2. Associations of MUC1 alleles with growth and carcass traits in Nellore cattle.

Trait[1]	Allele	Other alleles in the Model	Mean±Standard Error				
			Allele absent	N	Allele present	N	Pvalue
BW	1	None	27.77±0.90	18	29.13±0.36	277	0.13
	2	None	29.04±0.36	286	29.11±1.25	9	0.95
	3	None	29.02±0.36	271	29.32±0.80	24	0.69
	4	None	28.88±0.38	246	29.59±0.59	49	0.24
	5	None	29.05±0.36	288	28.40±1.43	7	0.64
	1	**2,3,4 and 5**	**28.11±1.15**	**18**	**30.41±1.21**	**277**	**0.02**
W_{210}	1	None	169.86±5.16	18	176.94±1.61	276	0.18
	2	None	176.33±1.57	285	179.09±7.44	9	0.71
	3	None	176.54±1.59	270	174.11±4.54	24	0.59
	4	None	176.58±1.81	245	175.78±3.13	49	0.82
	5	None	176.23±1.58	287	181.74±8.37	7	0.51
	1	**2,3,4 and 5**	**174.60±6.6**	**18**	**185.22±7.19**	**277**	**0.09**
W_{550}	1	None	287.64±6.12	18	293.51±2.05	276	0.34
	2	None	292.86±2.02	285	303.12±8.67	9	0.23
	3	**None**	**293.92±1.95**	**270**	**282.47±5.31**	**24**	**0.03**
	4	None	292.34±2.24	245	295.35±3.79	49	0.48
	5	None	292.85±2.00	288	302.68±10.69	6	0.36
	3	1,2,4 and 5	303.49±6.91	270	294.69±8.90	24	0.11
H_{550}	1	None	130.61±0.85	18	131.77±0.31	276	0.17
	2	None	131.67±0.31	285	132.39±1.18	9	0.53
	3	None	131.75±0.31	270	130.85±0.75	24	0.22
	4	None	131.70±0.34	245	131.63±0.54	49	0.90
	5	None	131.70±0.31	288	131.06±1.47	6	0.66
	1	2,3,4 and 5	130.73±1.11	18	132.05±1.20	276	0.19
LMA	1	None	45.53±1.29	18	46.55±0.53	267	0.43
	2	None	46.42±0.52	276	48.23±1.82	9	0.31
	3	None	46.51±0.52	261	45.70±1.16	24	0.47
	4	None	46.47±0.57	236	46.42±0.82	49	0.95
	5	None	46.45±0.52	279	46.54±2.22	6	0.96
BF	1	None	2.08±0.28	18	1.89±0.11	267	0.50
	2	None	1.90±0.11	276	2.33±0.40	9	0.26
	3	**None**	**1.94±0.11**	**261**	**1.53±0.25**	**24**	**0.09**

	4	None	1.87±0.12	236	2.00±0.18	49	0.52
	5	None	1.90±0.11	279	2.04±0.48	6	0.77
	3	**1,2,4 and 5**	**2.20±0.32**	**261**	**1.78±0.41**	**24**	**0.10**
RF	1	None	3.79±0.37	18	3.73±0.16	268	0.86
	2	None	3.72±0.15	277	4.53±0.52	9	0.11
	3	None	3.75±0.15	262	3.56±0.33	24	0.56
	4	None	3.74±0.17	237	3.71±0.24	49	0.92
	5	None	3.74±0.15	280	3.34±0.64	6	0.53
	2	1,3,4 and 5	3.45±0.37	277	4.24±0.65	9	0.14

[1]BW: birthweight; W_{210}: weaning weight; W_{550}: yearling weight; H_{550}: yearling height; LMA: Longissimus muscle area; BF: backfat thickness; RF: rump fat thickness.

Table 3. Nominal adjusted P values, least square means and standard errors obtained for the effects of the number of copies of each allele of the MUC1 gene polymorphism on growth and carcass traits.

Trait[1]	Allele	Other alleles in the Model	Mean ± Std Error						
			0 copy	N	1 copy	N	2 copies	N	Pvalue
BW	**1**	None	**27.52^{A}±0.90**	**18**	**29.72^{B}±0.40**	**66**	**28.75^{A}±0.37**	**211**	**0.01**
	3	None	29.02±0.36	271	29.64±0.85	21	27.22±2.06	3	0.50
	4	None	28.87±0.38	246	30.00±0.64	41	27.77±1.27	8	0.14
	1	2,3,4 and 5	27.80±1.11	18	29.48±1.35	66	28.77±1.82	211	0.49
	4	1,2,3 and 5	28.53^{A}±0.79	246	29.15^{A}±1.44	41	28.36^{A}±2.16	8	0.84
W_{210}	1	None	169.87±5.01	18	177.07±1.94	66	176.41±1.72	211	0.40
	3	None	176.54±1.59	270	176.48±4.85	21	158.14±12.42	3	0.33
	4	None	176.51±1.81	245	177.54±3.47	41	167.65±7.61	8	0.49
W_{550}	1	None	287.40±6.03	18	294.73±2.53	65	292.74±2.27	211	0.46
	3	None	**293.90^{A}±1.96**	**270**	**285.26^{A}±5.67**	**21**	**264.01^{B}±14.32**	**3**	**0.03**
	4	None	292.30±2.25	245	296.22±4.18	41	291.28±8.91	8	0.68
	3	1,2,4 and 5	**293.57^{A}± 3.41**	**270**	**275.57^{A}±10.23**	**21**	**257.25^{B}±18.70**	**3**	**0.05**
H_{550}	1	None	130.42±0.87	18	131.92±0.39	65	131.53±0.36	211	0.21
	3	None	131.75±0.31	270	131.06±0.80	21	129.44±1.96	3	0.35
	4	None	131.70±0.34	245	131.73±0.59	41	131.18±1.22	8	0.91
LMA	1	None	45.35±1.25	18	46.31±0.54	65	46.14±0.51	202	0.76
	3	None	46.53±0.52	261	46.30±1.24	21	42.06±3.00	3	0.32
	4	None	46.46±0.57	236	46.16±0.91	41	47.51±1.85	8	0.80
BF	1	None	2.08±0.27	18	1.86±0.11	65	1.87±0.11	202	0.75
	3	None	1.94±0.11	261	1.51±0.27	21	1.67±0.65	3	0.24
	4	None	1.87±0.12	236	1.92±0.19	41	2.35±0.40	8	0.51
RF	1	None	3.78±0.36	18	3.66±0.16	65	3.71±0.15	203	0.93
	3	None	3.75±0.15	262	3.54±0.36	22	3.70±0.86	3	0.83
	4	None	3.74±0.17	237	3.65±0.26	41	3.99±0.53	8	0.84

[1]BW: birthweight; W_{210}: weaning weight; W_{550}: yearling weight; H_{550}: yearling height; LMA: Longissimus muscle area; BF: backfat thickness; RF: rump fat thickness.
For the significant effects, values followed by different letters in the same row differ significantly ($p<0.05$)

Supplementary Material

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