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Technical note

Frequency of SNPs located in candidate genes for growth and their effect on live weight variables in beef cattle from Tamaulipas

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Abstract:

A full understanding of how specific genes affect live weight variables is required for their incorporation into genetic improvement programs. An analysis was done of the allelic frequencies of 28 single nucleotide polymorphisms (SNP) located in candidate genes for growth in cattle to identify their effect on live weight traits in Charolais and Simmental herds in the state of Tamaulipas, Mexico. Hair samples were collected from 313 animals and genotyped using the Sequenom MassARRAY system. Genotype analysis showed that all the markers were polymorphic in the evaluated populations and their allelic frequencies were significantly different between the two breeds ($P<0.05$). An association analysis found that in the Charolais population the marker PRL + 2723 had a significant effect ($P= 0.0350$) on birth weight and the marker GHR-6.1 affected

weaning weight (*P*= 0.0226). In the Simmental population GHR-6.1 was associated with yearling weight and the marker LEP-3100 (*P*=0.0249) had a significant effect on weaning weight. The tested 28 SNP panel is polymorphic in both breeds and three of the markers had a significant effect on the evaluated live weight parameters. They can therefore be potentially validated for use as tools in the selection and breeding of beef cattle in Tamaulipas.

Key words: Beef cattle, Somatotropic axis, SNP association, Allelic frequencies, Live weight.

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In cattle production, economically important traits are determined by various polygenic factors. One of the largest current challenges in animal genetics is to determine these trait's genetic architecture and thus incorporate genomic information into animal selection criteria[1].

Both birth weight and weaning weight impact livestock production and are relatively easy to record. They have been among the most studied data in traditional livestock genetics, and are currently important in genomic approaches based on marker-assisted selection applications in cattle[2,3].

Although many physiological processes regulate growth the somatotropic axis is the main regulator of development and growth in cattle[4,5]. Somatotropin, or growth hormone (GH), is secreted by the pituitary gland, is a potent regulator of physiological functions and plays a central role in growth. Release of GH is stimulated by growth hormone releasing hormone (GHRH), and is inhibited by somatostatin. In young animals GH is secreted at high levels during growth, but in adults, secretion levels vary. The most common stimulus for secretion in adults is reduction of plasma glucose concentrations. Although GH is the main regulator of the somatotropic axis, it acts in conjunction with other hormones, receptors and binding proteins. It stimulates release of somatomedins or insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), which exert a regulatory effect through negative feedback on the hypothalamus and adenohypophysis, and thus influence growth. Also secreted primarily by the pituitary gland, prolactin (PRL) is a polypeptide hormone that participates in multiple endocrine processes. However, it participates most frequently in lactation and reproduction in mammals through signaling pathways that involve the STAT5 transcription factors[6]. Other hormones do not directly influence growth but do affect weight; for example, leptin (LEP) regulates food intake and energy balance[7].

In cattle, the genes controlling the above peptides and hormones have been considered as candidates for productive traits. They have been widely characterized and in different breeds their polymorphisms have been associated with body weight[5,7,8], carcass weight[5,7,9], milk yield and fertility[10,11]. In Charolais herds in Mexico, for instance, associations have been identified between some molecular markers located in these candidate genes[2,12,13]. An important consideration is that the association patterns of these markers vary widely between breeds and even more so among populations. Evaluations are therefore needed before they can be employed in genetic selection and improvement programs.

The present study objective was to estimate and compare the allelic frequencies of a panel of SNP type markers and quantify its effect on live weight characteristics in Charolais and Simmental breed cattle in the state of Tamaulipas. Mexico.

From 2014 to 2015 samples were collected from 313 animals in beef cattle herds in municipalities in Tamaulipas. A total of 199 samples were collected from Charolais cattle in four herds located in three municipalities: San Fernando (2), Gustavo Díaz Ordaz (1) and Victoria (1). The sample consisted of 105 offspring born between 2012 and 2014 (55 females and 50 males), 77 dams and 17 sires. Verification of the filial relationship between offspring (n= 13) and the sampled dams and sires was only possible for one herd in San Fernando. Information on the filial relationship between offspring (n= 18) and three sampled sires in the Díaz Ordaz herd was also available. The sample for the Simmental breed was 114 animals, consisting of 32 offspring, 70 dams and 12 sires from a single herd in the municipality of Aldama. All the evaluated sires and offspring were registered animals.

For all five herds management and productive record data were available for birth weight (BW), weaning weight adjusted to 205d (WW) and yearling weight adjusted to 365d (YW). As a result, the data analyzed in the association analysis for each characteristic vary from those of the total studied sample.

Based on the existing literature a panel was designed of 28 SNP located in candidate genes for animal growth and weight. Using hair samples collected from each of the 313 animals, genotyping was run using the Sequenom MassARRAY System following specifications from GeneSeek Inc. (Lincoln, NE, USA).

Once the genotypes were obtained, the genotype and allele frequencies of the analyzed markers were estimated using the Allele Frequency Analysis module of the Cervus 3.0 program[14]. An exact test for Hardy-Weinberg equilibrium was run under the alternative hypothesis of heterozygous deficit (P>0.05) using the Genepop ver. 4.0.10 program[15]. In the genetic differentiation analysis, the null hypothesis tested was Ho = identical allelic distribution across populations. For the populations, the test was done using

population pairs contingency tables and unbiased estimation of the P value or Fisher's exact test for each locus[15].

With the genotype and productive data (BW, WW, YW) for the Charolais and Simmental breeds an association analysis was run for each breed. To isolate all the non-genetic effects of the genotypes each breed's linear model was adjusted as follows:

$$Y_{ijklm} = \mu + S_i + A_j + E_k + G_l + H_m + \beta_{ev} + \varepsilon_{ijklm},$$

Where:
$\mathbf{Y_{ijklm}}$= birth, weaning or yearling weight;
$\boldsymbol{\mu}$= general mean;
$\mathbf{S_i}$= fixed effect of i-th animal sex;
$\mathbf{A_j}$= fixed effect of j-th birth year;
$\mathbf{E_k}$ = fixed effect of k-th birth season;
$\mathbf{G_l}$= fixed effect of l-th genotype in analyzed SNP;
$\mathbf{H_m}$= fixed effect of m-th herd (only for Charolais animals);
$\boldsymbol{\beta_{ev}}$= mother's age linear covariable;
$\boldsymbol{\varepsilon_{ijklm}}$ = residual random error.

The model only included factors found significant in an exploratory analysis which considered evaluation of first-order interactions and analysis of the quadratic effect of mother's age. Subsequently, the least-squares means and standard error of the SNP genotype effects were estimated using the GLM procedure and compared using the PDIFF method with the Tukey-Kramer adjustment. All analyses were run with the SAS 9.0 statistical package (SAS Institute Inc., Cary, NC, USA).

Allelic frequency analysis of the 28 markers found them all to be polymorphic in the two evaluated populations (Table 1). Due to a heterozygous deficit, the LEP-1457 and PRL-RsaI markers deviated from Hardy-Weinberg equilibrium; the first in the Simmental breed ($P<0.0003$) and the second in the Charolais breed ($P<0.0001$). In both cases, sample size was the most probable explanation for the observed deviation, although another possible explanation was the level of inbreeding due to the limited number of sires used in the studied herds.

Table 1: Allelic frequencies of the evaluated 28 SNP panel for the Charolais and Simmental breeds

SNP	Charolais				Simmental			
	A	**C**	**G**	**T**	**A**	**C**	**G**	**T**
GHR-1.1	0.9020		0.0980		0.7479		0.2521	
GHR-1.4	0.5758		0.4242		0.4871		0.5129	
GHR-2.6		0.1106		0.8894		0.0304		0.9696
GHR-2.6	0.1106		0.8894		0.0302		0.9698	
GHR-4.			0.4444	0.5556			0.3803	0.6197
GHR-6.1	0.2186		0.7814		0.4786		0.5214	
GHR-A536	0.0354		0.9646		0.0302		0.9698	
GHR-F279	0.0528			0.9472	0.1043			0.8957
GHR-H54		0.6332		0.3668		0.7241		0.2759
GHR-N528	0.3995	0.6005			0.3319		0.6681	
GHR-S555	0.5452		0.4548		0.6034		0.3966	
GHRH+2279		0.3543		0.6457		0.2328		0.7672
GHRH-2298	0.3819	0.6181			0.4655	0.5345		
GHRH-4241	0.3593			0.6407	0.3922			0.6078
IGF1/*Sna* BI		0.6633		0.3367		0.5690		0.4310
LEP-1180		0.6106		0.3894		0.7802		0.2198
LEP-1457	0.5941		0.4059		0.6173		0.3827	
LEP-3100		0.7437		0.2563		0.8233		0.1767
LEP-3157	0.9898		0.0102		0.9957		0.0043	
LEP-3257		0.1658		0.8342		0.2586		0.7414
LEP-3272		0.1658		0.8342		0.2586		0.7414
LEP-978C		0.4975		0.5025		0.7802		0.2198
LEPY7FA	0.8065			0.1935	0.9914			0.0086
PRL/Rsa I	0.3081		0.6919		0.1121		0.8879	
PRL2723		0.2663		0.7337		0.2716		0.7284
STAT1-C213		0.9121		0.0879		0.9430		0.0570
STAT5A-12735		0.9564		0.0436		0.8190		0.1810
bGH/*Alu* I		0.7828	0.2172			0.7888	0.2112	

Comparison of allelic frequencies identified 25 markers that differ significantly between the two evaluated populations ($P<0.001$). Only three markers exhibited no differences between the populations: GHR-1.1 ($P>0.389$), LEP-3157 ($P>0.516$) and GH/*Alu* I ($P>0.649$).

Although all the evaluated markers can potentially associate with productive traits, the GH/*Alu* I, IGF1-*Sna* BI and LEP-1180 markers had particularly high frequencies because all three have been associated with productive traits such as animal weight, and carcass and meat quality in cattle populations of different breeds, and in both *Bos taurus taurus* and *Bos taurus indicus*[16-20].

The C/G transversion at nucleotide position 2141 of exon 5 of the GH gene (GH/*Alu* I marker) produces a change from valine (G allele) to leucine (C allele) at amino acid position 127 of the gene. Animals carrying a favorable C allele are usually associated with greater marbling and higher carcass weight[21,22]. For this marker, the evaluated breeds exhibited a higher frequency of the favorable allele. An analogous situation was observed with the IGF1/*Sna* B1 marker in the form of a T/C transition at position -472 of the non-coding 5' region of the IGF1 gene. Animals carrying the favorable C allele for this marker have been associated with greater gains in weight at weaning than carriers of the T allele[8,18].

The LEP-1180 marker is a non-synonymous transition from cytosine (C) to thymine (T) located in exon 2 of the leptin gene, which produces an amino acid change from arginine to cysteine[23]. The alleles of this marker have been associated with dorsal fat content and meat softness[16,23].

The studied populations exhibited a lower frequency of the T allele, considered favorable for the carcass characteristics mentioned above. This result may reflect the fact that selection pressure on beef breeds in Mexico has been skewed toward traits that are easily measured and recorded (e.g. live weight) rather than towards those associated with meat quality[2,12].

In the Charolais population the association analysis indicated that the PRL2723 marker located in the prolactin gene had a significant effect ($P= 0.0350$) on BW (Table 2). Mean weight in animals with the homozygous CC genotype (44.69 kg) was 5.66 kg higher than those with the homozygous TT genotype and 6.43 kg more than those with the heterozygous CT genotype.

Table 2: Markers with a significant effect on weight variables in the studied Charolais and Simmental populations

Breed *Loci* (*P*)	Genotype	n	LSM	SE	C
Charolais			BW		
PRL2723 (0.0350)	CC	7	44.69	2.64	a
	CT	42	38.23	1.27	b
	TT	54	39.03	1.22 b	b
			WW		
GHR-6.1 (0.0226)	AA*	0	-	-	
	AG	14	187.98	7.41	b
	GG	59	203.59	5.76	a
Simmental			WW		
LEP-3100 (0.0249)	CC	75	204.38	7.11	b
	CT	30	225.98	7.66	a
	TT*	0	-	-	
			YW		
GHR-6.1(0.0369)	AA	19	261.24	12.31 a	a
	AG	35	266.42	9.52 a	a
	GG	22	237.12	10.48 b	b

LSM = least-squares means; BW = Birth weight; WW = Weaning weight; YW = Yearling weight.
* Genotype excluded from association for lack of observations.
[ab] Means with a different lowercase letter are different ($P<0.04$). SE = standard error.

Due to its crucial role in mammary gland development, lactogenesis and regulation of important genes involved in milk production, the prolactin gene is a strong candidate for marker-assisted selection. Some SNPs in this gene have been associated with different traits. However, SNP PRL-2723, located in intron 1 of the prolactin gene, has been used in some studies, although to date this allelic substitution has no known positive or negative effects. In beef cattle, changes in milk production and composition can be reflected in the offspring[24].

A particularly relevant result of the association analysis was that the GHR6.1 marker had an effect on two live weight parameters in both evaluated breeds. In the Charolais population it significantly affected WW: average weight in animals with the homozygous GG genotype (203.59 kg) was 15.61 kg higher than in those with the heterozygous AG genotype (187.98 kg). Due to a lack of carriers (n = 0), genotype AA was excluded from the association analysis. In the Simmental population, the GHR-6.1 marker was associated with YW: animals with genotype AA and AG were heavier at one year than those with genotype GG (Table 2).

Like many other genes of the somatotropic axis, polymorphisms located in this gene have been associated with different productive traits, mainly in dairy cattle. In Mexican

beef cattle, in this case from Charolais herds in the states of Nuevo León and Sonora, this same marker (GHR6.1) is reported to explain approximately 9% of the genetic variance (*P*=0.0877) in birth weight, with an $\alpha_{G>A}$=0.509[12]. Charolais producers in Mexico commonly acquire genetic material from cattle farms in Nuevo León. Evaluation of these markers' effects on different live weight phenotypes will therefore validate this locus as a focal point in Charolais selection strategies in Mexico. This would also hold in the case of the Simmental breed since the GHR-6.1 marker was clearly associated with YW in the study population.

In the Simmental population the LEP-3100 marker was closely associated with WW. Mean WW for the CT genotypes (225.98 kg) was 21.6 kg higher than for the homozygous CC genotype. Due to the low number of carriers (n= 4) the TT genotype was excluded from the association analysis.

The leptin gene is among the most important biological candidates for study of body fat in animals and humans[19]. Leptin is one of many hormones which participate in regulation of intermediate metabolism through effector mechanisms involving growth factors such as IGF-1. The leptin gene has been extensively studied in cattle and different polymorphisms described that have been associated with productive traits involving energy metabolism, adiposity and reproduction. This gene has also been associated with regulation of body weight via mediation of weight gain metabolism[19].

The LEP-3100 polymorphism is a transition in exon 3 of the gene that causes an amino acid change of Ala>Val at position 80. This polymorphism has been positively associated with meat fatty acid composition; specifically, the C allele has been positively associated with C14:1 fatty acid content[20]. This is the first time the association of this marker with weaning weight has been tested; this variable is an important indicator of weight gain and productivity in meat production in the Simmental breed.

The 28 SNPs located in candidate genes for growth are polymorphic in the analyzed populations and exhibit significantly different allelic frequencies in the two evaluated breeds. In these breeds the association analysis identified three markers that significantly affect the evaluated live weight parameters.

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