Genome-wide association study on growth traits in Colombian creole breeds and crossbreeds with Zebu cattle

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ABSTRACT. Whole genome selection represents an important tool for improving parameters related to the production of livestock. In order to build genomic selection indexes within a particular breed, it is important to identify polymorphisms that have the most significant association with a desired trait. A genome-wide marker association approach based on the Illumina BovineSNP50 BeadChip™ was used to identify genomic regions affecting birth weight (BW), weaning weight (WW), and daily weight gain (DWG) in purebred and crossbred creole cattle populations. We genotyped 654 individuals of Blanco Orejinegro (BON), Romosinuano (ROMO) and Cebú breeds and the crossbreeds BON x Cebú and ROMO x Cebú, and tested 5 genetic control models. In total, 85 single nucleotide polymorphisms (SNPs) were related (P < 0.05) to the 3 evaluated traits; BW was associated with the highest number of SNPs. For statistical false-positive correction, Bonferroni correction was used. From the results, we identified 7, 6, and 4 SNPs with strong associations with BW, WW, and DWG, respectively. Many of these SNPs were located...
on important coding regions of the bovine genome; their ontology and interactions are discussed herein. The results could contribute to the identification of genes involved in the physiology of beef cattle growth and the development of new strategies for breeding management via genomic selection to improve the productivity of creole cattle herds.

**Key words:** Genome-wide association study; Growth traits; Single nucleotide polymorphism; Blanco Orejinegro; Romosinuano

**INTRODUCTION**

The selection of livestock for breeding and production has improved with the inclusion of genotypic information for the early identification of animals carrying desirable genetic variants of a given trait. Different segments of the DNA, such as chromosomal regions, genes, and specific polymorphisms, have been associated with relatively large effects on different measures of growth. Overlapping regions containing quantitative trait loci (QTL) for body weight (BW) and later measures of growth have been reported, as well as regions that may independently affect BW and pre- or postweaning BW gains (Kneeland et al., 2004; Snelling et al., 2010).

QTL have been detected on bovine chromosome 6 in different populations of animals and crossbred cattle. Studies such as Nkrumah et al. (2007) discovered a QTL at 42 cM for average daily gain. For other measures such as body length at birth and BW, some QTL have been identified at 38 and 39 Mb, respectively, in a Charolais x Holstein crossbred population (Gutiérrez-Gil et al., 2009). Several other QTL were detected for BW in this same region (Casas et al., 2000; Kneeland et al., 2004; Maltecca et al., 2009; McClure et al., 2010). Nevertheless, it is possible to note that this type of approach focuses only on those regions that are relatively certain to influence the trait of interest and does not account for the majority of the genetic variation present within the genome. Other methodologies are available that use DNA markers that are distributed throughout the entire genome.

The genome-wide association study (GWAS) is a new technique for the identification of causal genes for important traits in livestock that could increase the rate of genetic progress. It assesses sequence variations, mainly single nucleotide polymorphisms (SNPs), throughout the entire genome in conjunction with the phenotype and pedigree information to perform an association analysis and identify genes or regulatory elements that affect a desired trait (Zhang et al., 2012). The GWAS can be the first step in genomic selection studies and is becoming a popular tool in cattle breeding. It is based on breeding values predicted directly from dense sets of genetic markers; it is possible that each genetic marker from a SNP panel is potentially in linkage disequilibrium with QTL. Individually, small to moderate effects may not be meaningful; collectively, however, they may conform to a polygenic model and account for substantial genetic variation via genomic selection (Meuwissen et al., 2001; Goddard and Hayes, 2009).

The aim of this study was to use the GWAS approach to identify SNPs affecting growth traits such as BW, weaning weight (WW), and daily weight gain (DWG) in BON and ROMO breeds and their crosses with Zebu cattle in Colombia.
MATERIAL AND METHODS

Samples and DNA extraction

Blood samples were collected from 654 individuals from commercial cattle herds. Genetic groups included the breeds BON, ROMO, and CEBÚ, and the crossbreeds BON x Cebú and ROMO x Cebú. Data related to production parameters (i.e., BW, WW, and DWG) of individuals sampled were provided by the cattle owners.

Isolation of genomic DNA was carried out with a commercial extraction kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). The extracted DNA was quantified by spectrophotometric analysis at 260 nm using a NanoDrop ND-2000 Spectrophotometer (Thermo Fischer Scientific Inc., USA) and diluted to 50 ng/μL. The DNA quality was evaluated by estimating the ratio of absorbance at 260 and 280 nm. Samples were genotyped through Geneseeks on the BovineSNP50 genotyping BeadChip (Illumina Inc. San Diego, California, EUA).

Statistical analysis

The association analysis was performed using a regression approach implemented in the SNPassoc package of the R statistical software (CRAN, ver. 2.10.0, 2011, R Foundation for Statistical Computing) (González et al., 2003, 2007). Five genetic inheritance models were tested. Each SNP was considered a categorical variable with one level for each possible genotype (i.e., codominant model), one level for the dominant genotype and another level for the heterozygote and homozygote genotypes (i.e., dominant or recessive models), or one level for each additional copy of an allele (i.e., additive model). To estimate the association between phenotype Y (BW, WW, and DWG) and one SNP, a linear general model was used:

\[ Y_i = \alpha + \beta X_i + e_i \]  

(Equation 1)

where \( \alpha \) is the intercept, \( X_i \) is the \( i \)th subject’s genotype score for a given marker, and \( e_i \) is the error that has a normal distribution with an expectation 0 and variance \( \delta^2 \). Under an additive model, \( X_i \) indicates the number of minor alleles for the \( i \)th subject. Under the dominant model, \( X_i \) denotes, with coded values 1 and 0, whether the \( i \)th subject has at least one minor allele. In a similar way, under the recessive (or overdominant) model, \( X_i \) is codified as 1 and 0 depending on whether the \( i \)th subject has 2 minor alleles (or, in the overdominant model, 2 minor or 2 major alleles).

Proportions of probability, differences, and confidence intervals were estimated for each genetic model using the variance values for every single parameter. The statistical significance of each SNP was assessed comparing the polymorphism effect with one null model (which only included the intercept) through the likelihood ratio test as follows:

\[ \text{LRT} = 2(\log \text{Lik}_{null} - \log \text{Lik}_{other,other}) \]  

(Equation 2)

where “other” refers to the genetic model. When this test was capable of discriminating between models, the Akaike information criterion (AIC) was utilized to select the best-fit inheri-
tance model; the optimal model was defined as the model that yielded the lowest AIC.

\[
AIC = -2 \log \text{Lik} + 2q
\]  
(Equation 3)

where \( q \) denotes the number of parameters for the fitted model. Evaluation of the interaction among SNPs involves the simultaneous study of more than one SNP and can be estimated using the interaction P values, which is obtained by calculating the likelihoods underlying the null model \( \text{Lik}_{null} \), under each single-SNP \( \text{Lik}_i \), and under an additive SNP model \( \text{Lik}_{ad(i;j)} \), and under a full SNP model (including the SNP-SNP interaction; \( \text{Lik}_{full(i;j)} \)) for each pair of SNP \( i,j \). Here, SNPassoc uses the object-oriented features of R (classes and methods) to plot the interaction analysis. The upper triangle in the matrix contains the P values for the epistasis log-LRT:

\[
LRT_{ij} = -2(\log \text{Lik}_{full(i;j)} - \log \text{Lik}_{ad(i;j)})
\]  
(Equation 4)

The diagonal contains the LRT P values for the raw effect of each SNP:

\[
LRT_{ii} = -2(\log \text{Lik}_i - \log \text{Lik}_{null})
\]  
(Equation 5)

The lower triangle comprises the LRT P values comparing the two-SNP additive likelihood model to the best-fit single-SNP model:

\[
LRT_{ji} = -2[\log \text{Lik}_{ad(i;j)} - \log \max(\text{Lik}_i; \text{Lik}_j)]
\]  
(Equation 6)

RESULTS

Eighty-five markers showed meaningful associations; 26, 22, and 37 explained the phenotypic variation for BW, WW, and DWG, respectively. In order identify highly significant SNPs and eliminate false positives, a Bonferroni correction was used.

For BW, 7 SNPs located on chromosomes 1, 14, 19, and 23 showed significant associations. Figure 1 shows the association analysis for the markers related to BW within the entire bovine genome for 5 genetic control models (i.e., codominant, dominant, recessive, overdominant, and additive).

The markers are represented as vertical lines, and the horizontal lines represent threshold lines, which indicate the level of significance for each SNP. The lower line is the nominal value of probability (P value), and the upper line indicates the values that overtake the Bonferroni correction.
SNPs that exceeded the correction threshold line with the lowest P and AIC values were identified assuming each genetic model. The SNP ARS.BFGL.NGS.28289, with the lowest P value (P = 9.55E-3), showed a significant association under the dominant model. Table 1 shows the average, standard deviation, and the difference and significance values of the first 7 SNPs related to BW for each model.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Model</th>
<th>N</th>
<th>Genotype</th>
<th>Mean</th>
<th>SE</th>
<th>P</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARS.BFGL.NGS.28289</td>
<td>Dominant</td>
<td>235</td>
<td>A/A</td>
<td>27.79</td>
<td>0.23</td>
<td>9.55E-03</td>
<td>2262</td>
</tr>
<tr>
<td></td>
<td></td>
<td>176</td>
<td>B/A-B/B</td>
<td>30.22</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARS.BFGL.NGS.75663</td>
<td>Dominant</td>
<td>121</td>
<td>A/A</td>
<td>30.69</td>
<td>0.37</td>
<td>2.33E-02</td>
<td>2239</td>
</tr>
<tr>
<td></td>
<td></td>
<td>286</td>
<td>B/A-B/B</td>
<td>28.02</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARS.BFGL.NGS.2904</td>
<td>Dominant</td>
<td>175</td>
<td>B/B</td>
<td>30.23</td>
<td>0.30</td>
<td>9.42E-02</td>
<td>2273</td>
</tr>
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<td></td>
<td></td>
<td>238</td>
<td>B/A-A/A</td>
<td>27.80</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARS.BFGL.NGS.110427</td>
<td>Additive</td>
<td>121</td>
<td>A/A</td>
<td>27.79</td>
<td>0.23</td>
<td>6.60E-03</td>
<td>2264</td>
</tr>
<tr>
<td>ARS.BFGL.NGS.34390</td>
<td>Additive</td>
<td>175</td>
<td>B/A-B/B</td>
<td>30.22</td>
<td>0.30</td>
<td>7.33E-03</td>
<td>2244</td>
</tr>
<tr>
<td>ARS.BFGL.BAC.13422</td>
<td>Additive</td>
<td>238</td>
<td>B/A-A/A</td>
<td>27.80</td>
<td>0.23</td>
<td>1.13E-02</td>
<td>2184</td>
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<tr>
<td>ARS.BFGL.NGS.107530</td>
<td>Additive</td>
<td>238</td>
<td>B/A-A/A</td>
<td>27.80</td>
<td>0.23</td>
<td>7.86E-02</td>
<td>2246</td>
</tr>
</tbody>
</table>

AIC = Aikake information criteria.

In this case, individuals with genotype A/A exhibited an average of 27.7 ± 0.23 kg for BW, while the B/B and heterozygote genotypes exhibited greater BW, on average (i.e., 30.2 ± 0.3 kg). Likewise, the best-fit models for the ARS.BFGL.NGS.75663 and ARS.BFGL.NGS.2904 markers with respect to the dominant and higher values for BW were identified for individuals with the homozygous genotypes A/A and B/B, respectively.

The SNPs ARS.BFGL.NGS.110427, ARS.BFGL.NGS.34390, ARS.BFGL.BAC.13422, and ARS.BFGL.NGS.107530 exhibited highly significant associations with BW...
the additive model was assumed. Most of these SNPs also returned significant values under 2 additional models (i.e., codominant and recessive). However, in all cases, the AIC values indicated that the additive model was the best fit for genotypic variation of the trait analyzed for these markers.

Figure 2 displays the interaction plot analysis constructed using the first 21 markers with the highest significant effects ($P < 0.001$) on BW. Each plot contains the $P$ values obtained from different likelihood ratio tests.

![Interaction analysis for significant associated markers with body weight.](image)

A different color indicates different levels of statistical significance. The diagonal contains the $P$ values from LRT for the crude effect of each SNP. The upper triangle in the matrix contains the $P$ values for the interaction (epistasis) log-likelihood ratio test, and the lower triangle contains the $P$ values from LRT comparing the two-SNP additive likelihood to the best of the single-SNP models.

A considerable number of SNPs exhibited epistasis with moderate and high significance levels, as shown by the diagonal in Figure 2. It is possible to see a defined diagonal; however, not all interactions exhibited high $P$ values (color intensity at some points on the line are low). The SNPs ARS.BFGL.NGS.107530, ARS.BFGL.NGS.34390, ARS.BFGL.NGS.2904, ARS.BFGL.BAC.13422, and ARS.BFGL.NGS.28289 showed the highest significant interactions. Another group exhibiting a significant interaction was comprised of the SNPs ARS.BFGL.NGS.28289, Hapmap46986.BTA.34282, and ARS.BFGL.NGS.75663.

**WW**

After performing a Bonferroni correction, 6 SNPs located on chromosomes 5, 7, 8, 11, 16, and 24 were significantly associated with WW. Table 2 displays these polymorphisms.

BTB.01051906, ARS.BFGL.NGS.24866, and ARS.BFGL.NGS.12523 returned the lowest AIC and $P$ values under the dominant model. Conversely, using the recessive model, the marker ARS.BFGL.NGS.64868 yielded a lower $P$ value ($P < 3.28E-6$) with regard to differences in average WW (i.e., 143.1 ± 5.8 kg for the A/A genotypes and up to 165.4 ± 1.2 kg for the desirable genotypes B/B and B/A due to higher WW values).

Thus, the average WW for individuals with the B/B and B/A genotypes (i.e., desirable)
was 22.5 kg above the average value for individuals with the A/A genotype (i.e., undesirable). The markers Hapmap50042.BTA.58392 and ARS.BFGL.NGS.52586 showed highly significant additive effects, for which each additional, undesirable allele produced a decrease of 14.6 kg (1.33E-06) and 7.9 kg (7.70E-07) in the average WW of carrier animals, respectively.

An interaction analysis that included 20 SNPs with the most significant effects (P < 0.01) is shown in Figure 3.

Table 2. Genetic control for 5 polymorphisms associated with weaning weight in 2 Colombian creole cattle breeds.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Model</th>
<th>N</th>
<th>Genotype</th>
<th>Mean</th>
<th>SE</th>
<th>P</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTB.01051906</td>
<td>Dominant</td>
<td>312</td>
<td>B/B</td>
<td>167.20</td>
<td>1.20</td>
<td>5.54E-07</td>
<td>3660</td>
</tr>
<tr>
<td>ARS.BFGL.NGS.24866</td>
<td>Dominant</td>
<td>191</td>
<td>B/B</td>
<td>158.21</td>
<td>1.70</td>
<td>1.07E-07</td>
<td>3758</td>
</tr>
<tr>
<td>ARS.BFGL.NGS.12523</td>
<td>Dominant</td>
<td>151</td>
<td>A/A</td>
<td>157.01</td>
<td>2.24</td>
<td>4.17E-05</td>
<td>3751</td>
</tr>
<tr>
<td>ARS.BFGL.NGS.64868</td>
<td>Recessive</td>
<td>381</td>
<td>B/B-B/A</td>
<td>165.45</td>
<td>1.20</td>
<td>3.28E-06</td>
<td>3746</td>
</tr>
<tr>
<td></td>
<td>Additive</td>
<td>26</td>
<td>A/A</td>
<td>143.10</td>
<td>5.82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AIC = Akaike information criteria.

The darkest green coloration in the intersection among the markers indicates that a high proportion of the identified SNPs presented epistatic interactions (P < 0.001). The first 8 and other markers exhibited higher significant interactions (P > 0.001) along the diagonal than that might be associated with their functional effects.

**DWG**

Several markers were related to DWG under different models of genetic control. Figure 4 shows the SNP that exceeded the threshold line and exhibited the strongest association with the trait evaluated. The model that best explained the genetic variation with the highest number of SNPs was the dominant model, followed by the additive model.
Four SNPs were significantly associated (P < 1E-5) with DWG. The analysis indicates that the additive model best fit the variation explained by 2 of these polymorphisms (Table 3). The marker ARS.BFGL.NGS.12523 exhibited a highly significant association with DWG. In this case, individuals with the AA homozygote genotype exhibited lower average DWG and P values (P = 2.17E-5) than those of the B/A and B/B genotypes (580.9 ± 5.482 gr) under the dominant model. With regard to the SNP Hapmap27945.BTC.073459, which was explained by the recessive genetic model, DWG average values were higher in animals with the B/B homozygote or heterozygote genotypes (570.7 ± 4.89 gr) when compared to that of the A/A carrier individuals (P = 2.08E-5) (Table 3). The markers ARS.BFGL.NGS.12995 and Hapmap50042.BTA.58392 showed highly significant additive associations with DWG. Each additional copy of the undesirable allele (B) produced a negative difference of 32.4 g (P = 5.48E-6) and 58.1 g (P = 1.75E-6) on DWG, respectively. Two of these polymorphisms associated with the trait analyzed, ARS.BFGL.NGS.12523 and Hapmap50042.BTA.58392, also had significant effects on WW (Table 2).

Almost all the markers showed significant epistasis. The interaction plot for this trait is shown in Figure 5, which displays markers with highly significant interactions. In the diago-

Table 3. Genetic control for 5 polymorphisms associated with daily weight gain in 2 Colombian creole cattle breeds.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Model</th>
<th>N</th>
<th>Genotype</th>
<th>Mean</th>
<th>SE</th>
<th>P</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARS.BFGL.NGS.12523</td>
<td>Dominant</td>
<td>151</td>
<td>A/A</td>
<td>532.2</td>
<td>8.977</td>
<td>2.17E-05</td>
<td>4883</td>
</tr>
<tr>
<td>Hapmap27945.BTC.073459</td>
<td>Recessive</td>
<td>256</td>
<td>B/A-B/B</td>
<td>580.9</td>
<td>5.482</td>
<td>2.08E-05</td>
<td>4920</td>
</tr>
<tr>
<td>ARS.BFGL.NGS.12995</td>
<td>Additive</td>
<td>371</td>
<td>B/B-A/A</td>
<td>570.7</td>
<td>4.891</td>
<td>2.08E-05</td>
<td>4920</td>
</tr>
<tr>
<td>Hapmap50042.BTA.58392</td>
<td>Additive</td>
<td>39</td>
<td>A/A</td>
<td>489.5</td>
<td>19.048</td>
<td>5.48E-06</td>
<td>4927</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>1.75E-06</td>
<td>4939</td>
</tr>
</tbody>
</table>

AIC = Aikake information criteria.
nal, almost all boxes indicate significant associations, and the boxes below the diagonal show several SNPs with significant interactions with more than 4 different markers (dark green boxes, \( P > 0.001 \)).

Figure 5. Interaction analysis for significant associated markers with daily weight gain.

DISCUSSION

Candidate gene techniques have been widely used to discover and localize causative genes for quantitative traits or complex phenotypes. There are several ways to choose candidate genes to map QTL. The physiological approach is based on genes with known biological functions and actions related to the development or physiology of the trait of interest. The positional cloning approach considers genes that are located in the neighborhood of previously identified QTL regions. The third method is the comparative approach, which utilizes loci where polymorphisms are known to have a phenotypic effect in one species and explores them as candidates for similar variation in other species (Zhang et al., 2012).

In this study, we used a GWAS approach to identify markers or genes related to 3 growth traits (i.e., BW, WW, and DWG) in 2 Colombian creole cattle breeds. Significantly different effects were identified for several gene sets via 5 genetic inheritance models. Initially, 85 markers showed meaningful associations, of which only 26, 22, and 37 explained the phenotypic variation in BW, WW, and DWG, respectively. All of these polymorphisms corresponded to new genomic variations that have not yet been reported for the breeds assessed in the current study. The number of SNPs was lower than that reported by Snelling et al. (2010), who found 866 SNPs that were related to at least one of the components of growth when evaluated from conception to yearling; 524 and 14 of those SNPs were associated with BW and DGW, respectively, up to weaning. However, these authors utilized a different approach, including a model with additive effects of calf and dam SNP genotypes, year-sex-management contemporary groups, and covariates for calf and dam breed composition and heterosis. Moreover, calf and dam genotypes were simultaneously evaluated as fixed effect covariates. Conversely, we did not include the population genealogy in our model; this is the first approach using only productive and genomic information.

In order to identify the SNPs with highly significant effects and eliminate false positives, a Bonferroni correction was employed. Seven SNPs located on chromosomes 1, 14, 19, and 23 showed significant associations with BW. Some of those significant SNPs related
to the BW are described here. The SNP ARS.BFGL.NGS.107530 was related to BW and located in chromosome 1, which was 322.5 kb from the neural cell adhesion molecule 2 gene (NCAM2). NCAM2 is primarily expressed in the brain, where it is believed to stimulate neurite outgrowth and facilitate dendritic and axonal compartmentalization (Winther et al., 2012). A study by McIntyre et al. (2010) that characterized differences in gene expression of axon growth and guidance genes involved in neurogenesis of projection neurons showed that cell adhesion molecules (including Ncam2) were expressed by immature and/or mature olfactory sensory neurons.

Four SNPs on BTA 14 were related to BW; 2 were located in coding regions. The polymorphism ARS.BFGL.NGS.110427 was located within the XK, Kell blood group complex subunit-related family, member 4 gene (XKR4). Variations in this gene have been associated with subcutaneous rump fat thickness in indicine and composite cattle (Porto Neto et al., 2012). The GWAS constructed by Lindholm-Perry et al. (2012) showed that 5 markers within 22.79-22.84 Mb of this chromosome neighboring the XKR4 gene had highly significant associations with average daily feed intake and average daily gain. The other SNP located in the coding region was ARS.BFGL.NGS.34390, within the suppression of tumorigenicity 18 gene (ST18), which encodes a transcription factor that regulates proapoptotic and proinflammatory gene expression in fibroblasts. The remaining SNPs, ARS.BFGL.NGS.75663 and ARS.BFGL.BAC.13422, were both found in non-coding regions of BTA14. The former was 11.7 kb upstream of the neuropeptides B/W receptor 1 gene (NPBWR1), and the latter was 33.5 kb upstream of the metalloendopeptidase OMA1, mitochondrial-like gene (LOC100139328). One of these genes, NPBWR1, has been related to the homeostatic regulation of feeding (Takkenoya et al., 2010).

Other SNPs also associated with BW did not exhibit a clear relation with growth physiology. This was true for ARS.BFGL.NGS.28289, located in chromosome 15 within the FARS2 gene that encodes the mitochondrial enzyme phenylalanyl-tRNA synthetase 2 (mtPheRS), which charges transfer RNAs (tRNAs) with their cognate amino acids. Authors such as Elo et al. (2012) and Shamseldin et al. (2012) have shown that this gene is related to encephalopathies. They showed that some variants of this gene produce weak ATP bindings in the aminoacylation domain and disrupt synthetase function via interruption of the rotation of the tRNA anticodon stem-binding domain from a closed to an open form, thus affecting aminoacylation function and the stability of mtPheRS, and leading to a decrease in overall tRNA charging capacity. Similarly, the polymorphism ARS.BFGL.NGS.2904, within the SLC16A5 gene in chromosome BTA19, encodes the monocarboxylate transporter 6 (MCT6). According to Murakami et al. (2005), MCT6 could be involved in the disposition of various drugs, including loop diuretics. This gene is part of a differentially methylated region with frequent aberrant methylation patterns in oncogenic processes. In the study of Sugito et al. (2013), individuals exhibiting the hypomethylation of SLC16A5 and ZNF206 had significantly prolonged event-free survival when these 2 variables were analyzed together.

Six markers were significantly associated with WW, of which 2 were also related with DWG. ARS.BFGL.NGS.12523 and Hapmap50042.BTA.58392 were located in non-coding regions on the 11 and 24 BTA, respectively. The former was 16 bp downstream of an ankyrin repeat and the SOCS box containing 3 (ASB3) gene and 46 bp upstream of the endoplasmic reticulum lectin 1 (ERLEC1) gene. Using the same approach, the study conducted by Zhang et al. (2012) showed an association between carcass traits and fatty acid composition with 91
functional genes in Wagyu x Angus F1 cattle, suggesting that ASB3 can significantly affect the back fat trait. On the other hand, the ERLEC1 gene encodes a resident endoplasmic reticulum (ER) protein that functions in N-glycan recognition. This protein is thought to be involved in ER-associated degradation via interaction with the membrane-associated ubiquitin ligase complex. It also functions as a regulator of multiple cellular stress-response pathways in a manner that promotes metastatic cell survival (Yanagisawa et al., 2010).

Of the remaining 4 SNPs related to WW, only 3 were located in coding regions. The SNP BTB.01051906 was located within the ADAMTSL1 gene, which encodes a secreted protein that belongs to the ADAMTS (disintegrin and metalloprotease with thrombospondin repeats) family of proteases. This molecule may have important functions in the extracellular matrix and has been associated with gonad differentiation in the chicken embryo (Carré et al., 2011). On the other hand, the SNP ARS.BFGL.NGS.24866 was located in the DPYSL3 gene that encodes dihydroxyimidinase-like 3, which is expressed in various regions of the developing nervous system and has been involved in neuronal differentiation, axonal outgrowth, and the positioning of Rohon-Beard neurons and neural crest cells during neurulation in zebrafish (Tanaka et al., 2012); it is also expressed in immature olfactory sensory neurons, and some studies have demonstrated that this gene is a calpain substrate (Kowara et al., 2005; McIntyre et al., 2010). Finally, the last SNP associated with WW and located within a coding region was ARS.BFGL.NGS.64868, which was found within the HHAT gene that encodes an enzyme (hedgehog acyltransferase) that acts within the secretory pathway to catalyze amino-terminal palmitoylation of the hedgehog. Sonic hedgehog homolog (Shh) is a morphogene involved in regulating vertebrate organogenesis and growth during the embryonic period, such as neural development (Fuccillo et al., 2006), and, interestingly, palmitoylation of Shh is required for effective long- and short-range Shh-mediated signaling. A study by Buglino and Resh (2010) identified specific regions and multiple residues that regulate HHAT stability and catalysis.

Four polymorphisms were associated with the ADG trait; 2 are described above in relation to WW. The other 2 SNPs, Hapmap27945.BTC.073459 and ARS.BFGL.NGS.12995, were located in non-coding regions. The former was located close to the GRID2 gene and LOC783261 region, which are segments of DNA that encode an ionotropic glutamate receptor (delta 2) and a 60S ribosomal L7-like protein, respectively. The second SNP was located close to the uncharacterized region of LOC100847439.

WW and DWG had higher levels of interaction among the most significantly associated SNPs when compared to that for BW. This result may suggest a key pathway from important sets of markers close to genes that participate directly in the physiological networks related to growth at weaning. Additionally, we observed numerous polymorphisms with dominant actions and an interesting phenomenon in which crossbreeding might alter gene actions to fit the dominant and overdominant models, thus, explaining the origin of heterosis, which might clarify the action model in this study.

Several highly significant SNPs were identified, and their possible physiological functions have been described by their positions close to some functional genes, which may explain the relationships with the growth traits evaluated in this study. This study represents a starting point for genomic characterization that might be further studied to create new selection schemes and increase the weight of livestock animals while still maintaining their adaptations to tropical environments, a characteristic trait of Colombian creole cattle.
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REFERENCES


