



GENOME-WIDE ASSOCIATION STUDY FOR BIRTH, WEANING AND YEARLING WEIGHT IN COLOMBIAN BRAHMAN CATTLE

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Keyword:	Bos indicus, SNP, GWAS, growth

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**GENOME-WIDE ASSOCIATION STUDY FOR BIRTH, WEANING AND YEARLING
WEIGHT IN COLOMBIAN BRAHMAN CATTLE**

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Short Running title: GWAS for growth in Brahman cattle

Keywords: cattle, *Bos indicus*, SNP, QTL, GWAS, body weight

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ABSTRACT

Genotype data of 1,234 animals was analyzed to find genomic regions that potentially influence the birth weight, weaning weight at seven months of age and yearling weight of Colombian Brahman cattle. Multiple regions scattered across the genome were found to influence weights at different ages, also dependent on the trait component (direct or maternal). The most interesting regions were connected to previously identified QTLs and genes, such as ADAMTSL3, CAPN2, CAPN2, FABP6, ZEB2 influencing growth and weight traits. The identified regions will contribute to the development and refinement of genomic selection programs for Zebu Brahman cattle in Colombia.

INTRODUCTION

Weight traits are considered the most economically important production traits in beef cattle. The animals are weighed at predefined times to comply with the respective breeding scheme, where common measurements are taken at birth, on weaning and at market age. Apart from quantifying average daily gain, the birth weight is also associated with growth traits in general (Boligon *et al.*, 2009) as well as the mature weight (Meyer, 1995). The weaning weight is used as a criterion to select animals for further breeding (Guidolin *et al.*, 2012). With the availability of dense single nucleotide polymorphism (SNP) genotyping platforms, it is possible to study these traits at the genomic level. The information on regions influencing respective traits could be used as predictors of production potential at an early age, possibly complementing other (genomic) breeding values. Multiple genomic regions were identified by (Buzanskas *et al.*, 2014) influencing birth, weaning (210 days) and long-yearling weight (420 days) in Canchim beef cattle using genome-wide association techniques. In total, four regions were identified in connection with birth weight (BTA4 and BTA9), 12 regions for weaning weight (BTA4, BTA6 and BTA11) and ten regions for long-yearling weight (BTA7, BTA22, BTA25 and BTA27). A region influencing growth traits was found on BTA14 in Fleckvieh cattle, with followup links to calving ease (Pausch *et al.*, 2011). BTA14 was also highlighted in (Utsunomiya *et al.*, 2013) as the chromosome harboring the most important region for birth weight in Nellore cattle. In crossbred beef cattle, the genomic regions affecting weights of animals at birth, weaning and one year of age were scattered across the genome, with the majority of associations on BTA6, and to a lesser extent on BTA10, BTA11, BTA14 and BTA20 (Snelling *et al.*, 2010). To our knowledge, a similar examination of genomic regions influencing weight traits is so far missing for Brahman cattle. Therefore, the aim of our paper was to search for genome-wide associations influencing weight at different ages in Colombian Brahman cattle.

MATERIAL AND METHODS

Birth weight (BW), weaning weight (WW) and yearling weight (YW) for 1,234 genotyped animals from 35 Colombian herds and three regions were obtained from the historical databases of the National Association of Zebu Brahman Farmers. The single nucleotide polymorphisms (SNP) were genotyped using the Bovine Genomic profiler GGPHD 80K BeadChip (GeneSeek, Lincoln, NE), in the Animal Molecular Genetic Laboratory, of GENES DIFFUSION in Lille, France, and at the Molecular Genetic Laboratory of CORPOICA, Colombia, as described by (Matukumalli *et al.*, 2009). The initial genotype call rates averaged $99.4 \pm 0.06\%$ for 74,669 SNPs. In the followup procedure, the unplaced SNPs and those on the sex chromosomes were deleted. The remaining SNPs were required to meet the following criteria: call rate minimum 0.90, minor allele frequency minimum 0.05, animal call rate minimum 0.9, and pass parent-progeny test for Mendelian conflicts. After the quality control, a total of 63,971 SNPs and 1,562 genotyped animals remained for the analysis.

The single-trait model was:

$$y = Xb + Zu + Zm + e$$

where y is the vector of observations for BW, WW and YW; b is the vector of fixed effects including the contemporary group (region, herd, year, season, mating, sex, birth number) is the vector of random additive genetic effects, combining polygenic breeding values (based on pedigree) and genomic breeding values (based on genotypes) is the vector of maternal genetic effects; X and Z are incidence matrixes; e is the vector of random residuals. For genomic analysis, an ssGBLUP (Aguilar *et al.*, 2010) was used, where the A matrix was replaced by the H matrix. For the SNP estimation, the windows variance option was used to compute the proportion of variance explained by each marker.

RESULTS AND DISCUSSION

The most important regions influencing BW, WW and YW in Colombian Brahman cattle were identified based on the effect for each SNP. Both direct and maternal components were used to identify the region important for both, as well as any differences between them. The effect of SNPs was measured as the percentage of the variance explained in an SNP window of 4 SNPs. The regions were defined as the positions of the SNPs with the highest variance explained \pm 0.5Mb in each direction. The results for the whole genome are shown in Manhattan plots in Figures 1-3. The most important regions are more precisely identified in Tables 1-3, including the number of characterized protein-coding genes in the region (NCBI, annotation release 104) and any growth-related QTLs (CattleQTLdb, Release 27).

Birth Weight

For the BW, the highest SNP effects were found on BTA2 and BTA17, for maternal and direct effects, on BTA16 for the direct effect only, and on BTA1, BTA7 and BTA16 for the maternal effect only (Figure 1). Most of the regions were harboring QTLs connected with growth and development, although not all of those directly linked to birth weight. In addition, multiple genes were located in the identified regions (Table 1), although only a few could be connected to growth directly. The most important genes were the zinc finger E-box binding homeobox 2 (ZEB2) (Yamakoshi *et al.*, 2012) On chromosome 2 involved in pathways regulating early growth and development, fatty acid binding protein 6, (Zheng *et al.*, 2009) involved in skeletal muscle growth rates and calpain 2 (CAPN2) associated with growth in cattle (Zhang & Li, 2011).

Weaning weight

For this trait, the important regions were the same for both direct and maternal effects. The regions with the highest variance explained were found on BTA7, BTA8, BTA14, BTA20

and BTA28, shared in both direct and maternal effects (Figure 2). Interestingly, the magnitude of the effects was different in each, positive for direct and negative for maternal effects. The identified locations were matching to known QTL regions for growth on BTA7, BTA8 and BTA20, but not in the other chromosomes. In addition, there were QTLs for milk production in almost all identified regions. These QTLs might be connected to growth rate in the pre-weaning period, as the portion of the diet for young calves is based on milk. In addition to QTLs, there were a number of characterized genes in our regions, but none of them had an apparent strong link to the growth rate in cattle or other organisms.

Yearling weight

For the yearling weight, we have found signals on BTA1, BTA16 and BTA19 for direct and maternal effects, and on BTA1, BTA4, BTA 14, BTA21 and BTA28 for maternal effects only, all controlling between 0.10 to 0.18 of the variance (Figure 3). Again, most regions were harboring QTLs connected to growth. Interestingly, in one of the most important regions (BTA1, 155-156Mb), the only QTL was for “sheer force”. In addition, none of the four characterized genes had any apparent connection to growth. Upon closer examination, we found the calpain 7 (CAPN7) gene (BTA1, 154Mb), which was slightly out of our primary region, but with significant influence on growth in farm animals (Yang *et al.*, 2009). *et al.* Another interesting gene was the ADAMTS-like 3 (ADAMTSL3) on BTA21, with confirmed connections to body size and growth in cattle (Liu *et al.*, 2012) and humans (Lettre *et al.*, 2008).

In our study, we have used single-step genome-wide association study to identify regions connected to birth, weaning and yearling weight in Colombian Brahman cattle. The advantage of the method is that it uses phenotypes from both genotyped and non-genotyped individuals

by blending relationship matrixes (Wang *et al.*, 2012; Wang *et al.*, 2014). The regions with the highest proportion of variance explained for the respective trait were matching for the weaning weight, with some private regions of interest for birth and yearling weight in respect to direct and maternal EBV.

The regions denoted by SNPs with the highest variance explained were scattered across the genome (Figures 1 -3). Interestingly, none of these regions was identified in more than one trait. While some of the identified regions were on the same chromosome across traits (e.g. on BTA16 for birth and yearling weight), these were far enough from each other to distinguish them empirically as separate signals. In general, the identified regions corresponded to locations of previously identified QTLs related to growth and weight traits. The most interesting exception was the 57-58Mb region on BTA14 harboring no QTLs at all, despite an apparently rich QTL region downstream (BTA14: 51-52Mb) identified for yearling weights.

We have identified multiple genes in our regions, such as ADAMTSL3, CAPN2, CAPN2, FABP6, ZEB2 influencing growth and weight traits in cattle, or their homologous forms in other organisms. Contrary to the studies on Brazilian Nellore cattle (Utsunomiya *et al.*, 2013) the region harboring PLAG1 on BTA14 was not highly significant for any of the traits. Additional associations were reported on BTA9 for birth weight and BTA6, BTA11 for weaning weight, and BTA7, BTA22 and BTA27 for yearling weight (Buzanskas *et al.*, 2014; Lu *et al.*, 2014). None of these regions corresponded to our study. Thus, our findings warrant further investigations to search for common regions or to identify unique regions influencing weight traits in Colombian Brahman cattle.

CONCLUSION

In this study, we used a single-step genome-wide association study to identify the regions connected to birth, weaning and yearling weight in Colombian Brahman cattle. The regions with the highest proportion of variance explained (including a $\pm 0.5\text{Mb}$ buffer region) were identified as candidates influencing the weight traits. Signals were scattered across the genome, connected to previously identified QTL regions and genes such as ADAMTSL3, CAPN2, CAPN2, FABP6, ZEB2 influencing growth and weight traits. Similar regions were responsible for controlling birth, weaning and yearling weights, when direct and maternal effects were considered, but there was no overlap between traits. These results confirm that multiple different regions influence the weight traits, which should be considered during the development and refinement of genomic selection programs for Zebu Brahman cattle in Colombia.

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Figure 1. Manhattan plot for direct and maternal effects for birth weight in Zebu Brahman cattle in Colombia

Figure 2. Manhattan plot for direct and maternal effects for weaning weight at 7 months in Zebu Brahman cattle in Colombia

Figure 3. Manhattan plot for direct and maternal effects for yearling weight at 12 months in Zebu Brahman cattle in Colombia

Table 1: Most important regions for birth weight

Trait type	Chromosome	Region ^a	Number of characterized genes in the region ^b	Weight and growth-relevant QTLs
Direct and maternal	BTA2	17.34 – 18.42	5	<ul style="list-style-type: none"> • QTL:1549, "Body depth" • QTL:1550, "Body form composite index" • QTL:3491, "Hip height" • QTL:1553, "Stature" • QTL:1323, "Body weight (birth)"
		51.52 – 52.55	1	<ul style="list-style-type: none"> • QTL:10670, "Body weight (birth)" • QTL:10665, "Carcass weight" • QTL:1310, "Body weight (slaughter)"
	BTA17	49.40 – 53.40	5	<ul style="list-style-type: none"> • QTL:23867, "Metabolic body weight" • QTL:23868, "Metabolic body weight" • QTL:23870, "Body weight (weaning)"
Direct only	BTA16	27.83 – 28.86	9	<ul style="list-style-type: none"> • QTL:11025, "Body weight (birth)" • QTL:11026, "Body weight (weaning)" • QTL:4482, "Body weight (weaning)" • QTL:4486, "Pre-weaning average daily gain"
Maternal only	BTA1	1.62 – 2.65	9	<ul style="list-style-type: none"> • QTL:24744, "Weaning weight-maternal milk" • QTL:5265, "Residual feed intake"
	BTA7	73.22 – 74.28	10	<ul style="list-style-type: none"> • No weight and growth-related QTL
	BTA16	58.20 – 60.56	6	<ul style="list-style-type: none"> • No weight and growth-related QTL

^a The region is defined as the location of the most important SNPs \pm 0.5Mb

^b Source: NCBI, October 2015, annotation release 104

Table 2: Most important regions of DNA related to weaning weight in Zebu Brahman breed

Trait type	Chromosome	Region ^a	Number of characterized genes in the region ^b	Weight and growth-relevant QTLs
Direct and maternal	BTA7	56.96 – 57.99	2	<ul style="list-style-type: none">• QTL:10807, "Body weight (yearling)"• QTL:10806, "Longissimus muscle area"• QTL:10805, "Carcass weight"• QTL:10808, "Body weight (weaning)"• QTL:10810, "Body weight (birth)"
	BTA8	1.63 – 2.65	2	<ul style="list-style-type: none">• QTL:10821, "Body weight (weaning)"• QTL:10820, "Height (mature)"• QTL:10819, "Longissimus muscle area"• QTL:10817, "Carcass weight"
		78.35 – 79.40	7	<ul style="list-style-type: none">• No matching QTLs
	BTA14	57.26 – 58.34	4	<ul style="list-style-type: none">• No QTLs
	BTA20	2.70 – 3.74	6	<ul style="list-style-type: none">• QTL:1357, "Body weight (birth)"• QTL:11099, "Body weight (birth)"• QTL:11098, "Carcass weight"• QTL:11097, "Body weight (weaning)"• QTL:11094, "Carcass weight"• QTL:11096, "Body weight (yearling)"
	BTA28	1.91 – 2.97	4	<ul style="list-style-type: none">• No QTLs

^a The region is defined as the location of the most important SNPs ± 0.5Mb

^b Source: NCBI, October 2015, annotation release 104

Table 3: Most important regions of DNA related to yearling weight in Zebu Brahman breed

Trait type	Chromosome	Region ^a	Number of characterized genes in the region ^b	Weight and growth-relevant QTLs
Direct and maternal	BTA1	155.24 - 156.42	4	<ul style="list-style-type: none"> No matching QTLs
	BTA16	11.35 – 12.42	0	<ul style="list-style-type: none"> QTL:11019, "Body weight (yearling)" QTL:11022, "Carcass weight" QTL:11021, "Longissimus muscle area" QTL:11023, "Weaning weight-maternal milk" QTL:1355, "Carcass weight" TL:23153, "Body weight (weaning)"
	BTA19	9.39 – 10.49	17	<ul style="list-style-type: none"> QTL:11075, "Body weight (mature)" QTL:11076, "Body weight (yearling)"
Maternal only	BTA1	17.76 - 18.82	3	<ul style="list-style-type: none"> QTL:10639, "Weaning weight-maternal milk" QTL:10638, "Body weight (birth)"
	BTA14	51.05 – 52.15	1	<ul style="list-style-type: none"> QTL:23842, "Body weight (weaning)" QTL:23846, "Body weight (weaning)" QTL:23843, "Body weight (birth)" QTL:23844, "Body weight (birth)" QTL:23845, "Body weight (birth)" QTL:23265, "Carcass weight" QTL:23849, "Metabolic body weight" QTL:23850, "Metabolic body weight"
	BTA21	23.48 - 24.62	10	<ul style="list-style-type: none"> QTL:22801, "Average daily gain" QTL:11118, "Fat thickness at the 12th rib" QTL:2641, "Body weight (birth)" QTL:11123, "Body weight (mature)" QTL:11122, "Height (mature)" QTL:11120, "Height (yearling)"
	BTA28	42.28 – 43.47	11	<ul style="list-style-type: none"> QTL:22763, "Body weight (birth)"

^a The region is defined as the location of the most important SNPs \pm 0.5Mb

^b Source: NCBI, October 2015, annotation release 104

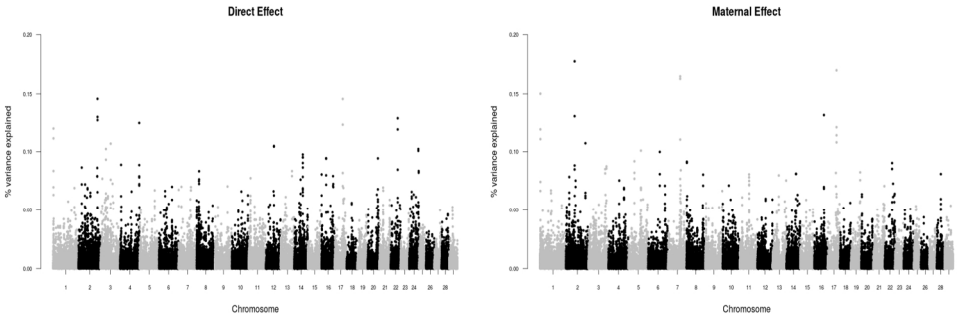


Figure 1. Manhattan plot for direct and maternal effects for birth weight in Zebu Brahman cattle in Colombia
254x190mm (200 x 200 DPI)

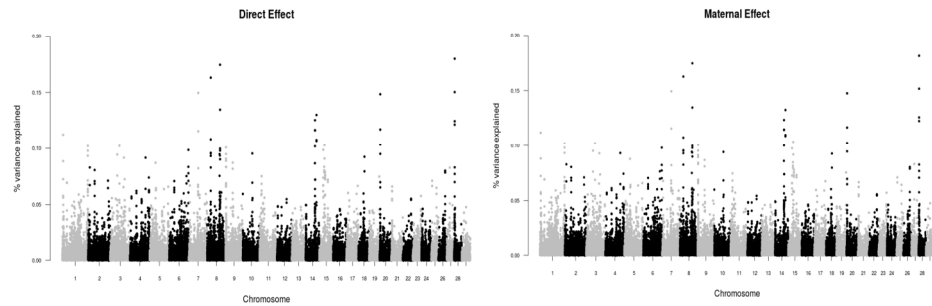


Figure 2. Manhattan plot for direct and maternal effects for weaning weight at 7 months in Zebu Brahman cattle in Colombia
254x190mm (200 x 200 DPI)

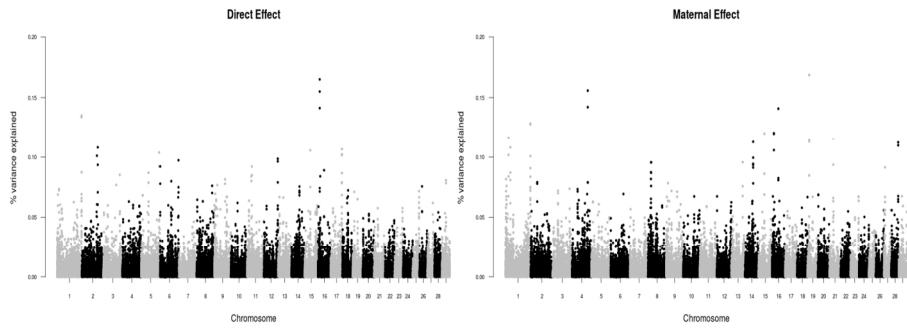


Figure 3. Manhattan plot for direct and maternal effects for yearling weight at 12 months in Zebu Brahman cattle in Colombia
254x190mm (200 x 200 DPI)