Vol.61: e18180177, 2018
http://dx.doi.org/10.1590/1678-4324-2018180177
ISSN 1678-4324 Online Edition

Biological and Applied Sciences
BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Signatures of Selection From Candidate Gene Polymorphisms Panel in Five Zebu Breeds

Gaspar Manuel Parra-Bracamonte[1*], Juan Carlos Martinez-Gonzalez[2], Ana María Sifuentes-Rincón[1], Xochitl Fabiola la Rosa-Reyna[1]
[1]Instituto Politecnico Nacional, Centro de Biotecnologia Genomica, Reynosa, Tamaulipas, Mexico; [2] Universidad Autonoma de Tamaulipas, Facultad de Ingenieria y Ciencias, Victoria, Tamaulipas, Mexico.

ABSTRACT

The signals of selection using candidate genes polymorphism were studied in five zebu breeds of Mexico. Three loci from GHRH and complementarily Steroyl Co Desaturase F762, Dopamine Beta Hydroxilase 17299, and LEP3272 were identified under selection. Findings depict Zebu selection pressure mainly on Brahman breed resulting in a divergent structure pattern.

Keywords: Cattle, population structure, PCA, selection signals

---

[*] Author for correspondence: gparra@ipn.mx, pabraman@hotmail.com

The Zebu cattle (*Bos taurus indicus*) is the second group of breeds most successful in terms of their worldwide distribution[1]. A significant proportion of the cattle raised in tropical and subtropical regions have the advantage of *Bos t. indicus* breeds physiological adaptability to thermal stress2 and their genetic resistance to diseases and parasites[1].

Diversity in Zebu cattle is related to their origin and use. For instance, Brahman has a synthetic breed origin and was created in the United States of America mostly with the influence of Guzerat and Nellore breeds[3]. Currently is the most popular zebu breed in Mexico. Nellore, Gyr, Guzerat and Indubrazil were introduced to Mexico from Brazil. Sardo Negro is the only one considered as Mexican cattle breed and was obtained through crosses between Zebu breed, from Gyr, Indubrazil and Brahman[4]. In Mexico, all Zebu breeds are used in crossbreeding strategies (with *B. t. taurus*) in systems with dual purpose. In general, all pure Zebu breeds are oriented to beef production, meanwhile, Gyr and Sardo Negro has also been oriented by selection to milk production.

Differences between productive orientation and breeding management of Zebu breeds throughout the years are expected to fix some genomic variants in loci under a different degree of selection. Theoretically, this loci fixation would allow the detection of some signatures of divergence[5]. The strategy of candidate genes is based on the physiological function, biochemical processes and metabolic pathways related to phenotypic traits[6]. This viewpoint has been focused on the seeking of genetic variation associated to traits of economical relevance[7]. Recently, Hernández et al.[7] using a selected candidate gene panel identified significantly associations of polymorphisms in Leptin and Growth hormone genes on live weight traits, supporting this approach on the searching of causative loci. Henceforth, candidate gene strategy might be effective to expose some specific variations that represent signals of unique and shared selection[5]. This study assesses the population structure of five Zebu breeds and the identification of signatures of divergent selection among them using a panel of candidate genes.

Five hundred and forty-eight samples from Brahman (n= 356; 40 herds), Guzerat (n= 20, 5 herds), Gyr (n= 40, 8 herds), Indubrazil (n= 24, 6 herds) and Sardo Negro (n=108, 10 herds) breeds were randomly sampled and genotyped by a custom 106 SNP panel (Appendix 1). Since very few samples were available, Nellore and Red Brahman breeds were formerly considered, but were later excluded from the analysis. All included animals were young males (age ≥365 d), sire candidates or already used as sires. All animals are included into the national genetic evaluations program of the Asociación Mexicana de Criadores de Cebú. Genetic evaluations have been performed since nearly 2003, and sire summaries are yearly published. In Mexico, the implementation of genetic improvement programs includes the use of genetic values for live-weight traits only. Notwithstanding, the Asociacion Mexicana de Criadores de Cebu from México was one of the first organizing registering field data in all their breeds since 1996, the organization of breeding programs and genetic evaluations begun until the early years of 2000's[8]. There is no documentation available for the animals sampled in the present manuscript on the breeding values use for selection; however, the traditional breed pattern selection is broadly used in these herds. For Brahman, the most popular Zebu breed in Mexico, there is evidence of introduction germplasm and active genetic flow from USA[8].

Genotyping was performed by GeneSeek Inc. (Neogen, Lincoln, NE, USA) using the Sequenom MassARRAY® system (iPLEX GOLD; Sequenom, San Diego, CA, USA). SNPs selected were located at candidate genes previously associated to different traits (i.e. live-weight, milk yield and composition, beef quality and temperament).

A population structure analysis was conducted using Bayesian coancestry analysis by STRUCTURE 2.3.4[9] considering 50,000 burning period length and 200,000 MCMC replicates after burning. Both, admixture and not admixture models were analyzed. STRUCTURE HARVESTER software[10] was later used to infer Delta K statistic based on the rate of change in the log probability of data between successive K values estimated in STRUCTURE, as suggested by Evanno et al.[11], the lower this value, was considered the most likely K. Additionally, a principal component analysis (PCA) of structure using PCADAPT package[12] was conducted in order to corroborate previous analysis without the Hardy Weinberg loci assumption (No migration, mutation, genetic drift nor selection) of STRUCTURE analysis. The same software of PCA was used to perform a population based analysis for the detection of the signal of selection was performed by using a stringent threshold to detect outliers (Alpha= 1.0 e-6.). An outlier locus detection analysis was complementarily performed by LOSITAN[13] considering a false discovery rate of 0.05 and 85 x 1000 simulations by an infinite allele mutation model. This approach considers Wright fixation index (Fst) outlier estimators relationships with expected heterozygosity from each locus that have excessively high or low Fst compared to neutral expectations and hence candidates for being subject to selection. A confirmation, of loci under selection was performed using a Bayesian approach by BAYESCAN[14] considering a burn-in period of 50,000 followed by 100,000 iterations.

According to the Evanno's test the most informative number of subpopulations estimated was K= 2 (Figure 1). The analysis of structure showed a group with Brahman breed, meanwhile Guzerat, Gyr, Indubrazil and Sardo Negro were grouped in a second cluster. Although some genetic information indicated resemblance of individuals among groups, in general, the graphic showed a well clustered classification. However, since Brahman cattle is one of the most popular Zebu breeds worldwide, the present results may suggest the relative artificial consequence of selection pressure mostly on this breed.

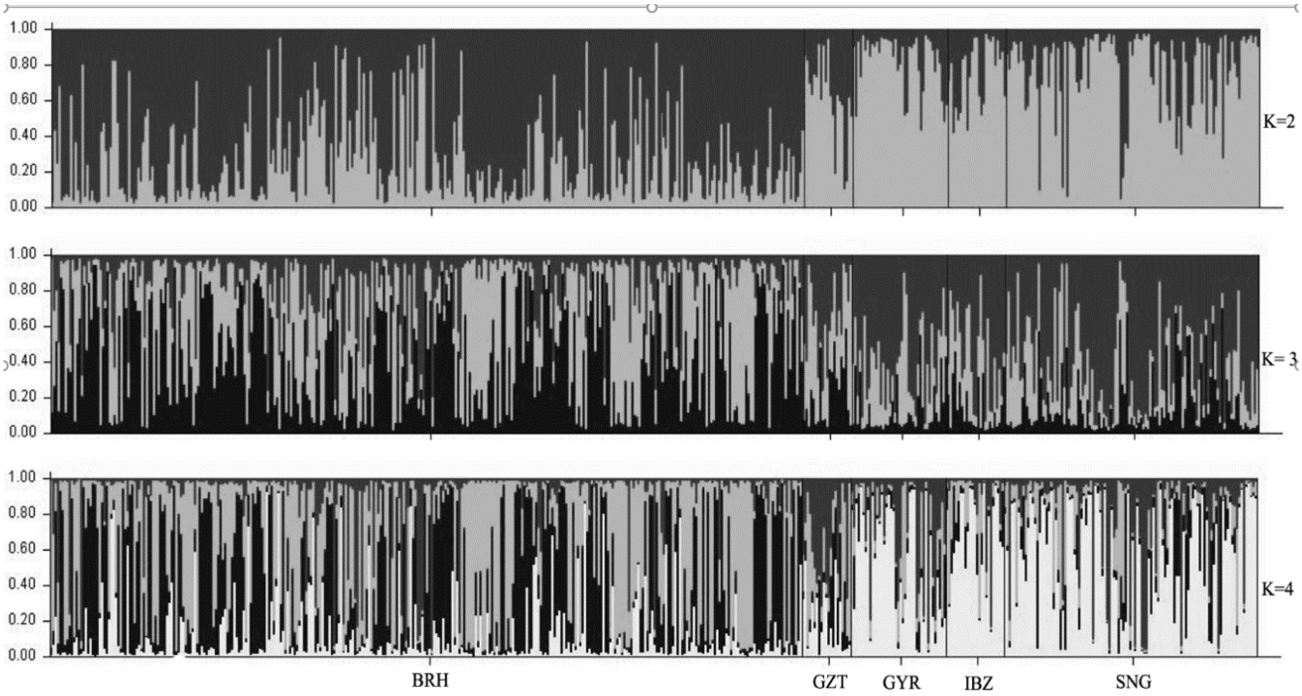

Figure 1. Population structure graphic showing K= 2 to K= 4 subpopulation of five Zebu breeds based in a candidate gene panel. BRH: Brahman, GZT: Guzerat, GYR: Gyr, IBZ: Indubrazil, SNG: Sardo Negro.

This did not imply the immediate implementation of the breeding values as a tool of selection for all the breeds, compared to their use, by example in Brahman breeding from United States; very likely conforming as observed a single cluster by the relationship of genetic flow. Nonetheless, as the results also suggested, no structure pattern of selection appears to have evidence for the other four Zebu breeds that suggest not selection pressure on loci evaluated has been applied. In the same way, PCA indicated that K=2 explained more than 8% of variance. Hence, candidate gene polymorphisms in candidate genes clustered apart Brahman population (Figure 2) indicating a disperse grouping among Guzerat, Gyr, Indubrazil and Sardo Negro.

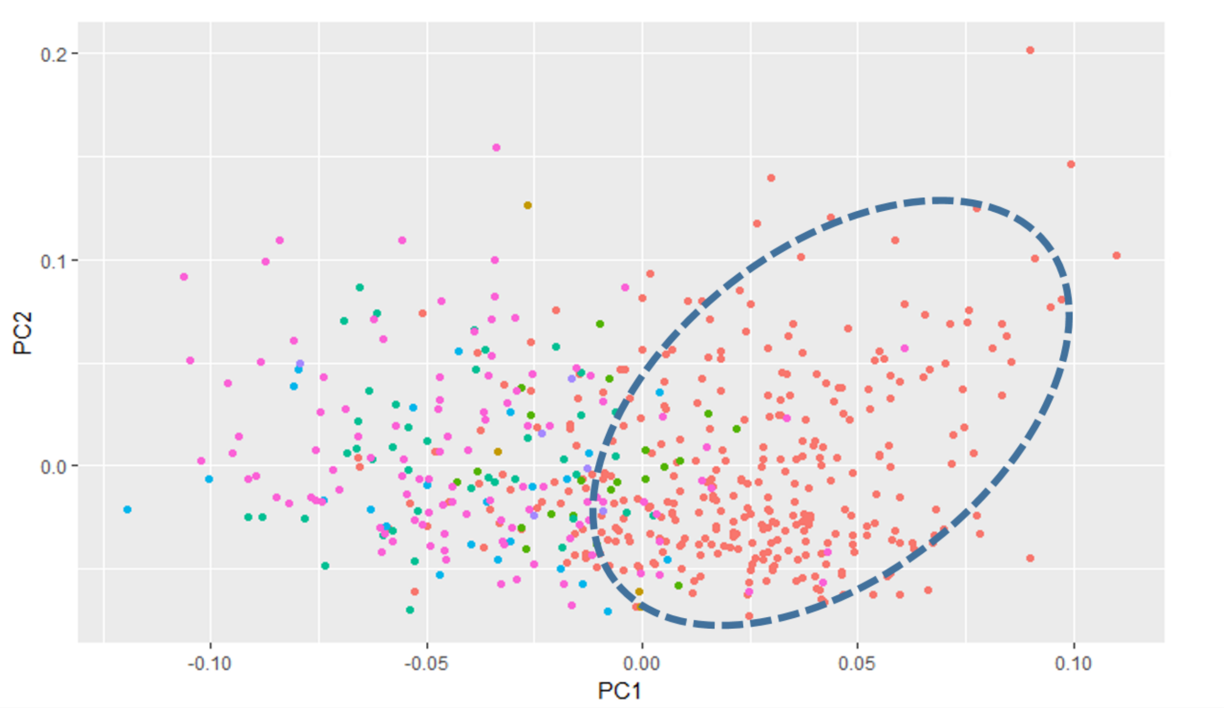

Figure 2. Principal component projection graphic showing Brahman divergent distribution from candidate gene polymorphisms.

The PCA analysis was performed ignoring 39 loci with minor allele frequency < 0.05. Outlier analysis based in PCA pointed out three loci in Growth Hormone-releasing hormone (GHRH) gene under selection (Figure 3). -4241A>T in 5’UTR region, -2298A>C from Intron I and +2279C>T from Intron 3 of GHRH were first reported by Cheong et al.[15]. They assessed this candidate gene in order to examine its relationship on meat production of Hanwoo cattle. They discovered statistical association only for -4241A>T. Although no linkage disequilibrium was identified between the segregation of these three loci, this finding is important, since GH is mediated by GHRH regulation. Furthermore, current genetic improvement programs on beef cattle breeds in Mexico including Brahman are related mostly on live weight gain selection pressure[7], henceforth, supporting the present outcome.

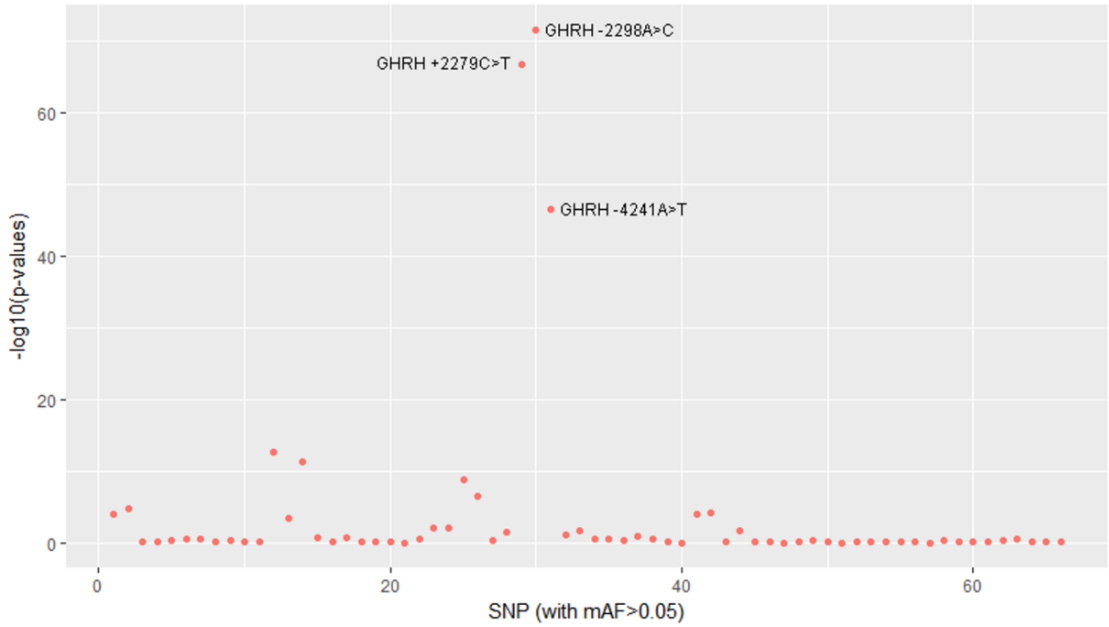

Figure 3. Manhattan plot of significantly outlier SNP positively related to selection by principal component analysis.

Complementary assessment by Fst outlier detection method, in order to distinguish the signatures of selection, indicated that three loci were under directional selection (Figure 4). Steroyl Co Desaturase F762 (SCD1 762), Dopamine Beta Hydroxilase 17299 (rs110864292), and LEP3272 (rs29004511) were the only loci that showed an outlier pattern. The Bayesian approach analysis confirmed Steroyl Co Desaturase F762 (SCD1 762), Dopamine Beta Hydroxilase 17299 (rs110864292) by their high Fst estimates (Table 1C, Complementary material).

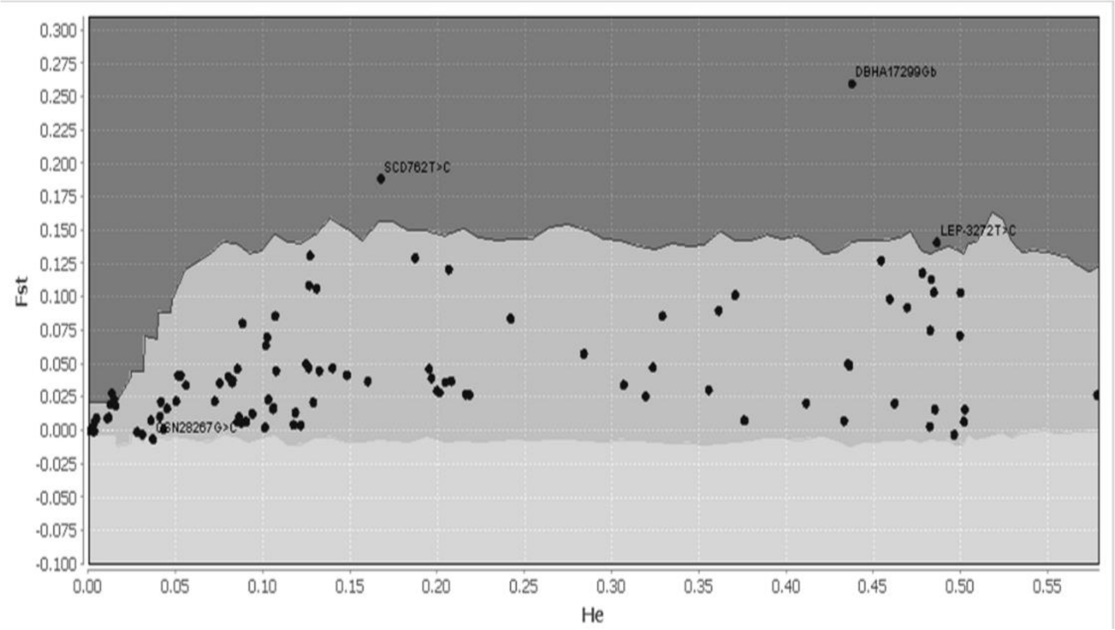

Figure 4. Graphics of Fst/He outlier positive selection loci in five Zebu breeds population.

The Steroyl Co Desaturase F762 polymorphism was reported by Taniguchi et al.[16], inside the open reading frame of the gene, and has been associated to fatty acid composition and melting point in Black Japanese cattle. Dopamine Beta Hydroxilase (DBH) rs110864292 polymorphism, was firstly reported by Lourenco et al.[17]. It is a non-synonym polymorphism located at exon XII and a functional effect of this change was suggested for Brahman breed. The distribution pattern of DBH polymorphisms amongst *Bos t.taurus* and *Bos t.indicus* breeds has been remarked by its potential association to temperament traits[17]. The Leptin rs29004511, is a synonimous polymorphism in exon 3 of this gene and was initially reported by Orrú et al.[18] in Simmental cattle. Leptin, an adipocyte derived hormone is related to different physiological functions and has been associated to some productive and reproductive traits in livestock[19]. It is considered as a predictor of live weight, scrotal circumference and reproductive hormones[20].

Some specific remarks on the performed analysis may include the similar output from population structure assessment by Bayesian and PCA analysis. Both analyses were able to distinguish Brahman population clustering, suggesting the very likely directional selection pressure on productive performance loci resulting in particular allele segregation during the breeding management through the decades. In particular, the breeding stock background of sampled animals could be considered as a real reflection of the current selection pressure on live weight and size correlated traits. Although, Guzerat, Gyr, Indubrazil and Sardo Negro are also subjected to breeding management by genetic evaluations of live weight traits, it is clear that Brahman population is one of the current most popular Zebu breeds, with an even active genetic flow from abroad.

Theoretically, the purpose of assess the number of nucleotide differences between taurine and zebu, and in this particular case, among zebu breeds, could be pointed out as the main issue of understanding domestication[21]. Approaches may include nuclear and mitochondrial genome variations and recently, the availability of large arrays have allowed the assessment of particular genomic variations that differentially are segregated in different populations. These variations can refer positive selection regions including genes determined by functional enrichment and ontology analysis. Here, the proposed evaluation includes candidate genes that theoretically have been associated in another cattle breeds populations, therefore, the proposed divergent signals explain directly a positive selection pressure. On the other hand, different studies support the inclusion of small sample sizes for some of the evaluated breeds when using the approaches of population structure and signatures of selection (e.g. n= 2-30) since availability of samples is scarce, compensating by randomly selection of samples and herds of origin when possible[22, 23, 24, 25, 26]. Here, all samples were from unrelated animals and herds, and small samples from Nellore and Red Brahman were excluded from the evaluation. However, future analyses might consider these breeds with substantially larger sample sizes confirming and proposing the present outcomes.

Finally, economic value of predictive polymorphisms under positive selection, such as those here reported, has been the focus of research for genetic improvement programs of beef and dairy cattle. Moreover, the understanding of the genetic architecture of traits that makes each breed unique is another perspective of these breed-specific indicators[5]. Currently the availability of large arrays of SNPs has made possible the genome wide exploration of these candidate hot spots and the partial understanding of evolutionary genetic architecture of some economical relevant traits. The present results could be confirmed using these genome wide approaches.

REFERENCES

1. Rischkowsky B, Pilling D. The state of the world's animal genetic resources for food and agriculture. Rome: Food and Agriculture Organization of the United Nations; 2007 [http://www.fao.org/docrep/010/a1250e/a1250e00.htm]

2. Hansen PJ. Physiological and cellular adaptations of Zebu cattle to thermal stress. *Anim Reprod Sci*. 2004; (82-83):349–60.

3. Sanders JO. History and development of Zebu cattle in the United States. *J Anim Sci*. 1980; (50):1188-1200.

4. Ocampo TJR Razas de ganado Cebú. Cebú Mexicano. 2004; (1):24-41.

5. Gutiérrez-Gil B, Arranz JJ, Wiener P. An interpretive review of selective sweep studies in Bos taurus cattle populations: identification of unique and shared selection signals across breeds. *Advan Farm Anim Gen Res*. 2016; (6):231.

6. Zhu M, Zhao S. Candidate gene identification approach: progress and challenges. *Int J Biol Sci*. 2007; (3):420-427.

7. Hernández N, Martínez-González JC, Parra-Bracamonte GM, Sifuentes-Rincón AM, López-Villalobos N, Morris ST, et al. Association of polymorphisms in growth hormone and leptin candidate genes with live weight traits of Brahman cattle. *Gen Mol Res*. 2016; (15):3.

8. Martínez J, Parra M. Edición y análisis de datos para la evaluación genética en ganado cebú. Taller "Evaluación del potencial para progreso genético en poblaciones de ganado rumiante en la región del Golfo". Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma de Yucatan–Universidad de Cornell, 2005: 16-18.

9. Pritchard, JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics.* 2000; (155): 945-959.

10. Earl DA, vonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Cons Genet Res. 2012; (4): 359-361.

11. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol*. 2005; (8): 2611-2620.

12. Luu K, Bazin E, Blum MG. pcadapt: an R package to perform genome scans for selection based on principal component analysis. *Mol Ecol Res*. 2017; (1): 67-77.

13. Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G. LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*. 2008; (9):323.

14. Foll M, Gaggiotti OE. A genome scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics* 2008; (180): 977-993

15. Cheong HS, Yoon DH, Kim LH, Park BL, Choi YH, Chung ER, et al. Growth hormone-releasing hormone (GHRH) polymorphisms associated with carcass traits of meat in Korean cattle. BMC genetics. 2006; (1): 35.

16. Taniguchi M, Utsugi T, Oyama K, Mannen H, Kobayashi M, Tanabe Y, et al. Genotype of Stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. *Mamm Genome.* 2004; (14): 142–148.

17. Lourenco-Jaramillo DL, Sifuentes-Rincón AM, Parra-Bracamonte, GM, Rosa-Reyna XFDL, Segura-Cabrera A, Arellano-Vera W. Genetic diversity of tyrosine hydroxylase (TH) and dopamine b-hydroxylase (DBH) genes in cattle breeds. *Genet Mol Biol*. 2012; (35): 435-440.

18. Orru L, Cifuni GF, Piasentier E, Corazzin M, Bovolenta S, Moioli B. Association analyses of single nucleotide polymorphisms in the LEP and SCD1 genes on the fatty acid profile of muscle fat in Simmental bulls. *Meat Sci*. 2011; (87): 344-348.

19. Máèajová M, Lamošová D, Zeman M. Role of leptin in farm animals: a Review. *J Vet Med A.* 2004; (51):157-166.

20. Thomas MG, Enns RM, Hallford DM, Keisler DH, Obeidat BS, Morrison CD, et al. Relationships of metabolic hormones and serum glucose to growth and reproductive development in performance tested Angus, Brangus, and Brahman bulls. *J Anim Sci*. 2002; (80): 757–767.

21. Magee DA, MacHugh DE, Edwards CJ. Interrogation of modern and ancient genomes reveals the complex domestic history of cattle. *Anim Front*. 2014; (3): 7-22.

22. Barendse W, Harrison BE, Bunch RJ, Thomas MB, Turner LB. Genome wide signatures of positive selection: the comparison of independent samples and the identification of regions associated to traits. *BMC genomics*. 2009; (1): 178.

23. Bovine HapMap Consortium. Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. Science. 2009; (5926): 528-532.

24. O'brien AMP, Utsunomiya YT, Mészáros G, Bickhart DM, Liu GE, Van Tassell CP, et al. Assessing signatures of selection through variation in linkage disequilibrium between taurine and indicine cattle. *Genet Sel Evol*. 2014; (1): 19.

25. Choi JW, Choi BH, Lee SH, Lee SS, Kim HC, Yu D, et al. Whole-genome resequencing analysis of Hanwoo and Yanbian cattle to identify genome-wide SNPs and signatures of selection. *Mol Cells*. 2015; (5): 466.

26. Bahbahani H, Tijjani A, Mukasa C, Wragg D, Almathen F, Nash O, et al. Signatures of Selection for Environmental Adaptation and Zebu× Taurine Hybrid Fitness in East African Shorthorn Zebu. *Front Genet*. 2017; (8): 68.

Received: April 09, 2018
Accepted: September 27, 2018