INSTITUTO POLITÉCNICO NACIONAL

CENTRO DE BIOTECNOLOGÍA GENÓMICA

"ANÁLISIS GENÉTICO-MOLECULAR DEL TEMPERAMENTO EN GANADO BOVINO: BÚSQUEDA Y ASOCIACIÓN DE POLIMORFISMOS EN GENES CANDIDATOS"

TESIS

QUE PARA OBTENER EL TÍTULO DE

DOCTORADO EN CIENCIAS EN BIOTECNOLOGÍA

PRESENTA

M. C. ESTELA GARZA BRENNER

REYNOSA, TAMPS. DICIEMBRE, 2017

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En la Ciudad de Reynosa, Tamps. siendo las 12:00 horas del día 05 del mes de Diciembre del 2017 se reunieron los miembros de la Comisión Revisora de la Tesis, designada por el Colegio de Profesores de Estudios de Posgrado e Investigación de CBG para examinar la tesis titulada:

Análisis genético molecular del temperamento en ganado bovino: búsqueda y asociación de polimorfismos en genes candidatos.

Presentada por el alumno:

Garza	Brenner	Estela
Apellido paterno	Apellido materno	Nombre(s)

Con registro: | B | 1 | 4 | 0 | 9 | 9 | 2 |

aspirante de:

Doctorado en Ciencias en Biotecnología

Después de intercambiar opiniones los miembros de la Comisión manifestaron ***APROBAR LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

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DEDICATORIA

A mis padres Magna y Jaime y a mi hermano, a quienes tanto amo y respeto, por motivarme y darme la mano cuando sentí que no iba a poder lograrlo. Por su apoyo incondicional y hacerme creer en mí y ver que es posible alcanzar lo que uno desea. Este escalón no se pudo haber logrado sin ustedes.

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ÍNDICE DE CONTENIDO

INDICE DE CONTENIDO ………………………………………… i

INDICE DE TABLAS ……………………………………………. ii

INDICE DE FIGURAS …………………………………………… iii

RESUMEN…………………………………………………………… iv

ABSTRACT………………………………………………………….. v

INTRODUCCIÓN GENERAL ……………………………………... 1

CAPITULO 1. Association of SNPs in dopamine and serotonin-pathway genes and their interacting genes with temperament traits in Charolais cows…………………………………………………….. 5

Resumen ………………………………………………….. 5

Introducción ……………………………………………… 7

Materiales y métodos ……………………………………….. 9

Resultados …………………………………………………... 13

Discusión ……………………………………………………. 18

Agradecimientos ……………………………………………… 23

Referencias …………………………………………………. 23

CAPITULO 2. Influence of genetic markers previously related to temperament on feeding behavior of yearling bulls in central test station ……………………………………………………………... 27

Resumen …………………………………………………….. 27

Introducción ………………………………………………… 30

Materiales y métodos ………………………………………. 31

Resultados ………………………………………………….. 34

Discusión…………………………………………………….. 36

Agradecimientos ………………………………………………. 39

Referencias …………………………………………………….. 39

CAPITULO 3. Markers associated with temperament also affect live weight traits in Charolais cows…….……………………... 42

Resumen …………………………………………………….. 42

Introducción ………………………………………………… 43

Materiales y métodos ………………………………………. 44

Resultados ………………………………………………….. 46

Discusión…………………………………………………….. 49

Agradecimientos ……………………………………………. 52

Referencias ………………………………………………….. 52

CONCLUSION GENERAL Y PERSPECTIVAS…………...………. 55

INDICE DE TABLAS

CAPITULO 1………………………………………………………………. 5

1.1 Distribution of cows from the four herds segregated into three temperament classifications…………………………………………….... 13

1.2 Allelic frequencies, change type and P-values of associated SNPs on temperament traits……………………………………………………. 17

CAPITULO 2…………………………………………………………… 27

2.1 Mean temperament and feeding behavior values ± standard error……………………………………………………………………… 34

2.2 Genotypic and allelic frequencies of rs43696138 and rs109576799 observed in beef cattle breeds…………………………………………… 35

CAPITULO 3 ……………………………………………………........ 42

3.1 Least squares mean for weight variables ± standard error……....... 46

3.2 Pearson coefficient correlations between temperament and growth…… 47

3.3 Allelic frequencies, change and probability values of associations with single nucleotide polymorphisms (SNPs) affecting growth……………. 48

INDICE DE FIGURAS

CAPITULO 1……………………………………………………… 5

1.1 Least squares means of exit velocity, pen score and temperament score by temperament groups in four herds of Charolais cattle……… 14

1.2 Prioritization of new genes as candidates for temperament in cattle.... 16

RESUMEN

La selección de animales con temperamento dócil para mejorar el bienestar y productividad dentro del hato se ha vuelto cada vez más importante. El temperamento del ganado es considerado un rasgo complejo y los estudios destinados a definir este rasgo son aun escasos. En este proyecto, se implementó una estrategia para la identificación de polimorfismos dentro de genes candidatos a asociarse con el temperamento bovino. La estrategia basada en redes de interacción proteína-proteína incluyó la búsqueda de nuevos genes candidatos los cuales, en conjunto con un grupo de 19 genes localizados en las vías dopaminérgica y serotoninérgica, permitieron llevar a cabo análisis de asociación fenotipo-genotipo. Para lograrlo, se evaluó, correlacionó y asoció el temperamento bovino con rasgos de eficiencia alimenticia y con el crecimiento (peso vivo) en diferentes poblaciones de ganado de carne que incluyeron a la raza Charolais, Brangus y Angus. El temperamento fue evaluado mediante dos pruebas estandarizadas: el comportamiento en el corral (PS, por sus siglas en inglés) y la velocidad de salida (EV). Se asignó un score de temperamento (TS) individual, calculado promediando el PS y EV (TS = (PS + EV/2)). De las 248 variaciones analizadas en este trabajo, seis mostraron efecto significativo sobre los tres parámetros de temperamento evaluados (PS, EV y TS). Dos de estos marcadores se incluyeron en un análisis para determinar su efecto sobre rasgos de eficiencia alimenticia en ganado Angus y Brangus. En esta última, se observó una asociación significativa en la media de ganancia diaria de peso y un marcador localizado en el gen HTR2A. Posteriormente, el panel completo se evaluó en una población de vacas de ganado Charolais y se encontró que cuatro de los marcadores previamente asociados con temperamento también se asociaron a rasgos de crecimiento.

Aunque estos resultados representan un importante progreso, ya que muestran que efectivamente la regulación del temperamento involucra múltiples genes con funciones divergentes, es necesario continuar con más estudios que permitan integrar rutas funcionales que integren todos los procesos biológicos que afectan el temperamento.

ABSTRACT

The selection of docile animals to improve welfare and productivity within the herd has become increasingly important. The cattle temperament is considered a complex trait, and studies aimed at defining this trait are still scarce. In this project, a strategy for the identification of polymorphisms within candidate genes associated with the bovine temperament was implemented. The strategy based on protein-protein interaction networks included the search for new candidate genes which, together with a 19 genes group located in the dopaminergic and serotoninergic pathways, allowed carrying out phenotype-genotype association analysis. To achieve this, the bovine temperament was evaluated, correlated and associated with feed efficiency traits and growth (live weight) in different beef cattle populations that include the Charolais, Brangus and Angus breeds. The temperament was evaluated by two standardized tests: the pen score (PS) and the exit velocity (EV). An individual temperament score (TS) was assigned, calculated by averaging PS and EV (TS=(PS+EV/2)). Of the 248 molecular variants analyzed in this work, six showed significant effect on the three indicators of temperament evaluated (PS, EV and TS). Two of these markers were included in an analysis to determine their effect on feed efficiency traits in Angus and Brangus cattle. In the latter, a significant association was observed in the mean of daily weight gain and a marker located in the HTR2A gene. Subsequently, the complete panel was evaluated in a population of Charolais cows and four of the markers previously associated with temperament were also associated with growth traits.

Although these results represent an important progress that shows that regulation of the temperament effectively involves multiple genes with divergent functions. It is necessary to continue with more studies that allow to integrate functional routes that integrate all the biological processes that affect the temperament.

IMPORTANCIA Y APORTACIÓN CIENTÍFICA DEL TRABAJO

El temperamento animal fue definido por Burrow (1997) como el comportamiento de un animal en respuesta a la manipulación por humanos, lo cual comprende un amplio rango de comportamientos, desde la docilidad hasta el miedo, el nerviosismo, la falta de respuesta, el intento de fuga y las conductas agresivas, donde son observables diversas condiciones como la actividad locomotriz general del animal y la reactividad al estrés (Fordyce *et al.,* 1982), siendo el resultado de la interacción hormonal, nerviosa y física del individuo. Es una característica importante para los sistemas de producción de ganado bovino, ya que tiene consecuencias tanto prácticas como económicas (Grandin, 1995), por lo que se considera como un rasgo económicamente relevante (ETR, por sus siglas en inglés).

El estudio del efecto del temperamento en los sistemas de producción ha demostrado que los bovinos productores de carne con un temperamento dócil tienen mejores parámetros de crecimiento, ya que se ha demostrado en estudios llevados a cabo en las etapas de finalización en la engorda, que los animales más dóciles exhiben ganancias de peso corporal hasta 14% mayores, en contraste con los animales más temperamentales (nerviosos o agresivos; Burrow *et al*., 1997), además de verse favorecidas otras características de interés productivo. Animales muy temperamentales se han asociado con diversos problemas que afectan su salud, ya que presentan mayores concentraciones de cortisol en sangre ($P<0.01$; 22.7 ± 1.0 *vs.* 17.8 ± 0.6 ng/mL) y mayor susceptibilidad a enfermedades, menores ganancias de peso ($P < 0.05$; menos del 14%), menor desempeño reproductivo al comparar edad a la pubertad ($P < 0.01$) y menores tasas de fertilidad bajo empadres controlados (88 % *vs.* 96 %), un menor rendimiento y baja calidad de la canal, afectando el pH ($pH < 5.4$) y la suavidad de la carne (fuerza de corte > 4.8 kg) , todo esto en contraste con animales más dóciles o calmados (Voisinet *et al.,* 1997b; Grandin, 2003; Fernandez *et al.*, 2008; Cooke *et al.,* 2014; Fiedrich *et al.*, 2015).

El rasgo de temperamento tiene influencia no sólo en el bienestar animal, sino también en quien lo maneja, además de jugar un papel importante en la productividad de las empresas ganaderas. Se considera que la selección genética de bovinos dóciles podría ser implementada y ser beneficiosa, ya que no hay efectos adversos aparentes sobre características productivas deseables y tiene el potencial de mejorar otros rasgos de comportamiento adaptativo (Boissy *et al.,* 1995).

El temperamento bovino es afectado por la edad, experiencia, sexo, manipulación de los animales, efectos maternos, factores ambientales, la herencia, la especie y la raza; por lo que su estudio es complejo (Burrow, 1997; Mormède, 2005).

Los esfuerzos para la identificación molecular de variantes genéticas que se relacionen con los rasgos de comportamiento han resultado en la descripción de algunos marcadores genéticos y genes candidato con potencial para la selección asistida para este rasgo. Por lo anterior, uno de los enfoques de las tecnologías moleculares ha sido la identificación de genes candidatos y variantes moleculares de los mismos, que se caracterizan por su habilidad para influir en la expresión de caracteres fenotípicos complejos a los que se asocian funcionalmente, o por su cercana localización a una región genómica asociada a dichos caracteres. Una gran ventaja de las tecnologías moleculares es la posibilidad de mejorar la selección para caracteres fenotípicos difíciles de evaluar por los métodos tradicionales de mejoramiento, es por ello que el objetivo general del proyecto fue: Identificar y asociar marcadores moleculares al temperamento del ganado bovino de carne y evaluar el efecto de éste sobre características económicamente relevantes.

En el Capítulo 1 de este estudio se describen los resultados de la aplicación del enfoque de redes de interacción, para identificar y priorizar nuevos genes de las vías dopaminérgicas y serotoninérgicas, como posibles candidatos a participar en la regulación del temperamento. Se identificaron nuevos genes con potencial de asociación al temperamento bovino, entre los que se incluyen a los genes POMC (Proopiomelanocortin), NPY (Neuropeptide Y), SLC18A2 [Solute carrier family 18 (vesicular monoamine transporter), member 2], SLC6A4 [Solute carrier family 6 (neurotransmitter transporter), member 4], y FOSFBJ (Murine osteosarcoma viral oncogene homolog). El análisis de asociación de SNPs reportados en estos 5 genes y de 19 genes adicionales que participan en las vías dopaminérgica y serotoninérgica, con el temperamento evaluado mediante la velocidad de salida y el comportamiento en el corral, permitió ubicar a seis SNPs con efecto significativo. Aunque estos resultados representan un importante progreso, ya que muestran que efectivamente la regulación del temperamento involucra múltiples genes con funciones divergentes, es necesario continuar con más estudios que permitan identificar rutas funcionales que integren todos los procesos biológicos que afectan el temperamento.

Una vez identificados los marcadores moleculares con el potencial de asociación con el temperamento, el objetivo del Capítulo 2 de este estudio fue analizar el efecto potencial del temperamento sobre la eficiencia alimenticia en tres razas de ganado bovino de carne (Angus, Brangus y Charolais) y evaluar el efecto de dos SNPs (rs109576799 y rs43696138) localizados en los genes DRD3 y HTR2A, respectivamente. Todas las muestras se tipificaron con ambos marcadores para posteriormente realizar un análisis de asociación de los genotipos con los rasgos evaluados, tanto para temperamento como para eficiencia alimenticia. No se confirmó la asociación de estos marcadores previamente reportados con el temperamento en ninguna de las razas evaluadas; sin embargo, en la raza Brangus se observó una asociación significativa en la media de ganancia diaria de peso (ADG, P = 0.049) con el marcador rs43696138, localizado en el gen HTR2A, resultando en mayores ganancias para los genotipos AA y GG (1.86 y 1.69 kg, respectivamente) en comparación con los toros heterocigóticos (AG = 1.55 kg). Se requieren más estudios para determinar el efecto de este y otros marcadores sobre el consumo residual de alimento (RFI) y el temperamento.

Finalmente, en el capítulo 3 se analizó la relación del temperamento bovino, medido como: velocidad de salida, comportamiento en corral y score de temperamento, con variables de crecimiento (peso al nacimiento, peso al destete y peso al año) de vacas de raza Charolais, siendo estos últimos indicadores fenotípicos de la capacidad de crecimiento del ganado vacuno. Algunos estudios han relacionado la capacidad de crecimiento con el temperamento. Es por ello que a partir del panel de marcadores previamente asociados al temperamento en bovinos Charolais (Capitulo 1), se evaluó el efecto de estos sobre el crecimiento, resultando en una asociación de diez SNPs localizados en seis genes candidatos (DRD2, DRD3, MAOA, POMC, TDO2 y HTR2A), para peso al nacimiento y peso al destete, cuatro de los cuales previamente habían presentado un efecto significativo sobre el temperamento.

Se debe prestar atención a aquellos polimorfismos que muestran asociaciones importantes con los rasgos estudiados y a la validación de sus efectos en otras poblaciones de ganado, especialmente cuando los mismos marcadores previamente asociados con el temperamento bovino, se asocien también con rasgos de eficiencia alimenticia o crecimiento, características de gran importancia económica en la explotación de bovinos de carne.

CAPITULO 1.

Association of SNPs in dopamine and serotonin-pathway genes and their interacting genes with temperament traits in Charolais cows*

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Abstract

Cattle temperament is a complex trait, and molecular studies aimed at defining this trait are scarce. We used an interaction networks approach to identify new genes (interacting genes) and to estimate their effects and those of 19 dopamine and serotonin-related genes on the temperament traits of Charolais cattle. The genes proopiomelanocortin (*POMC*), Neuropeptide Y (*NPY*), solute carrier family 18, member 2 (*SLC18A2*), and FBJ murine osteosarcoma viral oncogene homolog (*FOSFBJ*) were identified as new candidates. Their potential to be associated with temperament was estimated according to their reported biological activities, which included interactions with neural activity, receptor function, targeting or synthesis of neurotransmitters, and association with behaviour. Pen score (PS) and exit velocity (EV) measures were determined from 412 Charolais cows to calculate their temperament score (TS). Based on the TS, calm (n=55; TS, 1.09±0.33) and temperamental (n=58; TS, 2.27±0.639) cows were selected and genotyped using a 248 single-nucleotide variation (SNV) panel. Of the 248 variations in the panel, only 151 were confirmed to be polymorphic (single-nucleotide polymorphisms; SNPs) in the tested population. Single-marker association analyses between genotypes and temperament measures (EV, PS, and/or TS) indicated significant associations of six

SNPs from four candidate genes. The markers rs109576799 and rs43696138, located in the *DRD3* and *HTR2A* genes, respectively, were significantly associated with both EV and TS traits. Four markers, rs110365063 and rs137756569 from the *POMC* gene and rs110365063 and rs135155082 located in *SLC18A2* and *DRD2,* respectively, were associated with PS. The variant rs110365063 located in bovine SLC18A2 causes a change in the amino acid sequence from Ala to Thr.

Further studies are needed to confirm the association of genetic profile with cattle temperament; however, our study represents important progress in understanding the regulation of cattle temperament by different genes with divergent functions.

Keywords: Bovines, candidate genes, neurotransmitters, behaviour, BosNet.

Implications

The serotonin and dopamine signalling systems are essential for different behavioural phenotypes in both invertebrates and vertebrates. Studies focused on the genetic basis of behaviour in cattle are scarce. As enough evidence supports the importance of behavioural traits on the productivity of beef cattle of different breeds, the identification of intrinsic genetic sources of variation could be of fundamental application by facilitating the selection of appropriate temperament genotypes. Selecting livestock with greater docility has positive benefits for improving beef cattle performance, in addition to improving human safety and animal welfare.

Introduction

Basic behavioural traits of cattle include social behaviours such as aggression and temperament (Buchenauer, 1999). These traits may have a direct economic value and could be included in selection strategies (Mormède, 2005; Nkrumah *et al.*, 2007). Studies associating behaviour with production traits have found that nervous or aggressive animals have decreased milk flow and yield (Rushen, cited by Hiendleder, *et al.*, 2003). Temperament, defined as the animal's response to handling by humans (Burrow and Dillon, 1997), has been studied in different breeds and affects growth, health, performance, and carcass merit (Nkrumah *et al.*, 2007; Cooke, 2014).

At the molecular level, behavioural traits in cattle have been assessed using different approaches. Schmutz *et al.* (2001) reported the identification of quantitative trait loci (QTL) for response to isolation in beef cattle, while Hiendleder *et al.* (2003) reported the discovery of QTLs for milking speed. These studies uncovered the first evidence of QTLs associated with behavioural traits in dairy breeds. Genome-wide association studies (GWAS) to study temperament and meat quality in Nellore-Angus beef cattle found an association between response to social separation in a pen and a gene regulating sodium ion transport, indicating a difference in the responsiveness of the nervous system (Hulsman *et al.*, 2014).

Due to the influence of behavioural trait genes, the transporters and receptors of the serotonin- and dopamine-signalling pathways have been considered to harbour genetic variations that may be associated with variable behavioural responses (Momozawa *et al.*, 2005).

The serotonin and dopamine signalling systems are central to behavioural phenotypes in both invertebrates and vertebrates. In fact, defects in the noradrenergic system have been implicated in many mood, cognitive, and neurological disorders related with abnormal social behaviour (Våge and Lingaas; 2008). In cattle, Lühken *et al.* (2010) studied the genetic variation affecting the enzyme monoamine oxidase A (*MAOA*), and reported five single-nucleotide polymorphisms (SNPs), one of which, located in exon XV, was a non-synonymous mutation. In silico analysis did not show consistent results regarding the functional impact, and non-significant associations were found between this *MAOA* polymorphism and the behaviour-related scores analysed in the study. Glenske *et al.* (2011) found an association between a SNP located in an intron of the dopamine receptor gene (*DRD4*) and performance in a docility test. *DRD4* is involved in curiosity and novelty seeking in mammals (Rubinstein *et al.,* 1997).

Molecular studies supporting the genetic control of cattle temperament may lead to applications in production and animal welfare. Upon comparison of the different strategies employed, the candidate gene approach appears to be the most direct and effective; however, it is limited by the lack of a deep understanding of the molecular pathways involved in the genetic variability affecting these traits (Mormède, 2005). The search for molecular associations using the candidate gene approach may be improved by coupling different search strategies, such as interaction networks analyses, i.e., protein-protein interactions (PPIs) and the guilt-by-association strategy. The guilt-by-association strategy uses the biological information available in databases and statistical methods to identify potential candidate genes *in silico* (Paredes-Sánchez *et al.*, 2015). An advantage of this methodology is that it is not based exclusively on the biological information from the focal species; it actually uses all of the reference information available from other species, especially from better-understood model species, such as humans, mice, and *Drosophila* (Mormède, 2005).

In this study, we used an interaction networks approach to identify and prioritize new genes (interacting genes) and to estimate their effects and those of 19 dopamine- and serotonin-related genes on the temperament traits of Charolais cows.

Materials and methods

Data from 412 Charolais cows were included in the study. All were born between 2004 and 2013 and were managed in 4 herds located in Sonora (herd 1, n=50), Tamaulipas (herd 2, n=77), and Nuevo Leon (herd 3, n=145 and herd 4, n=140), Mexico. Animals from each herd were grouped by age into young cows (2-3 years of age) and mature cows (>4 years of age). Similar management objectives in the four herds were focused on breeding purebred Charolais cattle and the sale of breeding stock.

Temperament was assessed using pen score (PS) and exit velocity (EV). For PS, three evaluators assigned a score on a 5-point scale, where 1 = unalarmed and unexcited animal that walks slowly away from the evaluator, and 5 = very excited and moved aggressively toward the technician in a manner that requires evasive action to avoid contact between the technician and the cow, as described by Hammond *et al.* (1996). Exit velocity was assessed following the stimulus of hair sampling in the chute by measuring the rate of travel over a 1.83-m distance with an infrared sensor (FarmTek Inc., North Wylie, TX, USA). The velocity was calculated as EV = Distance (m) / Time (s). Individual temperament scores (TS) were calculated by averaging the PS and EV [TS = (PS + EV / 2)]. PS are considered subjective measures of temperament while EV is considered an objective measurement (Norris *et al.*, 2014). The combined score utilized multiple assessments (i.e., PS and EV) and may allow a more accurate classification of temperament (Burdick *et al.*, 2011).

As cattle temperament is influenced by age and handling management, among other environmental factors, animals were classified into three groups: calm, intermediate, and temperamental in each herd based on the herd-group average values (TS) ± 1 standard deviation (SD).

To examine the temperament differences of the segregated groups in relation to herd and age group factors, a linear model including the fixed effect of the interaction of the temperamental group with herd and the effects of the interactions of the temperament and age group was fitted. After this analysis, a least square means comparison was performed. SAS V.9 (SAS Statistical Analysis System, Cary, NC, USA) was used for all statistical analyses.

Identification and prioritization of candidate genes influencing temperament

A strategy for the identification of interacting genes was implemented using the BosNet approach. A list of reference genes was determined in accordance with the BosNet-user instructions (http://www.cbg.ipn.mx/Investigacion/Paginas/BosNet.aspx). A literature review was conducted to select the reference genes for temperament reported to be involved in the dopaminergic and serotonergic pathways in humans, horses, and dogs (Sifuentes-Rincón *et al.*, 2015). These candidates included all of the genes of the dopamine and serotonin pathways in cattle with reported sequences in NCBI Resources (http://www.ncbi.nlm.nih.gov/; Bovine Genome http://www.bovinegenome.org/; *DRD1*, Gene Id:281125; *DRD2*, Gene Id:281126; *DRD3*, Gene Id:537043; *DRD5*, Gene Id:526221; *DDC*, Gene Id:280762; *HTT*, Gene Id:615059; *HTR1A*, Gene Id:407137; *HTR1B*, Gene Id:317707; *HTR2A*, Gene Id:407230; *TDO2*, Gene Id:530397; *TH*, Gene Id:280707; *DBH*, Gene Id:280758;

ADRA2A, Gene Id:282135; *ADRA2B*, Gene Id:516422; *NET*, Gene Id:282363; *PNMT*, Gene Id:281413; *COMT*, Gene Id:618278; *MAOA*, Gene Id:281293; *MAOB*, Gene Id:338445; *TPH1*, Gene Id:781941; and *TPH2*, Gene Id:100336620).

To identify and prioritize candidate genes for each of the integrated networks, the interactions of the reference genes were extracted, and the degree of association with temperament (DAT) was calculated for each gene in the following subnet.

$$DAT = \sum_{j \in ref\ genes} W_{ij} \, . \sum_{j \in ref\ genes} P_{ij}$$

where W_{ij} is the linkage temperament connecting protein *i* and reference protein *j* and P_{ij} is the number of links connecting protein *i* and reference protein *j* (excluding itself). Thus, the probability that each of these proteins is associated with temperament or behaviour traits was evaluated based on its interaction with genes known to be functionally associated with this trait.

Using this information, the predictive power of each of the modelled networks for *Bos taurus* was evaluated, and the capacity of these networks to correctly identify genes associated with temperament was measured. This predictive power was characterized using receiver operating characteristic (ROC) curves. The area under the curve (AUC) was used as an indicator of the predictive power. AUC values ≤ 0.5 represent random predictions. AUC values > 0.5 represent predictions ranging from average to good.

For the selection of interacting candidate genes for temperament, the new score was used to calculate the positive predictive value (PPV), which indicates the likelihood of association between the gene and the target trait (Paredes-Sánchez *et al.*, 2015).

Genotyping and association analysis of selected SNPs affecting temperament

DNA was isolated from hair samples and genotyped using the Sequenom MassARRAY® platform (GeneSeek, Inc., Lincoln, NE, USA). The genotypic and allelic frequencies were estimated using Genepop® 4.0.10 software (Rousset, 2008).

Based on availability in the GenBank databases, we consider to include a 248 SNV (single-nucleotide variation) panel. These markers were defined as SNVs, due to with the exception of a few markers located at DRD4, TH and DBH genes, no frequency data in any population was available to be considered as a single nucleotide polymorphisms (SNP). The included SNVs were located mainly in the coding regions (71%) of 19 reference genes and 4 of their 5 interacting genes. The SNVs were represented by 48.27% synonymous and 23% non-synonymous variants. As expected, the number of SNVs included from each gene was different: the highest was 35 SNVs from the bovine *HTT* gene, and the lowest was one SNP in the *DRD4* gene. After genotyping and considering the frequencies (minor allele frequency > 10%) we defined them as SNPs.

The effects of selected SNP genotypes on the PS, EV, and TS were assessed from the selected animals dataset using a general linear model procedure that included the fixed effects of herd, age group, year of birth, and genotype in each studied SNP. In the model, $Y_{ijk} = \mu + HD_i + GE_j + G_k + \varepsilon_{ijk}$, where Y (EV, PS and TS) represents the dependent studies traits; HD, is the i-th herd effect (Herd 1… Herd 4); GE is the j-th age group effect (young and mature cows); G: is the effect of the k-th genotype in each individual SNP; and ε is the random error. Least square means of genotypes were estimated for the SNPs that demonstrated significant effects ($P < 0.05$), and a comparison of the means was performed with the PDIFF statement. All procedures were performed using SAS 9.0 software (SAS Institute Inc., Cary, NC, USA).

Results

Temperament assessment and contrasting population examination

The distribution of animals within each herd classified as calm, intermediate and temperamental is presented in Table 1. Both interactions between temperament classification were significant ($P = 0.0001$), indicating the differences in the classification categories among the studied herds and age groups.

Table 1. *Distribution of cows from the four herds segregated into three temperament classifications.*

Herd	Temperament Classification			Total
	Calm	Intermediate	Temperamental	
Herd 1	6	37	7	50
Herd 2	13	53	11	77
Herd 3	19	106	20	145
Herd 4	17	103	20	140
Total	55	299	58	412

In general, a gradient of greater temperament traits was observed in the young cow grouping compared with that in the older cows. Nonetheless, no significant differences were observed in the means for EV between the animals classified as calm in both age groups ($P = 0.068$). PS and TS showed similar patterns with greater means for young cows in the temperamental groups ($P \leq 0.04$) in comparison with the other levels of interaction, even with temperamental old cows (data not shown).

For all traits, the interactions between the temperamental groups and herd were significant ($P < 0.0001$), and the estimated means are illustrated in Figure 1. The cows in herd 4 showed greater differences in the assessment of EV, which was particularly evident in the cows belonging to the temperamental category, in relation to the intermediate and temperamental categories compared with those from the other herds analysed; additionally, herd 4 had the highest exit velocities (3.68 ± 0.12 m/s).

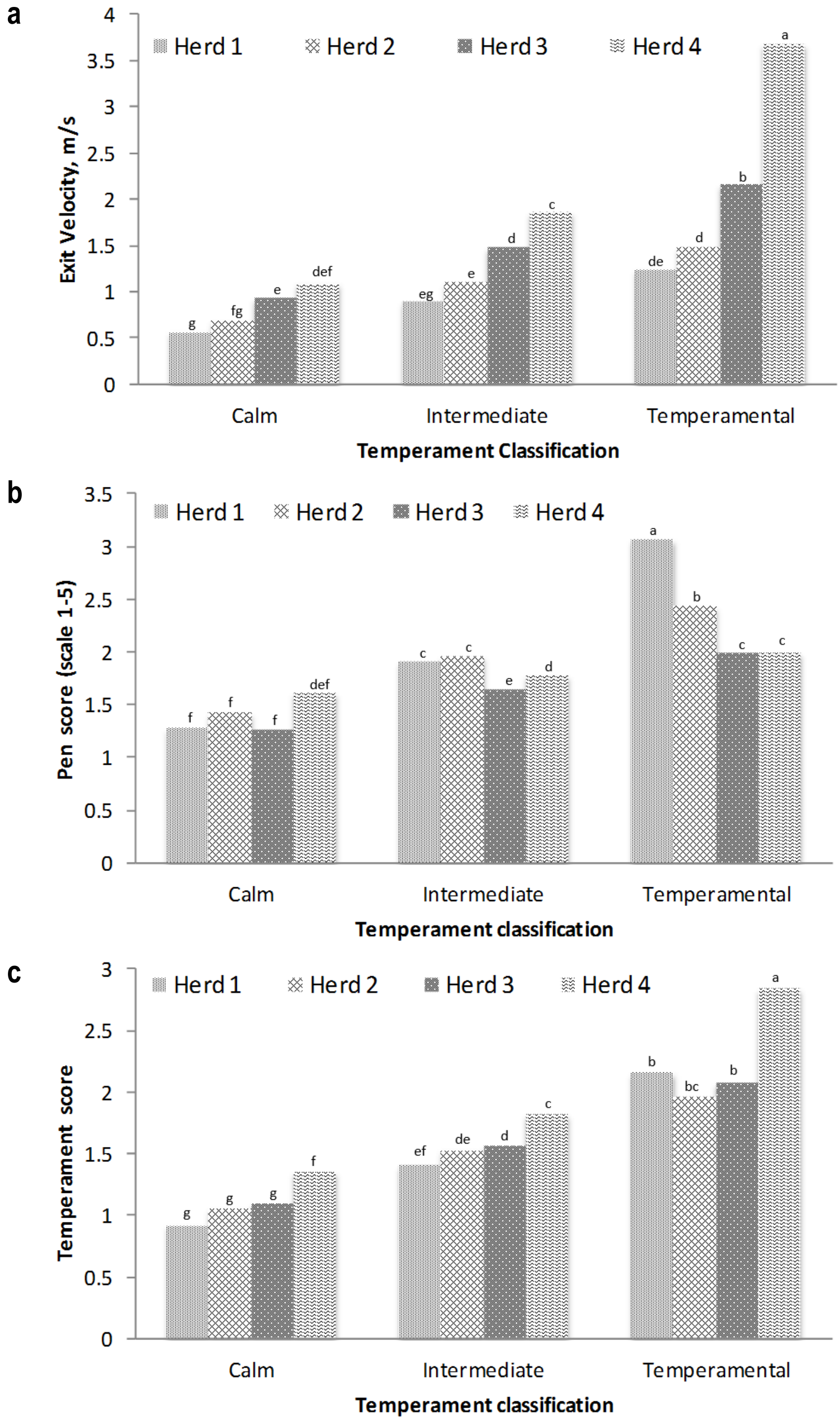

Figure 1. *Least-square means of exit velocity, pen score and temperament score by temperament groups in four herds of Charolais cattle.*
$^{a, b, c}$ *Different letters indicate significant differences (P<0.05).*

For PS, larger differences were observed in the temperamental cows of the different herds. Herd 1 had the largest mean EV (3.07 ± 0.14 points), while the intermediate and calm animals showed more equilibrated means among the herds producing compact groups (Figure 1b).

For TS, the mean values showing the interaction between the temperament classification and herd are depicted in Figure 1c. Similar to the pattern observed for EV, herd 4 exhibited a larger mean TS compared with the other herds in the same temperament classification ($P < 0.0001$); similarly, the calm groups of the herds showed more similarities in the mean TS (Figure 1c).

Association of SNPs located in the candidate genes with temperament

The selection criterion for the new interacting candidate genes to be associated with temperament was a PPV greater than 0.5 (genes with a greater than 50% probability). As shown in Figure 2, the BosNet-prioritized genes included *POMC* (Proopiomelanocortin; Gene ID: 504216), *NPY* (Neuropeptide Y; Gene ID: 504216), *SLC18A2* [Solute carrier family 18 (vesicular monoamine transporter), member 2; Gene ID: 282471], *SLC6A4* [Solute carrier family 6 (neurotransmitter transporter), member 4; Gene ID: 282365], and *FOSFBJ* (Murine osteosarcoma viral oncogene homolog; Gene ID: 280795); all genes showed PPVs of 0.58.

Animals from the 4 herds [n = 58 with the highest TS (temperamental group), n = 55 with the lowest TS (calm group)] were selected for the genotyping and association analyses.

From the tested panel, only 151 were confirmed to be polymorphic (SNPs) in the tested population (minor allele frequency > 10%).

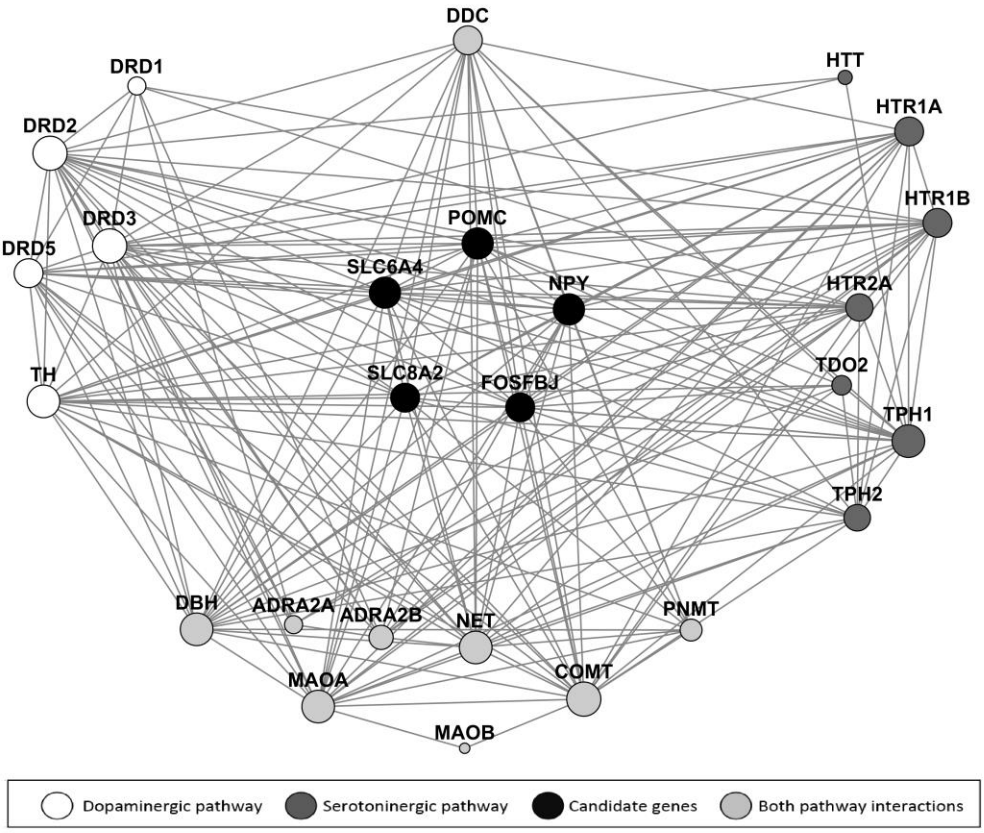

Figure 2. *Prioritization of new genes as candidates for temperament in cattle. Five genes (black) were found interacting with reference genes. The dot size of each represented gene indicates the strength of the interaction in the net.*

Table 2 shows the results of the association analysis. Six SNPs from four candidate genes were associated with one or more temperament traits (EV, PS, and/or TS). The markers rs109576799 and rs43696138, located in the *DRD3* and *HTR2A* genes, respectively, were significantly associated with both EV and TS traits. The CC genotype of the rs109576799 SNP had a significant effect on EV that was 1.06 and 1.03 m/s greater than the EVs of the heterozygous (CA, P = 0.0025) and homozygous (AA, P = 0.0018) genotypes; this locus was also significantly associated with TS. The CC genotype showed a TS that was 0.6 DS greater than those of the AA and CA genotypes (P = 0.0045 and P=0.0051, respectively).

Table 2. *Allelic frequencies, change type and P-values of associated SNPs on temperament traits.*

Gene	SNPId	Allele frequencies				Allele	Aminoacid Change	P-values		
		A	C	G	T			PS	EV	TS
DRD2	rs135155082			0.9264	0.0736	G/T	V [Val] → V [Val]	0.0263		
DRD3	rs109576799	0.6395	0.3605			A/C			0.0044	0.0103
HTR2A	rs43696138	0.2240		0.7760		A/G	L [Leu] → L [Leu]		0.0179	0.0444
POMC	rs134604486		0.3532		0.6468	C/T		0.0395		
	rs137756569	0.6604		0.3396		A/G		0.0498		
SLC18A2	rs110365063	0.3281		0.6719		A/G	A [Ala] → T [Thr]	0.0239		

PS: Pen score, EV: Exit velocity, TS: Total score

For the rs43696138 marker, the GA genotype had a significant effect on EV that was 0.09 and 1.21 m/s greater than the EVs of the homozygous (GG, P = 0.0053) and (AA, P = 0.06) genotypes. TS was also affected by the genotype of this marker. The heterozygous GA genotype exhibited a TS that was 0.66 DS greater than those of the AA and GG genotypes (P = 0.0393 and P = 0.0132, respectively).

Four markers, rs110365063 and rs137756569 from the *POMC* gene and rs110365063 and rs135155082 located in *SLC18A2* and *DRD2*, respectively, were associated with PS; the most significant markers were rs110365063 and rs134604486. In the former, the GG genotype increased the PS value by 0.338 points compared with those of the AA and AG genotypes (P = 0.05 and P = 0.01, respectively); meanwhile, the PS of the homozygous TT genotype of marker rs134604486 was 0.28 and 0.53 points greater than those of the CT (P = 0.06) and CC (P = 0.01) genotypes, respectively.

Discussion

Animal behavioural genetic studies have been challenged by the definition of phenotypes (Mormède, 2005). TS has been reported as a trait capable of accurately assigning beef cattle to calm, intermediate and temperamental classifications and has been considered as a selection tool that combines two of the most used temperament measures in cattle (i.e., EV and PS) (Burdick *et al.*, 2011); hence, we defined the groups of a Charolais cows' population based on their TS to identify the genetic variants potentially associated with this behavioural trait.

We determined the TS based on the evaluation of EV and PS, which indicated that the Charolais population in our study had temperament traits similar to previous reports in different cattle breeds, including *Bos taurus* and *Bos indicus* (i.e., Hereford, Angus, beef crosses Angus/Charolais and Brahman) (Nkrumah *et al.*, 2007; Schmidt *et al.*, 2014). As expected, the four herds studied had significant interactions with some environmental factors, with the most important being age and handling management. The segregation of animals based on their TS allowed us to develop a study population that included the temperament-based extreme animals from the four herds.

Cattle temperament has been described as a complex trait. According to Cooke, (2014) excitable temperaments have been positively associated with neuroendocrine stress reactions, regardless of the breed, age category, and production system. Hence, genes related to the stress response, which involves the activation of a complex range of pathways, including the endocrine, nervous, and immune systems, may potentially be associated with this trait (Smith and Vale, 2006).

Due to their influence on almost all biological functions and the availability of their sequences in public databases, 24 genes that encode different regulatory enzymes, transporters, and receptors of the cattle serotonin and dopamine signalling pathways were considered as the obligate candidate genes for exploring the genetic variations associated with temperament. In addition, in view of the low numbers of genes and genetic variations known in cattle and in an effort to explore the genetic complexity of this trait, we used a PPI approach to increase the number of candidate genes. The use of the BosNet tool resulted in the prioritization of five additional candidate genes: *POMC, NPY, SLC18A2, SLC6A4, and FOSFBJ.* Their potential to be associated with temperament was estimated based on their reported biological activities in humans and mice, which, with the exception of the *FOS FBJ* gene, included interactions with neural activity, receptor function, targeting or synthesis of neurotransmitters, and association with behaviour.

POMC is the precursor for several peptide hormones produced by posttranslational processing, some of which are involved in energy homeostasis, including alfa-melanocyte stimulating hormone (MSH), corticotropic hormone (ACTH), and β-endorphin. In cattle, this gene plays an important role in ingestive behaviour, energy homeostasis, and hot carcass and shipping weights (Zhang *et al.,* 2009; Deobald and Buchanan, 2011).

The *NPY* gene regulates appetite, feeding behaviour, and hormonal activity. Because of its role in feeding behaviour, some SNPs of this gene have been associated with growth traits in cattle (Sherman *et al.*, 2008).

Both *POMC* and *NPY* are key regulatory factors in the leptin/melanocortin pathway, which is considered one of the most important routes contributing to the regulation of energy metabolism; both are involved in the function of the hypothalamic-pituitary-adrenal axis, which plays an important role in the regulation of multiple physiological processes, including reproduction, anxiety, learning and memory, and cardiovascular function in both mammalian and non-mammalian species (Liu *et al.*, 2014).

In humans, the vesicular monoamine transporter 2 (VMAT2, SLC18A2) is a critical mediator of dopamine dynamics in the neuronal terminal. VMAT2 is an H+-ATPase antiporter that uses the vesicular electrochemical gradient to drive the packaging of cytosolic monoamines (dopamine, serotonin, norepinephrine, and histamine) into small synaptic and dense core vesicles (Lohr *et al.,* 2015). Bovine VMAT2 was cloned and characterized by Krejci *et al.* (1993); these authors reported that the bovine adrenal medulla sequence, bVMAT, is more closely related to the transporters from the human and rat brains than to that from the rat adrenal medulla; however, there are no additional reports about their function or association with any trait in cattle.

The *SLC6A4* gene is located on chromosome 19 and functions as a serotonin transmembrane transporter and in serotonin binding. The serotonin transporter is a member of a highly homologous family of sodium/chloride-dependent neurotransmitter transporters responsible for the reuptake of biogenic amines from the extracellular fluid. The serotonin transporter system is the pharmacological target of several clinically important antidepressants. Mortensen *et al.* (1999) reported the molecular cloning of the serotonin transporter from the bovine species, but no association studies have been reported in this species.

Finally, *FOSFBJ*, the human oncogene c-fos, is homologous to the Finkel-Biskis-Jinkins (FBJ) murine osteosarcoma virus oncogene. FOS was the first transcription factor identified that has a critical function in regulating the development of cells destined to form and maintain the skeleton. FOS is also a major component of the activator protein-1 (AP-1) transcription factor complex (Zhang *et al.,* 1998). According to the functions reported, the *FOSFBJ* gene might be expected to have a less specific effect on neural activity and behaviour than those genes targeting neurotransmitter synthesis and receptor function (*POMC*, *NPY*, *SLC6A4*, and *SLC18A2*); however, it may be associated with the known phenotypic effects of different temperaments, i.e., loss/gain of weight and growth (Burrow and Dillon, 1997; Cooke, 2014).

As mentioned previously, molecular markers associated with behavioural traits in cattle are scarce; only two studies examined the association of polymorphisms located in candidate genes with different behavioural traits (Lühken *et al.,* 2010; Glenske *et al.,* 2011). Herein, we used a selective genotyping strategy to identify the molecular markers associated with temperament in Charolais cattle. Although this strategy has been considered suitable for QTL identification in cattle (Moioli *et al.,* 2014), we justified the use of the temperament segregation for our association analysis with the multiple physiological studies that evaluated the effects of temperament on productive traits in cattle (King *et al.,* 2006; Cooke, 2014; Schmidt *et al.,* 2014). Most of the cited studies used the segregation population analysis.

From a panel of 151 tested SNPs, we identified the associations of six SNPs from four genes with three temperament traits. It is important to remark that we based the study on the genetic diversity previously reported in databases for each gene; however, an additional advantage of using the segregated population is that it may be used to discover new genetic and Charolais-specific variants of these genes via resequencing of the proposed candidate genes.

We found two SNPs (rs109576799 and rs43696138) located in the *DRD3* and *HTR2A* genes, respectively, that were significantly associated with EV and TS. The biological functions of both genes have not been reported in cattle; however, in humans, genetic variants of the dopamine D3 receptor (*DRD3*) gene have been implicated in schizophrenia, autism, and substance use disorders; the gene is also associated with emotional reactivity, executive function, and stress response (Wolf *et al.,* 2014). The bovine rs109576799 DRD3 marker is located in an intron of the *DRD3* gene; while this variation had no obvious functional implication on gene expression that could explain its effect on temperamental traits, the predicted role of the gene in emotional reactivity and the sensitivity of the dopamine system to environmental stressors may potentially explain its association with behaviour.

The coding SNP rs43696138 in the bovine serotonin 5-hydroxytryptamine receptor 2a (*HTR2A*) gene is a synonymous mutation (Lys/Lys) and may be considered inconsequential for primary protein structure and/or function; however, a growing number of studies have shown that synonymous mutations are also under evolutionary pressure and can be implicated in complex traits and diseases such as human cancer (Supek *et al.,* 2014). In humans, the *HTR2A* gene is a G-protein-coupled excitatory receptor exerting its influence through the activation of the secondary messenger phospholipase. Mutations in the human *HTR2A* gene are associated with susceptibility to schizophrenia and obsessive-compulsive disorder, and recently, polymorphisms of this gene have been proposed as a possible predisposition factor for aggressive traits (Banlaki *et al.,* 2015).

Four additional SNPs associated with PS were located in three candidate genes, two (*SLC18A2* and *POMC*) of which were prioritized by the interactive network approach, and their functions were discussed above. The SNP rs110365063, located in bovine *SLC18A2,* is a transition that causes a change in the amino acid sequence from Ala to Thr. Its association with PS in cattle should be validated in further studies to explore its effects on the gene expression and/or protein function of *SLC18A2*.

The human D2 dopamine receptor is a G-protein-coupled receptor centrally involved in reward-mediating mesocorticolimbic pathways (Neville *et al.,* 2004). Signalling through dopamine D2 receptors governs physiological functions related to locomotion, hormone production, and drug abuse. D2 receptors are also known targets of antipsychotic drugs that are used to treat neuropsychiatric disorders such as schizophrenia. The dopamine receptor D2 is located on bovine chromosome 15 and has been described to play a role in regulating prolactin secretion. A SNP in this gene has been proposed as a marker to identify cattle that are resistant to the effects of endophyte-derived alkaloids (Campbell, 2012). No studies have explored the role of this gene in cattle behaviour.

The extensive experimental evidence in humans and other non-human models supports our association results. Further attention must be paid to those polymorphisms showing important associations with temperament traits to validate their effects in other cattle populations.

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References

Banlaki Z, Elek Z, Nanasi T, Szekely A, Nemoda Z, Sasvari-Szekely M and Ronai Z 2015. Polymorphism in the Serotonin Receptor 2a (HTR2A) gene as possible predisposal factor for aggressive traits. PLoS ONE 10, e0117792. doi:10.1371/journal.pone.0117792.

Buchenauer D 1999. Genetics of behaviour in cattle. In The genetics of cattle (ed. R Fries and A Ruvinsky), pp. 365–390. CABI Publishing, Wallingford, UK.

Burdick NC, Agado B, White JC, Matheney KJ, Neuendorff DA, Riley DG, Vann RC, Welsh Jr TH and Randel RD 2011. Technical note: evolution of exit velocity in suckling Brahman calves. Journal of Animal Science 89, 233–236. doi:10.2527/jas.2010-2973.

Burrow HM and Dillon RD 1997. Relationships between temperament and growth in a feedlot and commercial carcass traits of bos indicus crossbreds. Australian Journal of Experimental Agriculture 37, 407–411. doi:10.1071/EA96148.

Campbell BT 2012. Improving beef cattle performance on tall fescue. PhD Dissertation. University of Tennessee, Knoxville, TN, USA. Retrieved on 23 february 2016 from http://trace.tennessee.edu/utk_graddiss/1277.

Cooke RF 2014. Bill E Kunkle interdisciplinary beef symposium: temperament and acclimation to human handling influence growth, health, and reproductive responses in cattle. Journal of Animal Science 92, 5325–5333.

Deobald HM and Buchanan FC 2011. Characterization of two pro-opiomelanocortin gene variants and their effects on carcass traits in beef cattle. BMC Genetics 12, 2. doi:10.1186/1471-2156-12-2.

Glenske K, Prinzenberg EM, Brandt H, Gauly M and Erhardt G 2011. A chromosome-wide QTL study on BTA29 affecting temperament traits in German Angus beef cattle and mapping of DRD4. Animal 5, 195-197. doi:10.1017/S1751731110001801.

Haegeman A, Williams JL, Law A, Zeveren AV and PeelmaN JL 2003. Characterization and mapping of bovine dopamine receptors 1 and 5. Animal Genetics 34, 290–293.

Hammond AC, Olson TA, Chase Jr CC, Bowers EJ, Randel RD, Murphy CN, Vogt DW and Tweolde A 1996. Heat tolerance in two tropically adapted Bos Taurus breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. Journal of Animal Science 74, 295–303.

Hiendleder S, Thomsen H, Reinsch N, Bennewitz J, Leyhe-Horn B, Looft C, Xu N, Medjugorac I, Russ I and Kühn C, Brockmann GA, Blümel J, Brenig B, Reinhardt F, Reents R, Averdunk G, Schwerin M, Förster M, Kalm E and Erhardt G 2003. Mapping of QTL for body conformation and behavior in cattle. Journal of Heredity 94, 496–506. doi:10.1093/jhered/esg090.

Hulsman HLL, Garrick DJ, Gill CA, Herring AD, Riggs PK and Miller RK 2014. Genome-wide association study of temperament and tenderness using Bayesian approaches in a Nellore-Angus cross-bred population. Livestock Science 161, 17–27.

Krejci E, Gasnier B, Botton D, Isambert MF, Sagné C, Gagnon J, Massoulié J and Henry JP 1993. Expression and regulation of the bovine vesicular monoamine transporter gene. FEBS Letters 335, 27-32. doi:10.1016/0014-5793(93)80432-T.

Liu WC, Chen SY, Jia XB, Wang J and Lai SJ 2014. Effects of variants in proopiomelanocortin and neuropeptide y genes on growth, carcass, and meat quality traits in rabbits. Asian-Australasian Journal of Animal Sciences 27, 609–615. doi:10.5713/ajas.2013.13807.

Lohr KM, Stout KA, Dunn AR, Wang M, Salahpour A, Guillot TS and Miller GW 2015. Increased vesicular monoamine Transporter 2 (VMAT2; Slc18a2) protects against methamphetamine toxicity. ACS Chemical Neuroscience 6, 790−799. doi:10.1021/acschemneuro.5b00010.

Lühken G, Glenske K, Brandt H and Erhardt G 2010. Genetic variation in monoamine oxidase A and analysis of association with behaviour traits in beef cattle. Journal of Animal Breeding and Genetics = Zeitschrift für Tierzüchtung und Züchtungsbiologie 127, 411–418. doi:10.1111/j.1439-0388.2010.00855.x.

Moioli B, Napolitano F and Catillo G 2014. A selective genotyping approach identifies QTL in a simulated population. BMC Proceedings 8 (suppl. 5), S5. doi:10.1186/1753-6561-8-S5-S5.

Momozawa Y, Takeuchi Y, Kusunose R, Kikusui T and Mori Y 2005. Association between equine temperament and polymorphisms in dopamine D4 receptor gene. Mammalian Genome 16, 538–544. doi:10.1007/s00335-005-0021-3.

Mormède P 2005. Molecular genetics of behaviour: Research strategies and perspectives for animal production. Livestock Production Science 93, 15–21. doi:10.1016/j.livprodsci.2004.11.002.

Mortensen OV, Kristensen AS, Rudnick G and Wiborg O 1999. Molecular cloning, expression and characterization of a bovine serotonin transporter. Molecular Brain Research 71, 120–126. doi:10.1016/S0169-328X(99)00178-3.

Neville MJ, Johnstone EC and Walton RT 2004. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Human Mutation 23, 540–545. doi:10.1002/humu.20039.

Nkrumah JD, Crews DH, Basarab JA, Price MA, Okine EK, Wang Z and Li C, Moore SS 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. Journal of Animal Science 85, 2382–2390. doi:10.2527/jas.2006-657.

Norris D, Ngambi JW, Mabelebele M, Alabi OJ and Benyi K 2014. Genetic selection for docility: a review. The Journal of Animal and Plant Sciences, 24(1):13-18.

Paredes-Sánchez FA, Sifuentes-Rincón AM, Segura Cabrera A, García Pérez CA, Parra Bracamonte GM and Ambriz Morales P 2015. Associations of SNPs located at candidate genes to bovine growth traits, prioritized with an interaction networks construction approach. BMC Genetics 16, 91. doi:10.1186/s12863-015-0247-3.

Rousset F 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8, 103–106. doi:10.1111/j.1471-8286.2007.01931.x.

Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson JL, McDougall JA, Chester JA, Saez C, Pugsley TA, Gershanik O, Low MJ and Grandy DK 1997. Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell 90, 991–1001. doi:10.1016/S0092-8674(00)80365-7.

Schmutz SM, Stookey JM, Winkelman-Sim DC, Waltz CS, Plante Y and Buchanan FC 2001. A QTL study of cattle behavioral traits in embryo transfer families. Journal of Heredity 92, 290–292. doi:10.1093/jhered/92.3.290.

Schmidt SE, Neuendorff DA, Riley DG, Vann RC, Willard ST, Welsh THJr and Randel RD 2014. Genetic Parameters of three methods of temperament evaluation of Brahman calves. Journal of Animal Science 92, 3082–3087.

Sherman EL, Nkrumah JD, Murdoch BM, Li C, Wang Z, Fu A and Moore SS 2008. Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. Journal of Animal Science 86, 1–16. doi:10.2527/jas.2006-799.

Sifuentes-Rincón AM, Trejo-Tapia AG, Randel RD, Ambriz-Morales P and Parra-Bracamonte GM 2016. Bovine dopamine receptors DRD1, DRD4, and DRD5:

genetic polymorphisms and diversities among ten cattle breeds. Genetics and Molecular Research 15, 1: gmr.15017725.

Smith SM and Vale WW 2006. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues in Clinical Neuroscience 8, 383–395.

Supek F, Miñana B, Valcárcel J, Gabaldón T and Lehner B 2014. Synonymous mutations frequently act as driver mutations in human cancers. Cell 156, 1324–1335. doi:10.1016/j.cell.2014.01.051.

Våge J and Lingaas F 2008. Single nucleotide polymorphisms (SNPs) in coding regions of canine dopamine- and serotonin-related genes. BMC Genetics 9, 10. doi:10.1186/1471-2156-9-10.

Wolf EJ, Mitchell KS, Logue MW, Baldwin CT, Reardon AF, Aiello A, Galea S, Koenen KC, Uddin M, Wildman D and Miller MW 2014. The dopamine D3 receptor gene and posttraumatic stress disorder. Journal of Traumatic Stress 27, 379–387. doi:10.1002/jts.21937.

Zhang CL, Wang YH, Chen H, Lei CZ, Fang XT, Wang JQ, Ma GB, Niu H and Xiao J 2009. [The polymorphism of bovine POMC gene and its association with the growth traits of Nanyang cattle]. Yi Chuan 31, 1221–1225. doi:10.3724/SP.J.1005.2009.01221.

Zhang Y, Feng XH and Derynck R 1998. Smad3 and Smad4 cooperate with c-Jun/c-Fos to mediate TGF-beta-induced transcription. Nature 394, 909–913. doi:10.1038/29814.

CAPITULO 2.

Influence of genetic markers previously related to temperament on feeding behavior of yearling bulls in central test station*

***Enviado con formato requerido a:** Revista Colombiana de Ciencias Pecuarias

Abstract

Background: Searching for gene and marker effects on economically important traits is based not only on the biological interest to understand the genetic architecture of complex traits but also on applying the information to practical breeding schemes.

Objective: In order to assess its effect on feed efficiency, two markers previously associated with bovine temperament were evaluated on Angus, Brangus and Charolais yearling bulls under centralized performance tests based on residual feed intake (RFI).

Methods: One hundred and thirty-six young beef bulls were included in a centralized feed efficiency performance tests based on residual feed intake (RFI). Besides feeding behavior traits, temperament was assessed using pen score (PS) and exit velocity (EV). All samples were genotyped with two markers located at HTR2A and DRD3 genes and an association analysis were conducted between these genotypes and the registered traits.

Results: In the Brangus breed, a significant association was observed for average daily gain (ADG; P=0.049) on the rs43696138 marker, located in HTR2A gene, resulting in higher gains for both AA and GG genotypes (1.86 y 1.69kg, respectively) when compared to the heterozygous bulls (AG = 1.55kg).

Conclusion: The previously reported association of these markers with temperament was not confirmed in the evaluated breeds, however rs43696138 marker showed an effect on a feeding behavior trait. Further studies are needed to determine the effect of this and other markers on both RFI and temperament.

Resumen

Antecedentes: La búsqueda de efectos genéticos y marcadores de rasgos económicamente relevantes no solo se basa en el interés biológico de comprender la arquitectura genética de rasgos complejos, sino también en aplicar la información en los esquemas prácticos de mejoramiento.

Objetivo: Evaluar el efecto sobre la eficiencia alimenticia de dos marcadores previamente asociados con el temperamento bovino en toros de un año de las razas Angus, Brangus y Charolais.

Métodos: Ciento treinta y seis toros fueron sometidos a una prueba de comportamiento alimenticio basada en el consumo residual de alimento (RFI). Además de los rasgos de comportamiento alimenticio, se evaluó el temperamento de los animales mediante la prueba de comportamiento en el corral (PS) y la velocidad de salida (EV). Todas las muestras se tipificaron con dos marcadores localizados en los genes DRD3 y HTR2A para posteriormente realizar un análisis de asociación de los genotipos con los rasgos evaluados.

Resultados: En la raza Brangus, se observó una asociación significativa en la media de ganancia diaria de peso (ADG, P = 0,049) en el marcador rs43696138, localizado en el gen HTR2A, resultando en mayores ganancias para los genotipos AA y GG (1.86 y 1.69kg, respectivamente) en comparación con los toros heterocigóticos (AG = 1.55 kg).

Conclusión: No se confirmó la asociación de estos marcadores previamente reportados con el temperamento en las razas evaluadas, sin embargo, el marcador rs43696138 mostro un efecto en un rasgo de comportamiento alimenticio. Se necesitan más estudios para determinar el efecto de este y otros marcadores con el consumo residual de alimento (RFI) y el temperamento.

Resumo

Antecedentes: A procura de efeitos de genes e marcadores em características economicamente importantes baseia-se não só no interesse biológico para compreender a arquitetura genética de traços complexos, mas também na aplicação da informação a esquemas de melhorias práticas.

Objetivo: Para avaliar o efeito sobre a eficiência alimentar, dois marcadores localizados nos genes DRD3 e HTR2A e previamente associados com o temperamento bovino foram avaliados em touros angulares de Angus, Brangus e Charolês sob testes de desempenho centralizados baseados na ingestão de alimento residual (RFI).

Métodos: Cento e trinta e seis novilhos jovens foram incluídos em testes centralizados de desempenho de eficiência alimentar, com base na ingestão de alimento residual (RFI), com um período de adaptação pré-julgamento de 20 de um teste de alimentação de 70 d. O temperamento foi avaliado usando pontuação da caneta (PS) e velocidade de saída (EV). As frequências alélicas e genotípicas foram estimadas e uma análise de associação foi realizada.

Resultados: Na raça Brangus, observou-se associação significativa para o ganho diário médio (ADG; P = 0,049) no marcador rs43696138, localizado no gene HTR2A, resultando em maiores ganhos tanto para genótipos AA quanto GG (1.86 e 1.69 kg, respectivamente) Quando comparado aos touros heterozigotos (AG = 1.55 kg).

Conclusão: A associação relatada anteriormente desses marcadores com temperamento não foi confirmada nas raças avaliadas, no entanto, o marcador rs43696138 mostrou um efeito sobre uma característica de comportamento alimentar. São necessários mais estudos para determinar o efeito deste e de outros marcadores tanto na RFI quanto no temperamento.

Key words: *beef cattle, feed efficiency, residual feed intake, single nucleotide polymorphism, temperament.*

Palabras clave: *bovinos de carne, eficiencia alimenticia, consumo residual de alimento, polimorfismo de un solo nucleótido, temperamento.*

Palabras clave: *gado de corte, eficiência alimentar, ingestão de alimentação residual, Polimorfismo de nucleotídeo único, temperamento.*

Introduction

The residual feed intake (RFI), it is defined as the difference between actual feed intake and the predicted based on mean requirements for body weight maintenance and the level of production, it was proposed for the first time for the evaluation of cattle by Koch *et al.* (1983). Cattle with a lower residual feed intake consume less food, and therefore the maintenance requirements are lower, while the growth seems to be unaffected (Koch *et al.,* 1963; Crews *et al.,* 2005). Studies have reported some moderate heritabilities for this trait, Crews *et al.* (2005) estimated values from 0.26 to 0.30, Arthur *et al.* (2001b) and Moore *et al.* (2007) from 0.39 to 0.43; thus, it is expected that selection for RFI results in a genetic change relatively comparable to those obtained with other moderately heritable traits, such as growth traits.

More excitable cattle may be more easily stressed and less efficient (Lindholm-Perry *et al.,* 2014; Friedrich *et al.,* 2015). Furthermore, temperamental animals usually present less body weight gain, reduced reproductive efficiency, inferior meat quality and higher disease susceptibility, thus animal temperament impacts the economic efficiency in any production system (Friedrich *et al.,* 2015). Negative correlations between daily weight gain and temperament traits such as flight speed, chute score and others, have been found (Haskell *et al.,* 2014) and these results encouraged the interest to include bovine temperament as a strategy to select docile and, therefore, more efficient animals (Lindholm-Perry *et al.,* 2014).

To contribute to the discovery of molecular markers that better assist in selection of animals with higher feed efficiency and docile temperament, the objective of this study was to analyze the effect of two temperament related SNPs on feed efficiency in Mexican beef cattle.

Material and methods

Population of study

The present study did not require ethical clearance from the Animal Care and Use Committee because the data were extracted from existing herd books and the biological samples and genetic materials had been obtained for previous studies and were stored in germplasm banks.

One hundred and thirty-six young bulls, including the Angus (AN), Brangus (BR) and Charolais (CH) breeds, were included in the study. All of them had an average initial age and body weight of 273±38 d and 272±38 kg, respectively, at the time they were included in the first (April 2016; AN=42, BR=29, CH=19) and second (July 2016; AN=21, BR=12, CH=13) centralized feed efficiency performance tests based on residual feed intake (RFI) in northern Mexico (Regional Beef Cattlemen Union of Chihuahua, UGRCH, for its acronym in Spanish).

Feeding and temperament evaluation

Animals were subjected to the same feeding protocol; including a pre-trial adaptation period of 20 d and a feeding trial of 70 d. Animals were weighed at two consecutive days at the beginning and the end of the trial, and every 14 d of the test. Individual daily feed intake and feeding behavior were measured and recorded using the GrowSafe System (GrowSafe System Ltd., Airdrie, AB, Canada). Calculated traits also included residual feed intake (RFI) as described by Koch et al. (1963), dry matter feed intake (DMFI), feed to gain ratio (F:G) and average daily gain (ADG) .

Temperament was assessed using pen score (PS) and exit velocity (EV). For PS, three evaluators assigned a score on a 5-point scale, where 1 = unalarmed and unexcited animal that walks slowly away from the evaluator, and 5 = very excited and aggressive toward the technician in a manner that requires evasive action to avoid contact between the technician and the animal, as described by Hammond et al. (1996), being evaluated at the beginning and at the end of trial. Exit velocity was assessed following the stimulus of hair sampling in the chute by measuring the rate of travel over a 1.83-m (6 ft) distance with an infrared sensor (FarmTek Inc., North Wylie, TX, USA). The velocity was calculated as EV = Distance (m) / Time (s) [8,13]. All animals' first measures (PS, EV, weighted and collected hair samples) were achieved at the time they arrived to the UGRCH facilities.

Genotyping and association

Hair samples collected during temperament testing were used for DNA isolation using Genelute Mammalian Genomic DNA kit (Cat. G1N350, Sigma-Aldrich Co. LLC, St. Louis, Missouri, USA).

Genotyping of the rs43696138 marker located in the HTR2A gene was performed using allelic discrimination in the ABI PRISM 7500 Sequence Detection System, following the manufacturer's instructions (Applied Biosystems). Assay conditions were 2 min at 50°C, 10 min at 95°C, and 40 two-step cycles of 92°C for 15s and 60°C for 1 min. The SNP assay was set up using SDS software by the allelic discrimination settings (ABI Prism 7500 Real-Time Sequence Detection Software).

To genotype for the rs109576799 marker (transversion A/C, located at intronic region, Chromosome 1: 59343757 of DRD3 gene), a PCR/RFLP assay was designed using the sequence and SNPID rs109576799; primers (F6799 5'-CTGGAGGCCCGGGGAAGAATCA-3'; R6799 5'-GCCCGCCCACACGCCTACTAC-3') were used for PCR. The reaction mixes comprised 20–100 ng genomic DNA, 1.5 or 2.5 mM MgCl2, 0.1 µM of each primer, 0.4 mM dNTPs and 2.5 U Go Taq polymerase (Promega Corporation, Madison, WI, USA). A touchdown method was used with the amplification profile of an initial denaturation step of 95 °C for 10 min, five three-step cycles of 45 sec at 95 °C, 45 sec at 68 °C (the temperature was reduced 2 °C each cycle), then twenty five cycles of 45 sec at 95 °C, 45 sec at 65 °C and finally one cycle of 45 sec at 72 °C. PCR fragments were separated by electrophoresis in a 1.5% agarose gel, stained with SYBR Gold and visualized by UV irradiation. After PCR, digestion reactions were done following the manufacturer's protocol for the Fok1 enzyme and digestion fragments were analyzed on a 2.5% agarose gel (Sigma-Aldrich).

Allelic and genotypic frequencies were estimated using GENEPOP 4.0.10 software. The effects of selected SNP genotypes on analyzed variables were assessed adjusting a general linear model with the GLM procedure of SAS v. 9.4 (Statistical Analysis System Inc., Cary, NC, USA) which included the fixed effects of the number of performance test and genotype for each studied SNP. In the model, $Y_{ij} = \mu + G_i + H_j +$

ε_{ij}, where Y= dependent variable (EV, PS, ADG, DMFI, RFI, F:G), G is the effect of the i-th genotype for each individual SNP, H= effect of the j-th performance test (1, 2) and ε_{ij}= random error. Least squares mean of genotypes were estimated for the SNPs that demonstrated significant effects ($p < 0.05$) and comparisons of the means were performed with the PDIFF option in the LSMEANS statement of the GLM procedure.

Results

Phenotypic trait descriptions

The mean values for temperament (pen score=PS and exit velocity=EV) and the feeding behavior traits within each breed are presented in Table 1. Except for PS values, Brangus was identified as the most temperamental breed and Angus the most docile.

Table 1. Mean temperament and feeding behavior values ± standard error

Breed	[1]PS±SE	[2]EV±SE	DMFI (kg)	F:G	ADG (kg)	RFI (kg)
Angus	1.91±0.05b	1.79±0.07c	10.24±0.14a	6.69±0.10a	1.55±0.02a	0.002±0.09a
Brangus	2.28±0.06a	2.38±0.09a	9.94±0.17a	6.15±0.13b	1.63±0.03a	-0.011±0.11a
Charolais	2.05±0.07b	2.03±0.11b	9.00±0.20b	5.91±0.15b	1.53±0.04a	-0.165±0.13a

[1] PS: Pen Score, was assigned to animals to describe behavior when approached by a human; scores ranged from 1 (calm) to 5 (most temperamental).
[2] EV: Exit Velocity, was calculated as velocity=distance(m)/ time(s).
DMFI: dry matter feed intake; F:G feed to gain ratio; ADG: average daily gain; RFI: residual feed intake.
a,b,c: The different letters indicate significant differences ($P < 0.05$)

The mean value for DMFI was significantly different between Charolais and the other two tested breeds, with a lower value of dry matter feed intake compared to Angus and Brangus. Meanwhile, for F:G Angus presented the highest values compared with Charolais and Brangus breeds. No significant differences were observed on average daily gain and residual feed intake among breeds.

Effect of temperament markers on feed efficiency traits

Table 2 shows the allelic frequencies of the two markers evaluated in the three beef cattle breeds. As reported (Garza-Brenner *et al.*, 2017), the three populations evaluated had the highest frequency of the G allele for the marker rs43696138 and the A allele in the case of the rs109576799 marker.

For the two markers, no significant associations were found for the temperament variables (PS and EV) in the breeds evaluated.

Table 2. Genotypic and allelic frequencies of rs43696138 and rs109576799 observed in beef cattle breeds.

Marker	Genotypes/ alleles	Angus (n=63)	Brangus (n=41)	Charolais (n=32)
rs43696138	GG	0.57	0.46	0.78
	GA	0.38	0.49	0.22
	AA	0.05	0.05	0.00
	G	0.76	0.72	0.89
	A	0.24	0.28	0.11
rs109576799	AA	0.13	0.02	0.31
	AC	0.72	0.88	0.56
	CC	0.15	0.10	0.12
	A	0.49	0.46	0.59
	C	0.51	0.54	0.41

rs43696138 marker located in HTR2A gene; rs109576799 marker located in DRD3 gene.

Estimation of the two marker's effect on efficiency parameters showed that only for Brangus there was a significant association for average daily gain (ADG; *P*=0.049) to the rs43696138 marker, resulting in higher gains for both homozygous AA and GG genotypes (1.86 y 1.69 kg, respectively) when compared to the heterozygous genotype (AG = 1.55 kg).

Discussion

On beef cattle systems, feed costs represent around two thirds (50 – 75 %) of total production costs. Implementation of feed efficiency evaluation in cattle has been a primary goal in beef cattle systems to reduce feeding costs and the negative impacts on the environment (Crews *et al.*, 2005). Both, RFI and temperament, have been described as complex traits and some studies have proposed to find the genes that explain and regulate their expression (Crews *et al.,* 2005; Lindholm-Perry *et al.,* 2014). In order to find genetic markers associated with both traits, Lindholm-Perry *et al*. (2014) found that two markers previously associated with cattle ADG/ADFI (average daily gain/average daily feed intake) and frame size were also associated with flight speed.

Recently, Garza-Brenner *et al.* (2017) also identified two new SNPs: rs109576799 and rs43696138, in DRD3 and HTR2A genes, respectively, that were significantly associated with exit velocity (EV) and temperament score (TS) in Charolais cows. Both genes have been related to dietary efficiency, playing an important role in the control of appetite in humans (Zhang *et al.,* 2004; Leung *et al.,* 2009) but in beef cattle there are no previous studies that support their biological function (Garza-Brenner *et al.,* 2017).

Phenotypic measures of temperament indicated, as it was expected, that Brangus yearling bulls had the highest values for the temperament variables. Genetic background is an important factor influencing temperament. *Bos indicus* and *Bos indicus*-crosses tend to be more temperamental than *Bos taurus* (Burdick *et al.,* 2011). Temperament is most often measured at weaning, and therefore most of the published literature has focused on the effects of temperament during preweaning and posweaning periods (Burdick et al., 2011). Even when temperament measures were taken at an average age past, but close, to weaning of the studied animals, the mean values of the temperament evaluations (i.e, exit velocity) were in a range considered as docile in the literature (Burrow *et al*., 1997; Burdick *et al*., 2011). Burrow *et al*. (2011) reported that cattle with an EV > 2.4 m/s are temperamental while cattle with an EV < 1.9 m/s are calm.

RFI is a feed efficiency measure that is calculated as the difference between the actual dry matter intake of an animal and its expected predicted dry matter intake for a given level of maintenance and production (Llonch *et al.*, 2016). Cattle with calm temperament have been found to have greater average daily gain (ADG; Burrow *et al.*, 1997), even so, no significant differences were observed for RFI and ADG between breeds.

The results obtained for the effects of the two markers on temperament contrast with those previously obtained in Charolais cows (Garza-Brenner *et al.*, 2017), where the two SNPs (rs109576799 and rs43696138) were previously associated with temperament, in case of the present study we did not observed any association with temperament and these markers. The sample size could be an important factor contributing to this result. It has been proposed that when a marker association discovery occurs in the Gene-SNP/trait, different factors could affect the association. For example, the genetic background of the animals studied, in this case *Bos taurus vs. Bos indicus,* comparing Charolais and Angus with Brangus breed; another factor that could affect the association is the historic selection pressure on the trait. Each producer in Mexico selects the phenotypic characteristics that they consider appropriated by the breeding and production system. Different frequencies in the founder animals and random genetic drift could be other factors (Machado *et al.*, 2003).

As is described by Garza Brenner *et al*. (2017), in cattle, the biological functions of HTR2A and DRD3 genes have not been previously reported; however, according with studies in humans, an effect on behavior traits is expected (Supek *et al.*, 2014; Wolf *et al.*, 2014; Banlaki *et al.*, 2015; Wolf *et al.*, 2014).

Recently, Dos Santos *et al*. (2017) identified candidate genes for reactivity in Guzerat (*Bos indicus)* cattle. One of those genes was ZBTB20, a transcriptional factor with the potential for association with behavioral traits such as anxiety and a strong candidate for reactivity in cattle. The authors also found that 70kb upstream of ZBTB20 gene is the dopamine receptor 3 (DRD3) gene. Therefore, it is important to continue with studies to establish the role played by the marker rs109576799 evaluated in this work both in the expression of bovine temperament and in parameters of feed efficiency, as well as to study the linkage disequilibrium of this marker with the intronic marker located in the ZBTB20 gene.

In a study by Llonch *et al*. (2016), the association of temperament and stress response with productivity, feed efficiency, and methane emissions in cattle, was evaluated. No significant associations were found between temperament and feed efficiency; however, they observed that a greater response to stress was associated with reduction in feed intake. Calmer cattle therefore ingest larger amounts of food and this will be reflected in increased growth and fat deposition compared to more temperamental animals. Likewise, in the study carried out by Lindholm-Perry *et al*. (2014), no association was found for the tested markers. However, these authors also analyzed SNPs on BTA6:38–39 Mb, a region previously associated with average daily gain (ADG) and average daily feed intake (ADFI) by themselves, and several of those SNPs were associated with flight speed ($P \leq 0.005$). The study of the genetic architecture of complex traits such as temperament and feed efficiency are a major challenge. Whenever the use of genomic approaches allows elucidating this complexity involving one by one genes that have an influence on these traits.

As a final remark, it is important to mention that the number of individuals studied plays an important role in association studies and having a greater number of individuals by genotypes evaluated allows more accurate and reliable estimation. Further research on these and related genes could clarify and validate their proposed effect on temperament and feed efficiency.

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Conflicts of interest. The authors declare they have no conflicts of interest with regard to the work presented in this report.

References

Arthur PF, Renand G and Krauss D. Genetic and phenotypic relationships among different measures of growth and efficiency in young Charolais bulls. Livest Prod Sci 2001b;68:131-139.

Banlaki Z, Elek Z, Nanasi T, Szekely A, Nemoda Z, Sasvari-Szekely M, Ronai Z. Polymorphism in the Serotonin Receptor 2a (HTR2A) gene as possible predisposal factor for aggressive traits. PLoS One 2015;10, e0117792. doi:10.1371/journal.pone.0117792.

Burdick NC, Randel RD, Carrol JA, Welsh TH Jr. Review article: Interactions between Temperament, Stress, and Immune Function in cattle. Int J Zool 2011;1-9.

Burrow HM, Dillon RD. Relationships between temperament and growth in a feedlot and commercial carcass traits of Bos indicus crossbreds. Aust J Exp Agr 1997;37:(4)407–411.

Crews DH “Denny” Jr. Genetics of efficient feed utilization and national cattle evaluation: a review. Genet Mol Res 2005;4(2):152-165.

Dos Santos FC, Peixoto MGCD, Fonseca PadS, Pires MdFÁ, Ventura RV, Rosse IdC., et al. Identification of candidate genes for Reactivity in Guzerat (*bos indicus)* Catte: A Genome-Wide Association Study. PloS ONE 2017;12(1): e0169163. doi:10.1371/journal.pone0169163.

Friedrich J, Brand B, Schwerin M. Genetics of cattle temperament and its impact on livestock production and breeding – a review. Arch Anim Breed 2015;58:13–21.

Garza-Brenner E, Sifuentes-Rincón AM, Randel RD, Paredes-Sánchez FA, Parra-Bracamonte GM, Arellano Vera W, Rodríguez Almeida FA, Segura Cabrera A. Association of SNPs in dopamine- and serotonin-pathway genes and their interacting genes with temperament traits in Charolais cattle. J App Genet 2017;58(3):363-371.

Hammond AC, Olson TA, Chase Jr CC, Bowers EJ, Randel RD, Murphy CN, Vogt DW and Tweolde A. Heat tolerance in two tropically adapted *bos taurus* breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. J Anim Sci 1996;74:295–303.

Haskell MJ, Simm G, Turner SP. Genetic selection for temperament traits in dairy and beef cattle. Front Genet 2014;5:1–18.

Koch RM, Swiger LA, Chambers D, Gregory KE. Efficiency of feed use in beef cattle. J Anim Sci 1963;22:486-494.

Leung AK. Encyclopedia of Molecular Mechanisms of Disease, F. Lang, 2009, Ed., pp. 103, Springer, Berlin, Germany.

Lindholm-Perry AK, Kuehn LA, Freetly HC, Snelling WM. Genetic markers that influence feed efficiency phenotypes also affect cattle temperament as measured by flight speed. Anim Genet. 2014;46:60–64.

Llonch P, Somarriba M, Duthie C-A, Haskell MJ, Rooke JA, Troy S, Roehe R and Turner SP. Association of Temperament and Acute Stress Responsiveness with Productivity, Feed Eficiency, and Methane Emissions in Beef Cattle: An Observational Study. Front Vet Sci 2016;3:43. doi: 10.3389/fvets.2016.00043.

Machado MBB, Alencar MM, Pereira AP, Oliveira HN, Casas E, Countinho LL and Regitano LCA. QTL affecting body weight in a candidate region of cattle chromosome 5. Genet Mol Biol 2003;26:259-265.

Moore SS, Mujibi FB, and Sherman EL. Molecular basis for residual feed intake in beef cattle. J Anim Sci 2007;87(E. Suppl.):E41–E47. doi:10.2527/jas.2008-1418.

Supek F, Miñana B, Valcárcel J, Gabaldón T, Lehner B. Synonymous mutations frequently act as driver mutations in human cancers. Cell. 2014;156:1324–1335. doi:10.1016/j.cell.2014.01.051

Wolf EJ, Mitchell KS, Logue MW, Baldwin CT, Reardon AF, Aiello A, Galea S, Koenen KC, Uddin M, Wildman D, Miller MW. The dopamine D3 receptor gene and posttraumatic stress disorder. J Trauma Stress 2014;27:379–387. doi:10.1002/jts.21937.

Zhang XN1, Jiang SD, He XH, Zhang LN. 102T/C SNP in the 5-hydroxytryptamine receptor 2A (HTR2A) gene and schizophrenia in two southern Han Chinese populations: lack of association. Am J Med Genet B Neuropsychiatr Genet 2004;126B(1):16-8.

CAPITULO 3.

Markers associated with temperament also affect live weight traits in Charolais cows*

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ABSTRACT

Background: Birth, weaning and yearling live weights are phenotypic indicators of growth ability in beef cattle, and previous studies have related growth ability to temperament. Thus, we investigated whether a panel of molecular markers previously associated with temperament had an effect on the growth characteristics (birth weight, weaning weight and yearling weight) of Charolais cows.

Results: Significant Pearson correlations ($p<0.05$) between two weight traits (birth weight (BW) and weaning weight (WW)) and two temperament measurements (exit velocity (EV) and temperament score (TS)) were observed in the young cow group, whereas no significant correlations were observed between the temperament measurements and weight traits for the mature cow group. Significant effects of ten SNPs (single nucleotide polymorphisms) on BW and WW were observed.

Conclusions: Four of the same markers previously associated with temperament were associated with growth traits. Further studies aimed at validating our results could determine whether the four studied markers had a pleiotropic effect on temperament and live weight traits.

Key words: beef cattle, body weight, SNPs, temperament.

Introduction

One of the most important characteristics for cattle production, especially for beef cattle, is growth, especially birth weight, which represents the first phenotypic variable of a new individual in a population and is also considered an indicator of calving ease [1]. Furthermore, for beef cattle producers, calves with greater weights at weaning have the advantage of being more morphologically developed and better equipped to successfully cope with the environment [2].

Growth is one of the most studied genomic complex traits in cattle. Databases with phenotypic records that describe growth include birth weight, weaning weight and yearling weight. The expression of this trait is influenced by multiple environmental and genetic factors, and innumerable interactions of this trait with other biological factors, such as temperament, have been described.

Different studies have found that cattle with slower flight speeds gain weight more rapidly than those with faster flight speeds [3,4,5] and temperamental cattle have lower live weights than docile cattle [6]. Petherick *et al.* [7] found a negative correlation between exit velocity and daily weight gain. Similarly, in a study with many animals, unfavorable genetic and phenotypic relationships were observed between weaning weight and exit velocity in *Bos indicus* (Nellore) cattle, demonstrating that animals with high exit speeds had lower body weights [8]. Similarly, a study with *Bos taurus* cattle reported that animals exhibiting more excitable temperaments were phenotypically more likely to have a lower body weight at entry into the feedlot than more docile cattle [9]. Thus, investigators and producers have increasingly focused on the reaction of livestock during management using diverse methods to determine the animal's docility [10]. Such research is based on evidence from the literature, where docility is correlated with not only the ease of managing cattle but also economically relevant traits.

Although many growth-influencing genes [2] with a degree of influence on temperament [11,12] have been identified, previous reports have not determined the effect of these genes on both characteristics. The aim of this study was to determine how a panel of molecular markers previously associated with temperament affect growth characteristics (birth weight, weaning weight and yearling weight) in Charolais cows.

Materials and methods

Evaluated animals

Data of 412 Charolais cows from herds located in the states of Sonora (herd 1; n=51), Tamaulipas (herd 2, n=77 and herd 3; n=144) and Nuevo León (herd 4; n=140) in México were used for this study. The cows were categorized into two groups according to age (adult cows ≥ 4 years old and young cows aged 2-3 years). The four evaluated herds were from similar backgrounds regarding the management practices and objectives for the breeding and sale of purebred Charolais cattle. Data on the birth weight (BW), weaning weight (WW) and yearling weight (YW) were obtained from the Charolais Herd Book de México A.C. © (consulted July of 2017) online database (http://www.amcgr.com/charolais/BúsquedaAnimales.aspx) using the registration number for each cow.

Evaluation of temperament

An individual pen score (PS) was assigned to each animal by three evaluators using a five-point scale (1=docile to 5=very temperamental) [13]. The exit velocity (EV) was defined as the time required to traverse 6 feet (1.83 m) after exiting a squeeze chute as described by Burdick *et al.* [14], and the temperament score (TS) was obtained for each animal using the formula ((EV+PS)/2). Because PS and EV have similar ranges and distributions, the TS trait has been suggested to provide a more useful measure of temperament [15,16].

Statistical analysis

The genotypic and allelic frequencies were estimated using Genepop® 4.0.10 software [17].

Using a panel of markers previously associated with bovine temperament [12], an association analysis was performed with the growth characteristics, such as BW (kg), WW (kg) and YW (kg). Only 80 cows of 412 were genotyped for this study.

A general linear model was fitted for BW, WW and YW using the following model: $Yijk = \mu + HD_i + GE_j + G_k + \varepsilon_{ijk}$, where Y_{ijk} = BW, WW and YW, which represent the dependent traits of this study; μ = the overall mean value; HD_i = the *i*th herd effect (Herd 1, Herd 2, ... Herd 4); GE_j = the *j*th age group effect (young and mature cows); G_k = the effect of the *k*th genotype in each individual SNP, and ε_{ijk} = the random error.

The least squares means of the genotypes were estimated for the SNPs that demonstrated significant effects ($p<0.05$), and the means were compared using the PDIFF statement. Pearson correlation coefficients were determined and all other procedures were performed using SAS software (SAS Inst. Inc., Cary, NC, USA; version 9.4).

Results

The least squares mean values for BW, WW and YW from both cow groups are presented in Table 1.

Table 1. Least square mean values for weight variables±standard error

Herd	Group	[1]BW±SE	[2]WW±SE	[3]YW±SE
1	Mature cows[4]	34.80±0.95	202.84±6.72	-
	Young cows[5]	36.25±0.99	210.50±7.19	331.50±29.9
2	Mature cows	40.52±0.75a	249.59±5.40	326.33±7.36
	Young cows	35.85±1.23b	244.70±5.93	338.15±9.16
3	Mature cows	33.88±0.49a	203.35±3.85	276.68±5.20
	Young cows	30.67±0.39b	201.89±4.71	285.96±6.91
4	Mature cows	41.03±0.77b	231.02±4.60b	336.47±5.80a
	Young cows	45.97±0.97a	248.85±4.24a	311.66±4.77b

[1]BW=birth weight; [2]WW= Weaning weight; [3]YW= Yearly weight; [4]More than 4 calving; [5]Less than three calving.
a,b: The different letters indicate significant differences ($P < 0.05$)

The general Pearson correlation coefficient results for the two Charolais age groups tested in this study are presented in Table 2. In the young cow group, low and moderate but significant correlations with the variables BW ($p<0.0001$) and WW ($p<0.03$) were found only for two temperament measurements (EV and TS). However, the variable YW was not correlated with any temperament measurements (PS, EV and TS). In the mature cow group, significant correlations were not observed between the temperament and weight traits.

Table 2. Pearson coefficient correlations between temperament and growth.

		PS	EV	TS
Mature cows	BW	[1]0.055	-0.008	0.033
		[2]0.428	0.901	0.635
	WW	0.031	-0.021	0.007
		0.652	0.760	0.920
	YW	0.021	-0.013	0.004
		0.796	0.867	0.953
Young cows	BW	0.146	0.514	0.521
		0.088	<0.0001	<0.0001
	WW	0.064	0.196	0.201
		0.455	0.021	0.018
	YW	-0.018	-0.143	-0.139
		0.842	0.118	0.127

BW=birth weight, WW= weaning weight, PS= pen score, EV= exit velocity, TS= temperament score.

[1] Pearson correlation coefficient.

[2] Probability value

Association with molecular markers

Table 3 presents the results of the association analysis, in which 10 SNPs on 6 genes were associated with one or two of the growth measures (nine for BW and three for WW). Significant associations were not found for YW.

Seven markers (rs41749779 (DRD2); rs134256715 (MAOA; monoamine oxidase A); rs134604486, rs136809285, rs137756569, rs41257366 (POMC); and rs43696138 (HTR2A)) were associated with BW, with the most significant associations observed for markers rs41749779 and rs134256715 (*p*=0.008 and *p*=0.002, respectively). The marker ra17871686 located on the POMC gene was significantly associated with WW.

The rs109576799 and rs385054562 markers, which were located on the DRD3 and tryptophan 2,3-dioxygenase (TDO2) genes, respectively, were significantly associated with BW and WW.

Cows with genotype CC of marker rs385054562 had a higher birth weight than cows with the TT genotype (39.36 *vs.* 36.74 kg, *p*=0.042); similarly, for WW, the CC genotype had higher WWs than cows with the TT genotype (240.19 *vs.* 224.49 kg, *p*=0.043). Cows with the CC genotype of marker rs109576799 had higher BWs and were 5.32 kg (*p*=0.004) and 4.59 kg (*p*=0.014) heavier than the AA and CA genotypes, respectively.

Table 3. Allelic frequencies, change and probability values of associations with single nucleotide polymorphisms (SNPs) affecting growth.

Gene	SNP ID	Allele frequencies				Allele	Amino acid change	*P*-Value	
		A	C	G	T			BW	WW
DRD2	rs41749779	0.482	0.517			A/C		0.008	
DRD3	rs109576799	0.639	0.360			A/C		0.015	0.025
MAOA	rs134256715	0.893	0.107			A/C	Cys→Trp	0.002	
POMC	rs134604486		0.353		0.646	C/T		0.030	
	rs136809285		0.598		0.401	C/T		0.026	
	rs137756569	0.660		0.339		C/T		0.027	
	rs41257366	0.400		0.600		A/G		0.021	
	rs17871681		0.316		0.683	C/T	Phe→Phe		0.085
TDO2	rs385054562		0.205		0.795	C/T		0.042	0.043
HTR2A	rs43696138	0.224		0.776		A/G	Leu→Leu	0.027	

BW= birth weight, WW= weaning weight.

Discussion

Among complex and productive traits of interest, growth has been extensively studied and its genetic architecture is currently under investigation [19]; however, economically important traits are greatly influenced by environmental and biological factors. Thus, the interactions between growth and traits that are strongly influenced by environmental and biological factors, such as temperament, must be investigated.

The Pearson coefficient correlation results presented in Table 2 in this study are inconsistent with those described in other studies focused on correlating temperament with growth because all the correlations found here were positive. This finding could be related to the time at which temperament was registered [14,16]. Temperament is usually registered in animals around weaning time, either pre- or post-weaning. The mature cows likely did not show a correlation with the temperament traits because of their age; therefore, age could have had an influence because older cows are acclimated to the location and environment in which they have lived in for years.

From a molecular perspective, the use of candidate genes that affect economically important traits has been shown to be a direct method of understanding biological functions that focuses on the trait of interest. This approach consists of exploring genes that are implicated in known biological pathways [18]. Most associated studies are based on specific polymorphisms of a gene. Using this approach, investigations have been performed to elucidate the molecular basis of bovine temperament [11,19]. Recently, Garza-Brenner *et al.* [12] conducted a study of genes and markers associated with bovine temperament. The selected markers are candidate genes primarily associated with the dopamine and serotonergic pathways as well as five protein-protein interacting genes.

Using the 151 SNP panel of Garza-Brenner *et al.* [12], we identified an association with ten SNPs located on 6 genes with two of the three recorded growth measures (BW and WW). Interestingly, four of the six genes associated in this study (DRD2, DRD3, POMC and HTR2A) have been previously described [12].

The rs109576799 (DRD3), rs134604468, rs137756569 (POMC) and rs43696138 (HTR2A) markers have been associated with bovine temperament [12], and in the present study, these markers were also associated with live weight traits (BW and WW).

Recently, Lindhom-Perry *et al.* [20] explored the role of certain markers for two different traits and found that two markers previously associated with certain phenotypical and efficiency traits, such as frame size, average daily gain and average daily feed intake, were also associated with flight speed.

Research focusing on this pleiotropic interaction has recently increased because of the availability of dense SNPs arrays and the development of statistical methodologies capable of probing the multitrait-marker interactions [21]. Further studies analyzing our results could determine whether the four studied markers had a pleiotropic effect on temperament and live weight traits.

The specific association analysis of live weight traits showed that marker rs134256715, which is located on the MAOA gene, is important because is a non-synonymous SNP (Cys/Trp). The MAOA gene plays an important role in the inter-individual variable aggressiveness, impulsive response, and serotoninergic response capacity of the central nervous system (CNS) as well as in complex behavior regulation. This gene has been termed the “warrior gene” because of its association with the response to aggression in certain behavioral studies [22,23]. Lühken *et al.* [19] studied the genetic variation of the gene in two cattle breeds (Angus and Simmental), which are known for their behavior during management, and evaluated 5 SNPs; however, they did not observe a significant association between the polymorphism and the recorded behavioral scores.

The TDO2 gene does not have any reported associations in cattle. However, in studies conducted in humans, a potential candidate gene for autism [24] and its mechanisms in signaling pathways have been reported because this gene is crucial in the synthesis of serotonin and tryptophan, which is an amino acid involved in energy metabolism, and it is also a modulator of disease behaviors associated with inflammatory states, psychiatric disorders, behavioral modulation and cognitive functioning [25,26]. Previous reports have not described any associations between the genetic markers in the present study and body weight except for the POMC gene, in which an association was observed ($p<0.05$) with the BWs of Nanyang cattle at 6 months of age and with weight gain [27].

Further attention should be focused on polymorphisms that have shown important associations with the studied traits to validate their effects on other populations of cattle, especially because four of the same markers previously associated with temperament have also been associated with growth traits.

In conclusion, polymorphisms previously associated with temperament showed to be associated with growth, which indicates that temperament may directly influence productivity at the genetic level and is expressed in the animal phenotype. This report presents the first findings in which markers associated with temperament have been correlated with growth-related traits.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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References

[1]. Canellas LC, Barcellos JOJ, Nunes LN, Oliveira TE, Prates ÊR, Darde DCH. Post-weaning weight gain and pregnancy rate of beef heifers bred at 18 months of age: a meta-analysis approach. R Bras Zootec 2012; 41:1632-1637.

[2]. Jahuey-Martínez FJ, Parra-Bracamonte GM, Sifuentes-rincón AM, Martínez-González JC, Gondro C, García-Pérez CA and lópez-Bustamante LA. Genomewide association analysis of growth traits in charolais beef cattle. J Anim Sci 2016;94:4570–4582. doi:10.2527/jas2016-0359

[3]. Burrow HM, Dillon RD. Relationships between temperament and growth in a feedlot and commercial carcass traits of Bos indicus crossbreds. Aust J Exp Agr 1997;37:(4)407–411.

[4]. Voisinet BD, Grandin T, Tatum JD, O'Connor SF, Struthers JJ. (1997). Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim Sci. vol. 75, no. 4, pp. 892–896.

[5]. Cafe LM, Robinson DL, Ferguson DM, McIntyre BL, Geesink GH, and Greenwood PL. Cattle temperament: Persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. J Anim Sci 2011;89:1452–1465.

[6]. Fordyce G, Goddard ME, Tyler R, Williams G, Toleman MA. Temperament and bruising of Bos indicus cross cattle. Aust J Exp Agric 1985; 25:283–288.

[7]. Petherick JC, Holroyd RG, Doogan VJ, and Venus BK. Productivity, carcass and meat quality of lot-fed Bos indicus cross steers grouped according to temperament. Anim Prod Sci 2002;42:389-398.

[8]. Sant'Anna AC, Paranhos da Costa MJR, Baldi F, Rueda PM, and Albuquerque LG. Genetic associations between high speed and growth traits in Nellore cattle. J Anim Sci 2012;90:3427–3432.

[9]. Reinhardt CD, Busby WD and Corah LR. Relationship of various incoming cattle traits with feedlot performance and carcass traits. J Anim Sci 2009;87:3030–3042. doi:10.2527/ jas.2008-1293

[10]. Haskell MJ, Simm G, Turner SP. Genetic selection for temperament traits in dairy and beef cattle. Front Genet 2014;5:1–18.

[11]. Glenske K, Prinzenberg EM, Brandt H, Gauly M, Erhardt G. A chromosome-wide QTL study on BTA29 affecting temperament traits in German Angus beef cattle and mapping of DRD4. Animal 2011;5:195–197. doi:10.1017/S1751731110001801

[12]. Garza-Brenner E, Sifuentes-Rincón AM, Randel RD, Paredes-Sánchez FA, Parra-Bracamonte GM, Arellano Vera W, Rodríguez Almeida FA, Segura Cabrera A. Association of SNPs in dopamine- and serotonin-pathway genes and their interacting genes with temperament traits in Charolais cattle. J App Genet 2017;58(3):363-371.

[13]. Hammond AC, Olson TA, Chase Jr CC, Bowers EJ, Randel RD, Murphy CN, Vogt DW and Tweolde A. Heat tolerance in two tropically adapted Bos Taurus breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. J Anim Sci 1996;74, 295–303.

[14]. Curley KO Jr, Neuendorff DA, Lewis AW, Cleere JJ, Welsh Jr TH and Randel RD. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. Horm Behav 2008;53:20-27.

[15]. Burdick NC, Randel RD, Carrol JA, Welsh TH Jr. Review article: Interactions between Temperament, Stress, and Immune Function in cattle. Int J Zool 2011;1-9.

[16]. Rousset F. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 2008;8: 103–106.

[17]. Schmidt SE, Neuendorff DA, Riley DG, Vann RC, Willard ST, Welsh THJr and Randel RD. Genetic Parameters of three methods of temperament evaluation of Brahman calves. J Anim Sci 2014;92:3082–3087.

[18]. Mormède P. Molecular genetics of behaviour: Research strategies and perspectives for animal production. Livest Prod Sci 2005;93:15–21.

[19]. Lühken G, Glenske K, Brandt H, Erhardt G. Genetic variation in monoamine oxidase A and analysis of association with behaviour traits in beef cattle. J Anim Breed Genet 2010;127:411–418. doi:10.1111 /j.1439-0388.2010.00855.x

[20]. Lindholm-Perry AK, Kuehn LA, Freetly HC, Snelling WM. Genetic markers that influence feed efficiency phenotypes also affect cattle temperament as measured by flight speed. Anim Genet. 2014;46:60–64.

[21]. Gianola D, de los Campos G, Toro MA, Naya H, Schön CC, Sorensen D. Do molecular markers inform about pleiotropy? Genetics 2015;201, 23–29.

[22]. Kim-Cohen J, Caspi A, Taylor A et al. MAOA, maltreatment, and gene–environment interaction predicting children's mental health: new evidence and a meta-analysis. Mol Psychiatry 2006;11:903–913.

[23]. McDermott R, Tingley D, Cowden J, Frazzetto G, Johnson DDP. Monoamine oxidase A gene (MAOA) predicts behavioral aggression following provocation. Proceedings of the National Academy of Sciences, 2009;106(7):2118-2123. doi: 10.1073/pnas.0808376106

[24]. Nabi R, Serajee FJ, Chugani DC, Zhong H, Huq AH. Association of tryptophan 2,3 dioxygenase gene polymorphism with autism. Am J Med Genet B Neuropsychiatr Genet 2004;15,125B(1):63-8.

[25]. Too LK, Li KM, Suarna C, Maghzal GJ, Stocker R, McGregor IS and Hunt NH. Deletion of TDO2, IDO-1 and IDO-2 differentially affects mouse behavior and cognitive function. Behav Brain Res 2016;312:102−17.

[26]. Sorgdrager FJH, Doornbos B, Penninx BWJH, de Jonge P, Kema IP. The association between the hypothalamic pituitary adrenal axis and tryptophan metabolism in persons with recurrent major depressive disorder and healthy controls. J Affect Disord 2017;222:32-39. doi: 10.1016/j.jad.2017.06.052.

[27]. Zhang CL, Wang YH, Chen H, Lei CZ, Fang XT, Wang JQ, Ma GB, Niu H, Xiao J. The polymorphism of bovine POMC gene and its association with the growth traits of Nanyang cattle. Yi Chuan 2009;31:1221–1225. doi:10.3724/SP. J.1005.2009.01221.

CONCLUSION GENERAL Y PERSPECTIVAS

En el Capítulo 1, los genes POMC, NPY, SLC18A2, SLC6A4 y FOSFBJ fueron identificados como genes candidatos a asociarse al rasgo de temperamento bovino, mediante una búsqueda guiada por red funcional de interacción. Los marcadores rs109576799 y rs43696138, localizados en los genes *DRD3* y *HTR2A*, respectivamente, resultaron significativamente asociados con variables de temperamento (velocidad de salida y score de temperamento).

Con base a la disponibilidad en bases de datos de variaciones de un solo nucleótido (SNV´s), los cinco genes priorizados y 21 genes de referencia adicionales que codifican diferentes enzimas reguladoras, transportadores y receptores de las vías de señalización de la serotonina y dopamina pueden ser considerados como genes candidatos para explorar las variaciones genéticas asociadas con el temperamento.

Para el Capítulo 2, los marcadores en los genes DRD3 y HTR2A, previamente asociados al temperamento fueron considerados para ser probados en tres razas de toretes sometidos a pruebas de comportamiento, pero no habían sido asociados con características de eficiencia alimenticia, sólo en el marcador rs43696138, localizado en el gen HTR2A, resulto una asociación significativa entre la raza Brangus para la variable de media de ganancia diaria de peso, no fue posible confirmar la asociación de estos dos marcadores con las variables de temperamento, sin embargo se mostró un efecto en un rasgo de comportamiento alimenticio, se requiere de más estudios para poder determinar el efecto de estos y otros marcadores con el potencial de estar asociados con eficiencia alimenticia y temperamento bovino.

El Capítulo 3, considero la población de estudio de las vacas Charolais evaluadas en el Capítulo 1, donde se buscó correlacionar el crecimiento con las variables de temperamento (VS, PS, ST), donde solo se presentaron correlaciones significativas en el grupo de vacas jóvenes. Cuatro de los marcadores previamente asociados con temperamento bovino, resultaron ser asociados también con rasgos de crecimiento. Otros estudios dirigidos a validar nuestros resultados podrían determinar si los cuatro marcadores estudiados presentan un efecto pleiotrópico sobre temperamento y rasgos de peso vivo, así como con características de eficiencia.