Genomic insights for feeding behavior traits in beef cattle

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Summary

Understanding of animal behavior is an important step to promote sustainable beef production, improving feed efficiency and animal welfare. The objective of this study was to identify candidate genes associated with feeding behavior traits including feeding event frequency (FREQ) and daily feeding duration (DUR) in crossbred beef cattle. The phenotypes were collected using the GrowSafe system during performance tests carried out between 2003 and 2013. The average of FREQ and DUR were calculated for 3,509 and 3,511 animals, respectively. After quality control, 3,529 genotyped animals and 37,298 SNPs remained. The genome-wide association analyses were performed using the weighted single-step GBLUP method. The results were reported as proportion of additive genetic variance explained by each window of 20 consecutive SNPs. The top 10 SNP-windows with the major effects for each trait were located on chromosomes 1, 2, 6, 7, 9, 12, 13, 14 and 16 for FREQ and 1, 3, 4, 5, 8, 13, 20, 23 and 28 for DUR. These regions explained 12.96% and 10.29% of the additive genetic variance and contain 17 and 163 positional candidate genes for FREQ and DUR, respectively. Potential genomic regions and candidate genes were identified and may improve the biological understanding of feeding behavior traits in beef cattle.

Keywords: feeding behavior, genome-wide association study

Introduction

Feed efficiency and welfare traits are both of considerable importance for the development of sustainable beef cattle breeding programs (Broom, 2010). The individual variability in feed efficiency is influenced by several extrinsic and intrinsic factors including diet content, physiological process (e.g. appetite, digestion, body composition, metabolism, activity and thermoregulation) and feeding behavior (Reyer *et al*., 2017).

Regarding individual feeding behavior, which is largely consistent and repeatable over time, studies reported that low feed efficient animals have a higher frequency of bunk visits, longer feeding duration and head down feeding time (Chen *et al*. 2014). Some attempts to estimate genetic parameters for feeding behavior traits, as well as, genetic and phenotypic relationships with performance, feed efficiency, feed intake and carcass merit traits have been reported and ranged from low to moderate in magnitude (Nkrumah *et al*., 2007; Chen *et al*., 2014). However, there is a lack of scientific literature pertaining to genome-wide association studies (GWAS) for feeding behavior traits in beef and dairy cattle. Results from such studies can increase our knowledge of genetic and biological mechanisms underlying feeding behavior traits and their association with feed efficiency, performance and carcass traits. The first initiatives were conducted for other species, such as pigs (Ding *et al*., 2017) and chicken

(Mignon-Grasteau *et al*., 2017). Therefore, the objective of this study was to identify genomic regions and candidate genes associated with feeding behavior traits in crossbred beef cattle.

Material and methods

The feeding behavior traits analyzed in the present study were collected using an automated GrowSafe feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada), during performance tests carried out between 2003 and 2013 on crossbred beef cattle. Average test duration was 80 ± 6 days, immediately after a 21- to 28-d adaptation to the feedlot facilities and diet. Phenotype collection was described in detail by Basarab *et al*. (2003) and behavioral traits were feeding event frequency (FREQ) and daily feeding duration (DUR) available for 3,509 and 3,511 animals, respectively. FREQ was defined as the sum of the total number of feed events divided by the number of performance test days, in which each event was considered as a bunk visit with feed consume greater than 0 kg and intervals between the last 2 readings of the same transponder greater than 300s. The event also finished when the animal was detected at a different bunk or a new animal was detected at the bunk. DUR was defined as a sum of the total feed duration time, recorded as the difference between the feeding event end and start times, divided by the number of days throughout the performance test. The mean of FREQ, DUR and live weight at the beginning of the tests were 55.94 ± 29.92 events/d, 100.89 ± 37.53 min/d and 373 ± 74 kg, respectively.

Animals were genotyped using BovineSNP50 BeadChip array (Illumina, San Diego, California, USA). Quality control was performed to remove SNPs with MAF < 0.01, call rate < 0.90 and those not in HWE (p-value < 1^{-6}). Also, SNPs with heterozygosity deviating more than 15% from the expected value as well as non-autosomal SNPs were excluded. Samples with call rate less than 0.90 were also excluded. After quality control, a total of 37,298 SNPs genotypes on 3,529 animals were available for further analyses.

Genome-wide association analyses

The GWAS were performed using a weighted single-step GBLUP (WssGBLUP) methodology (Wang *et al*., 2012) with a Bayesian approach utilizing Gibbs sampling. The BLUPF90 programs (Misztal *et al*., 2016) were used to calculate SNP effects by applying the following single-trait animal model:

where: is the vector of observations; is the vector of fixed effects; is the vector of direct additive genetic effects, assuming , where is the combined pedigree and genomic relationship matrix and genetic additive variance; is the corresponding incidence matrix for the fixed effects; is the incidence matrix of the random additive direct genetic effect; and is the vector of the residual effect in which , where is the identity matrix and is the residual variance. The model included fixed effects of contemporary groups (CG) defined as gender, herd of origin, birth year, diet and management group. Animal weight at the beginning of test and breed composition were included as covariates with linear effect. Records with ±3 SD of the CG mean and CGs having less than 5 animals were excluded.

The iterative process described by Wang *et al*. (2014) was used to estimate SNP effects and SNP weights for GWAS, as follow: 1. = **,** where is a weight matrix for SNP; 2. Calculate **(**according to Van Raden, 2008); 3. Calculate GEBVs for all animals in the data set

using WssGBLUP; 4. Convert GEBVs to SNP effects: ; 5. Calculate weight for each marker: , where *i* is the *i*-th SNP; 6. Normalize SNP weight to remain the genetic variance constant; 7. Exit or loop to step 2. This process was repeated twice from step 2 to 7. The percentage of additive genetic variance explained by each window of 20 non-overlapping consecutive SNPs were used to identify genomic regions with major effect on each trait. The UMD3.1 *Bos taurus* genome assembly available at Ensembl Genome Browser (http://www.ensembl.org/index.html) was used by biomaRt R package (Durinck *et al*., 2009) to identify candidate genes located inside the top 10 SNP-windows. The DAVID software (Huang *et al*., 2009) was used to identify biological process and pathways linked with candidate genes.

Results and discussion

The percentage of additive genetic variance explained by non-overlapping windows of 20 consecutive SNPs is shown in Manhattan plots (Figure 1). The top 10 SNP-windows were located on *Bos taurus* chromosomes (BTA) 1, 2, 6, 7, 9, 12, 13, 14 and 16 for FREQ and on BTA 1, 3, 4, 5, 8, 13, 20, 23 and 28 for DUR (Table 1). These regions explained a total of 12.96% and 10.29% of the additive genetic variance, respectively and 17 and 163 annotated genes were identified inside the top windows for FREQ and DUR, respectively. In addition, previously reported QTLs, available on QTLdb database (Hu *et al*., 2013), for feed efficiency related traits, such as average daily gain, residual feed intake and feed conversion rate overlapped with the top SNP windows for FREQ and DUR.

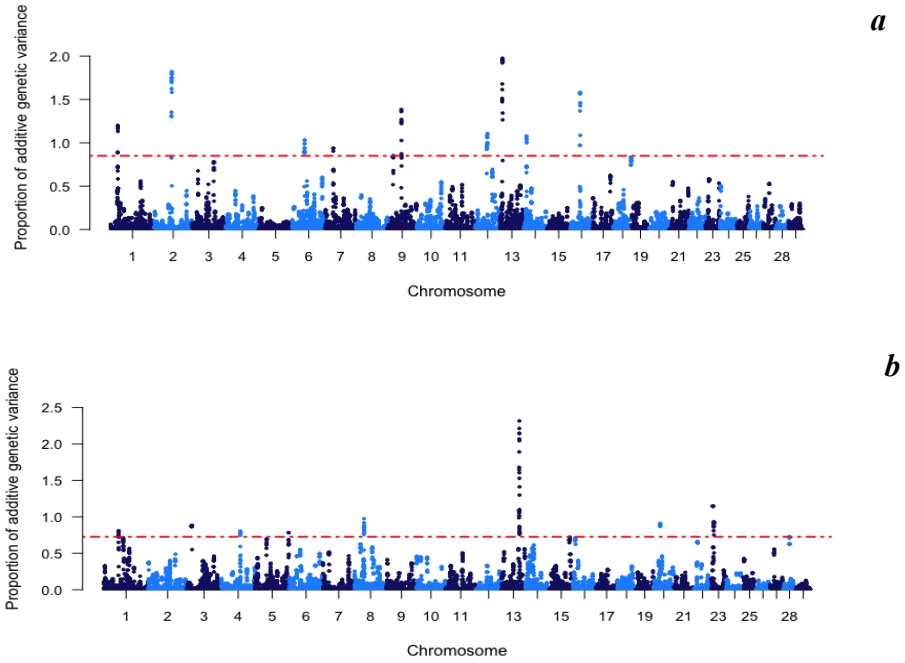

Figure 1. Manhattan plot of additive genetic variance explained by windows of 20 adjacent SNPs for a) feeding event frequency (events/day) and b) daily feeding duration (min/day).

Although BTA 1 and 13 were associated with both FREQ and DUR, the location of major windows was different for each trait and no pleiotropic regions were identified. The peroxysome proliferator-activated receptor-gamma coactivator-1alpha (*PPARGC1A*) was

identified as a potential candidate gene on BTA 6 (44 Mb) for FREQ. This gene plays a physiological role to modulate the feed intake (GO: 0002021) and was associated with intramuscular fat deposition, productive and reproductive traits in beef cattle (Ramayo-Caldas *et al*., 2014).

Table 1. Summary of top 10 SNP-windows with major effects on feeding behavior traits.

Feeding event frequency			Daily feeding duration		
BTA[1]	**Location (bp)**	**Var (%)[2]**	**BTA[1]**	**Location (bp)**	**Var (%)[2]**
1	25,685,179 - 27,465,349	1.20	1	52,748,123 - 53,656,600	0.80
2	63,516,470 - 65,069,037	1.82	3	18,520,392 - 19,860,064	0.88
6	44,622,597 - 45,412,181	1.03	4	68,528,799 - 69,831,612	0.80
7	29,579,813 - 31,136,178	0.94	5	119,261,609 - 120,378,417	0.78
9	20,247,290 - 21,490,009	0.85	8	33,747,904 - 34,714,805	0.97
9	49,865,815 - 50,804,011	1.38	13	63,257,337 - 65,006,713	2.32
12	45,002,070 - 45,952,853	1.11	20	22,823,334 - 24,228,836	0.91
13	8,347,568 - 9,556,357	1.97	23	5,896,623 - 7,276,902	1.15
14	11,154,590 - 11,983,913	1.08	23	9,453,816 - 10,688,252	0.93
16	35,723,500 - 36,565,106	1.58	28	22,760,601 - 23,742,925	0.73

[1]BTA = *Bos taurus* chromosomes
[2]Var = Proportion of additive genetic variance

For DUR, some genes located on BTA 3 (*RORC*, *TNFAIP8L2*), 13 (*BPIFB1*) and 23 (*DBS*, *PSMB8*, *PSMB9*, *BOLA-DMA*, *BOLA-DMB*, *BOLA-DOA*, *BOLA-DOB*, *BOLA-DYB*) were linked to immunity (UP_KEYWORDS). In addition, *RORC*, *BOLA-DMA*, *BOLA-DMB*, *BOLA-DOA*, *BOLA-DOB* and *BOLA-DYB* genes were related to inflammatory bowel disease (KEGG_PATHWAY), which is a chronic inflammation of the intestinal tract leading to lack of appetite with consequent weight loss and reduced feed efficiency (Shafran *et al*., 2015). Moreover, the *RORC* (retinoic acid receptor-related orphan receptor C) gene was also associated with intramuscular fat, marbling and carcass weight in different cattle breeds (Barendse *et al*., 2010). These results reinforce the associations between feeding behavior and feed efficiency related traits, corroborating the genetic correlations previously reported (Chen *et al*. 2014).

The present study identified major SNP windows and potential candidate genes, providing new insights for the biological understanding of feeding behavior in beef cattle. The results corroborate the possibility to use animal behavior as tool to detect individual health status (Tucker *et al*., 2015), performance and carcass merit. However, further functional genomic studies are important to better understand the relevant pleiotropic pathways underlying feeding behavior and feed efficiency traits.

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