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Genome-wide association study for carcass traits in a composite beef cattle breed☆

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ABSTRACT

Improvement of carcass traits is highly emphasized in beef cattle production in order to meet consumer demands. Discovering and understanding genes and genetic variants that control these traits is of paramount importance. In this study, different genome wide association approaches (single step GBLUP GWAS, Bayes A and Bayes B) were implemented and compared for three ultrasound carcass traits: fat thickness (FAT), intramuscular fat (IMF) and ribeye area (REA) of a composite beef cattle breed. The results showed different SNP marker windows associated with carcass traits explaining a small percentage of the genetic variance. The SNP marker window with the highest percentage of genetic variance (1.83%) associated with FAT was located on BTA14 in position 24 Mb. Surveying candidate genes in the regions associated with these traits revealed genes such as *LYPLA*, and *LYN* genes which have been associated with feed intake and growth in beef cattle. This study supported previous results from GWAS of carcass traits and revealed additional regions in the bovine genome associated with these economically important traits. Comparing the top 5 SNP windows for each trait across the GWAS methods revealed that only a few of these windows overlap.

1. Introduction

Beef cattle are marketed based on carcass characteristics. This has generated a great interest in improving carcass merit through genetic selection (Schroeder and Mark, 2000). Carcass phenotypes such as intra-muscular fat, fat thickness and ribeye area are all moderately to highly heritable allowing for accurate genetic merit prediction and overall higher genetic gain (Pariacote et al., 1998). The amount of intramuscular fat is a key factor in determining meat quality and palatability (Koch et al., 1993; Wheeler et al., 1994) and back fat is responsible in determining cutability (Herring et al., 1994).

Discovery of genes and quantitative trait loci (QTLs) is of great importance since they can be directly used in a marker assisted selection. One approach is genome wide association study (GWAS), a powerful tool to detect genetic variants affecting economically important traits (Goddard and Hayes, 2009). With the decreasing cost of genotyping, GWAS is becoming a routine. Numerous approaches of implementing GWAS in animal agriculture have been proposed consisting of linear and non-linear models. The single step GBLUP GWAS is a linear model which assumes a normal distribution of SNP effects and back solves SNP marker effects from genomic estimated breeding values (GEBVs) (Aguilar et al., 2010; Wang et al., 2012). Non-linear approaches such as Bayes A and Bayes B assume a heavy tail prior distribution for SNP effects and use Markov Chain Monte Carlo (MCMC) to sample from the posterior distribution (Meuwissen et al., 2001; Kizilkaya et al., 2010). In this study, linear and non- linear approaches were tested.

Several genome-wide association studies of carcass traits using single nucleotide polymorphism (SNP) arrays have been conducted. Lu et al. (2013) found several SNP markers associated with back fat thickness, Longissimus dorsi muscle area or ribeye area and marbling scores in a dataset consisting of different cattle breed. Karim et al. (2011) reported two QTLs associated with bovine stature on BTA 14 which mapped to the *PLAG1–CHCHD7* gene. Moreover, Barendse et al. (2007) have reported several QTLs associated with feed efficiency traits. Recently, Silva et al. (2016) conducted a GWAS of

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Table 1
Summary statistics of the dataset for fat thickness (FAT), ribeye area (REA), and intra muscular fat (IMF).

Trait	*n*	Mean	SD
FAT	2892	0.34	0.13
REA	2891	10.07	1.97
IMF	2893	3.23	0.66

carcass traits in Nellore cattle population using a high density SNP chip and found several SNP windows explaining a small percentage of the additive genetic variance. The majority of these QTLs identified in these studies have not been validated. This issue is associated with the complexity of the traits of interest. Complex polygenic traits are often under the control of genetic and environmental factors and their interaction. Thus, detecting genetic variants associated with these traits is challenging especially when these variants have moderate to small effects (Todd et al., 2007; Hindorff et al., 2009). Classical genome wide association studies (GWAS) suffer from the high dimensionality of the parameter space leading to high false discovery rate (Balding, 2006; Pe'er et al., 2008). Also, high linkage disequilibrium between a given quantitative trait locus (QTL) and several markers, sometimes within the same gene, leads to small effects to each one of these markers and ultimately lack of statistical power to declare any of them as being significant. This lack of statistical power has made the replication of GWAS results difficult. In fact, there is a substantial literature in the field of animal agriculture and human medicine on the inability to replicate large portion of GWAS results (Visscher et al., 2012). Although an increase in sample size will improve the statistical power and help alleviate the problem, this alternative is costly, time consuming and often not possible due to several reasons including the unavailability of biological samples.

Few genome wide association studies have been conducted in composite beef cattle populations. The objectives of this study are to conduct and evaluate linear (single step GBLUP GWAS) and non-linear approaches (Bayes A and Bayes B) of genome-wide association studies using carcass traits of a composite beef cattle breed and also detect additional variants associated with these traits.

2. Materials and methods

2.1. Data

Data for this study consisted of 3020 animals from a composite beef cattle breed (50% Red Angus, 25% Charolais, 25% Tarentaise) born between 2002 and 2011 at USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT. Cows were randomly assigned to be fed two levels of harvested supplemental feed from December to March of each year. First level is adequate winter supplemental feed as recommended by the industry (ADEQ) and second is marginal supplemental feed (MARG) which is approximately 61% of the supplemental feed provided to ADEQ. At weaning, offspring from these cows were randomly assigned to either ad libitum (CONTROL) or restricted (RESTRICTED; 80% of control at a common body weight basis) access to feeding for 140-d development post weaning. Average daily growth resulting from the CONTROL and RESTRICTED levels of feeding were 0.67 and 0.51 kg/d for females and 0.94 and 0.62 kg for males. Additional information is available in Newman et al. (1993a, 1993b) and Roberts et al. (2016). The pedigree file consisted of 5374 animals including 128 sires and 1723 dams. Ultrasound carcass phenotypes considered in this study are fat thickness (FAT), intramuscular fat (IMF) and ribeye area (REA). These phenotypes were collected as described previously (Roberts et al., 2007). Summary statistics of the phenotypes used is presented in Table 1.

Animals were genotyped using a mixture of low density SNP 3k panel and high density Illumina Bovine50k (Illumina, San Diego, CA). Animals genotyped with low density (LD) panel were imputed to the 50 K SNP panel using FImpute software (Sargolzaei et al., 2011) where population and pedigree information were used simultaneously. The average allelic R^2 was 0.94 which indicates high imputation accuracy of the missing genotypes.

Quality control was performed which consisted of excluding SNP markers with minor allele frequency less than 0.05 and SNPs with Call Rate (CR_{SNP}) < 0.90 and Fisher's exact test *P*-value for Hardy–Weinberg Equilibrium (HWE) < 1×10^{-5}. After quality control, the number of SNP genotypes consisted of 41,694 SNP markers.

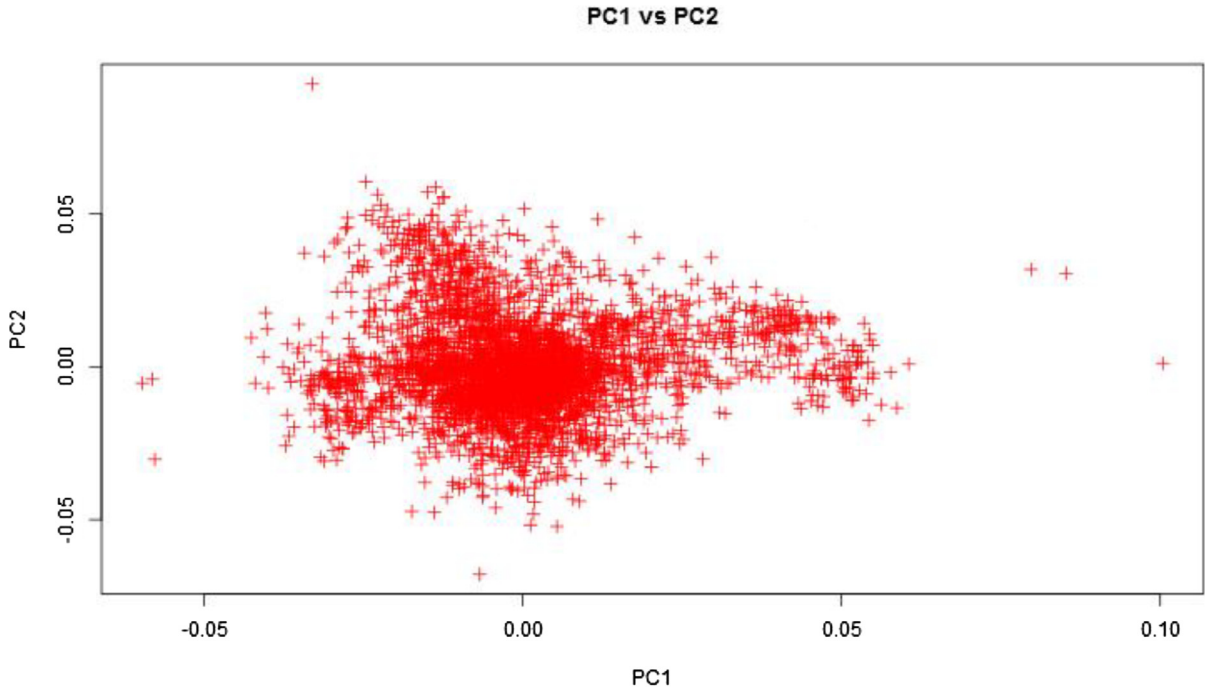

Fig. 1. Distribution of animals using the first two principal components of the genomic relationship matrix.

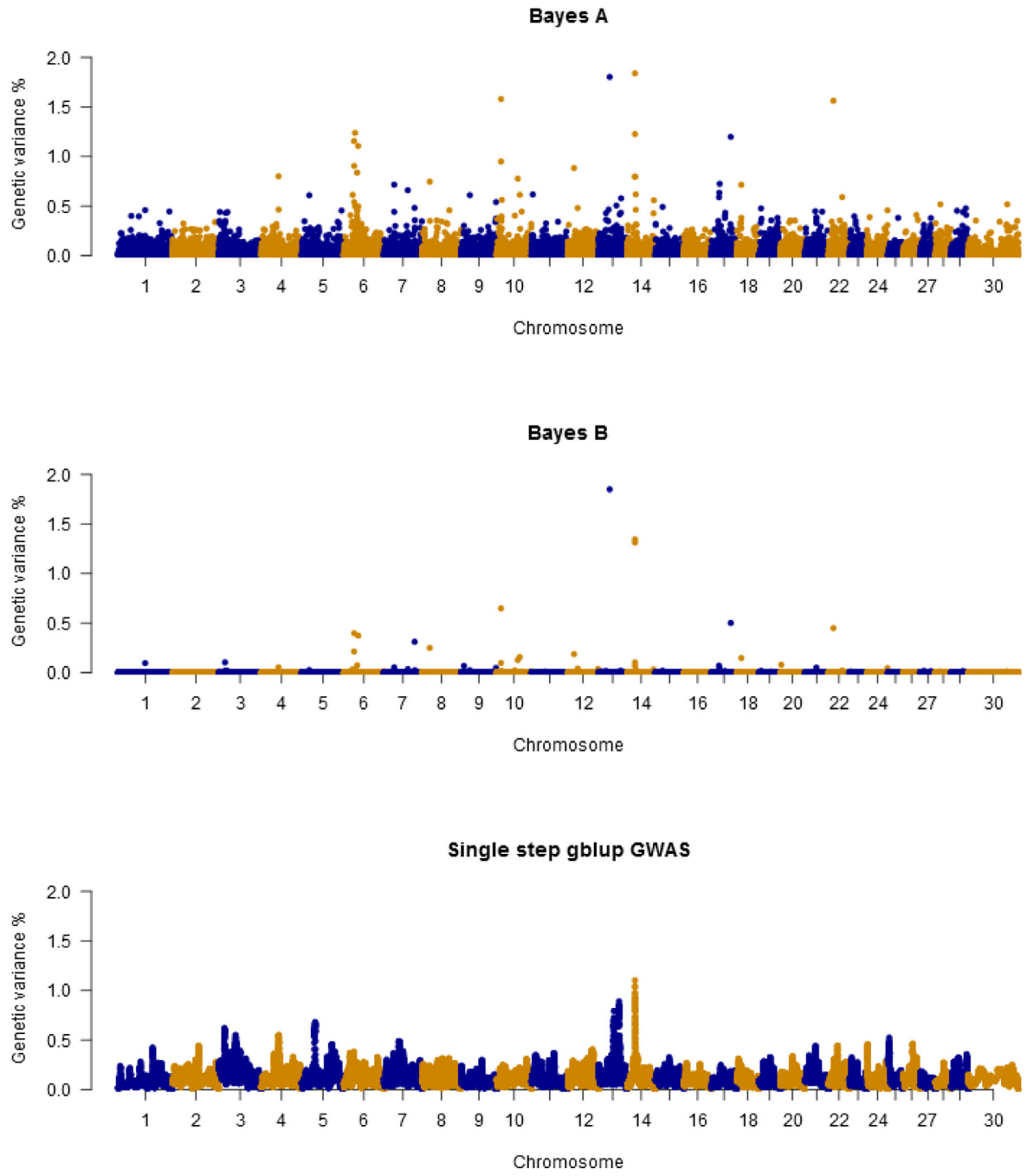

Fig. 2. Manhattan plots of the percentage of additive genetic variance explained by windows of 20 adjacent SNPs for fat thickness (FAT) using three different methods.

Table 2
Variance components estimate of fat thickness (FAT), ribeye area (REA) and intramuscular fat (IMF)[a].

Trait	σ_a^2	σ_e^2	h^2
FAT	0.00073 (0.0001)	0.0014 (0.0007)	0.34 (0.06)
REA	0.51 (0.13)	1.08 (0.19)	0.32 (0.08)
IMF	0.12 (0.03)	0.14 (0.06)	0.46 (0.04)

σ_a^2: additive genetic variance; σ_e^2 = residual variance; h^2: heritability.
[a] Numbers in parenthesis are standard errors of estimates of variance components.

2.2. Variance component estimation

A linear model was adopted to estimate variance components for the three carcass traits using REML. A single trait analysis was carried out using BLUPF90 software package (Misztal et al., 2002). The model was as the following:

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Zu} + \boldsymbol{e},$$

where y is the vector of phenotypes, X is an incidence matrix relating phenotypes to fixed effects which included age of the animals at the moment of ultrasound, sex effect, treatment effect (CONTROL, RESTRICTED), dam treatment effect (ADEQ and MARG) as described before and also contemporary group effect (year and age-of-dam subclasses), b is the vector of fixed effects solutions, Z is an incidence matrix that

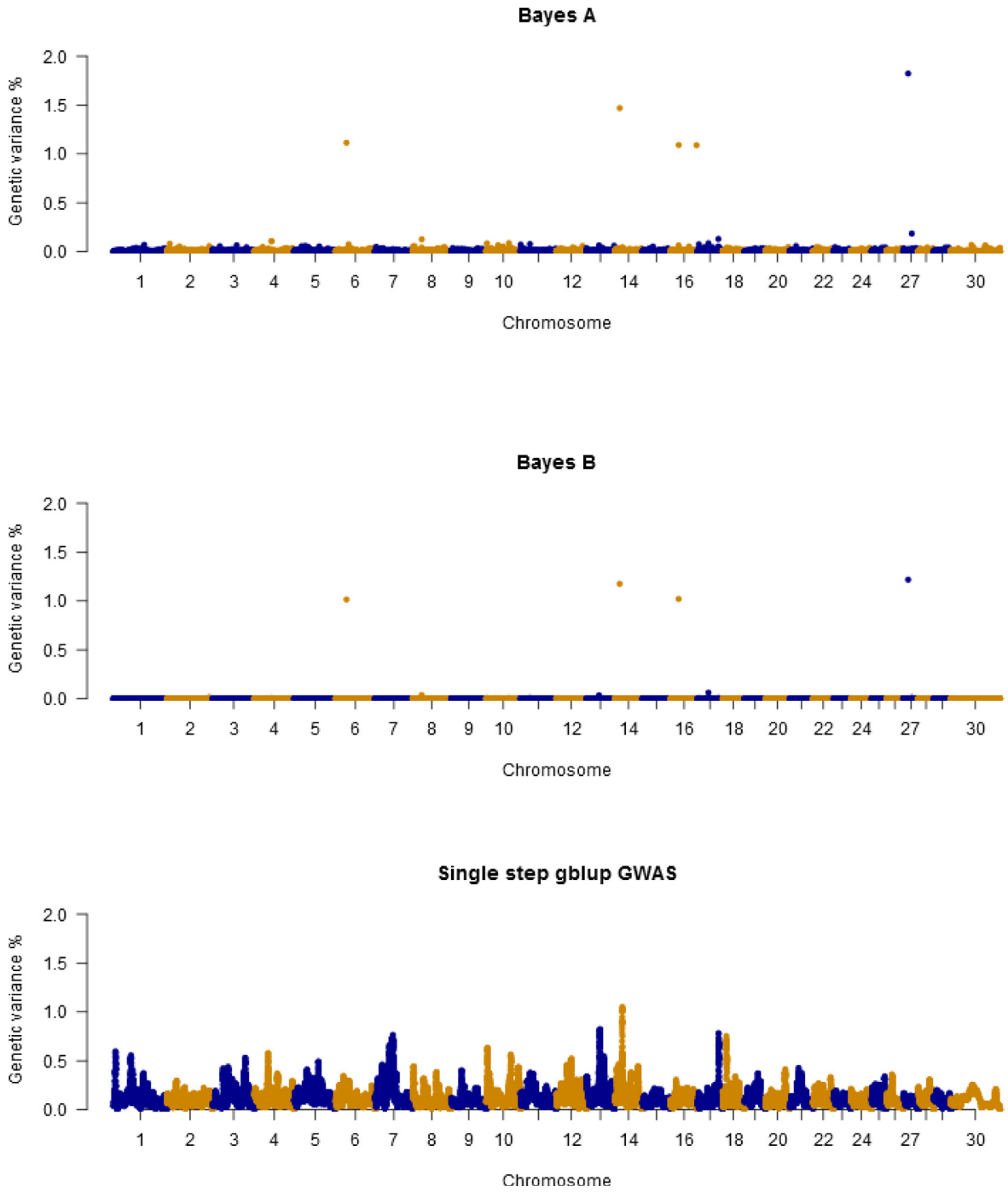

Fig. 3. Manhattan plots of the percentage of additive genetic variance explained by windows of 20 adjacent SNPs for intra muscular fat (IMF) using three different methods.

relates animals to phenotypes, u is the vector of breeding values and e is the vector of random residuals.

A principal component analysis on the genomic relationship matrix (G) was conducted in order to investigate the population structure. The analysis revealed no population substructure (Fig. 1), therefore principal components were not included in the model.

2.3. Bayesian GWAS

The first GWAS approach implemented is the Bayesian GWAS. Phenotypes were corrected for fixed effects using single trait model. The fixed effects consisted of age of the animals at the moment of ultrasound, sex effect, treatment effect (CONTROL, RESTRICTED), dam treatment effect (ADEQ and MARG) as described before and also contemporary group effect (year and age-of-dam subclasses). This method fits all SNP markers simultaneously utilizing prior information and information from the data. Bayes A and Bayes B models (Meuwissen et al., 2001) were used. The Bayes A model is the following:

$$\mathbf{y} = \mu + \sum_{j=1}^{n} z_j \alpha_j + \mathbf{e},$$

where $\mathbf{y}$ is the vector of corrected phenotypes, μ is the overall mean, n is the number of SNPs, z_j is the genotype covariate of the jth SNP coded according to the additive model (0, 1 and 2) α_j is the allelic substitution

Table 3
Results of genome wide association of fat thickness (FAT) in a composite beef cattle breed using different methods.

Chr[a]	Position[b]	Genes[c]	% (σ_a^2)[d]
Single step GBLUP **GWAS**			
BTA14	24,524,205	*LYN, LYPLA1, MRPL15, PENK, RGS20, RP1, RPS20*	1.10
BTA14	24,437,778	*ATP6V1H, CHCHD,SDR16C5, SDR16C6, SOX17, TGS1, TMEM68*	1.04
BTA14	24,573,257	*MRPL15, PENK, RGS20, RP1, RPS20, SDR16C5, SDR16C6, SOX17*	1.03
BTA14	24,407,125	*CHCHD7, IMPAD1, LYN, LYPLA, TCEA1, TGS1, TMEM68*	0.97
BTA14	24,607,527	*CHCHD7, FAM110B, IMPAD1, UBXN2B*	0.96
Bayes A			
BTA14	24,524,205	*LYN, LYPLA1, MRPL15, PENK, RGS20, RP1, RPS20*	1.83
BTA13	33,626,917	*ARHGAP12, CACNB2, EPC1, KIF5B, NSUN6, SLC39A12, ZEB1, ZNF438*	1.80
BTA10	13,436,362	*AAGAB, DENND4A, DIS3L, LCTL, MAP2K1, MAP2K5, MIR2290, RAB11A, RPL4, SLC24A1, SMAD3, SMAD6, SNAPC5, TIPIN, VWA9, ZWILCH*	1.57
BTA22	14,133,039	*ABHD5, CTNNB1, EIF1B, ENTPD3, MIR138-1, RPL14, TRAK1, ZNF621*	1.56
BTA6	33,353,299	*NOT_FOUND*	1.23
Bayes B			
BTA13	33,626,917	*ARHGAP12, CACNB2, EPC1, KIF5B, NSUN6, SLC39A12, ZEB1, ZNF438*	1.84
BTA14	24,573,257	*CHCHD7, FAM110B, IMPAD1, UBXN2B*	1.34
BTA14	24,524,205	*LYN, LYPLA1, MRPL15, PENK, RGS20, RP1, RPS20*	1.31
BTA10	13,436,362	*AAGAB, DENND4A, DIS3L, LCTL, MAP2K1, MAP2K5, MIR2290, RAB11A, RPL4, SLC24A1, SMAD3, SMAD6, SNAPC5, TIPIN, VWA9, ZWILCH*	0.64
BTA17	57,732,465	*ACAD10, BRAP, CCDC63, CUX2, HSPB8, HVCN1, MYL2, PPP1CC, PRKAB1*	0.50

[a] Bovine chromosome.
[b] Position in base pair based on UMD3.1 bovine genome assembly.
[c] Genes identified according to genome assembly UMD_3.1.
[d] Percentage of genetic variance explained by 20 SNP windows.

Table 4
Results of genome wide association of rib eye area (REA) in a composite beef cattle breed using different methods.

Chr[a]	Position[b]	Genes[c]	% (σ_a^2)[d]
Single step GBLUP **GWAS**			
BTA6	64,853,128	*GNPDA2, GUF1, KCTD8, YIPF7*	0.74
BTA16	47,443,681	*ACOT7, DNAJC11, HES2, KCNAB2, KLHL21*	0.72
BTA6	65,502,276	*LAP3, GABRA2, GABRG1, GNPDA2*	0.72
BTA16	47,558,131	*NOL9, PHF13, RNF207, THAP3, TNFRSF25, ZBTB48*	0.71
BTA6	65,749,899	*GUF1, KCTD8, YIPF7*	0.71
Bayes A			
BTA18	2,220,037	*AARS, ADAT1, BCAR1, BCNT2, CFDP1, CFDP2, CHST6, COG4, CTRB1, DDX19A, DDX19B, EXOSC6, FA2H, FUK, GABARAPL2, GLG1, IL34, KARS, LDHD, LOC618826, MIR2324, MRCL, PDPR, SF3B3, ST3GAL2*	1.72
BTA16	63,803,806	*ACBD6, CACNA1E, LHX4, MR1, QSOX1, STX6, XPR1*	1.65
BTA6	99,128,925	*AGPAT9, COPS4, COQ2, ENOPH1, FAM175A, HNRNPD, HNRPDL, HPSE, LIN54, MIR2446, MIR2447, MRPS18C, PLAC8, SCD5, SEC31A, THAP9, TMEM150C*	1.47
BTA20	2,951,322	*FGF18, GABRP, KCNIP1, KCNMB1, LCP2, NPM1, STK10, TLX3, UBTD2*	1.36
BTA17	21,889,966	*NOT_FOUND*	1.18
Bayes B			
BTA12	40,073,676	*NOT_FOUND*	1.46
BTA20	2,951,322	*FGF18, GABRP, KCNIP1, KCNMB1, LCP2, NPM1, STK10, TLX3, UBTD2*	1.16
BTA6	99,150,246	*AGPAT9, COPS4, COQ2, ENOPH1, FAM175A, HNRNPD, HNRPDL, HPSE, LIN54, MIR2446, MIR2447*	1.07
BTA6	99,128,925	*MRPS18C, PLAC8, SCD5, SEC31A, THAP9, TMEM150C*	1.07
BTA17	21,889,966	*NOT_FOUND*	0.71

[a] Bovine chromosome.
[b] Position in base pair based on UMD3.1 bovine genome assembly.
[c] Genes identified according to genome assembly UMD_3.1.
[d] Percentage of genetic variance explained by 20 SNP windows.

effect of SNP$_j$, and **e** is the vector of random residuals. The Bayes B is similar to the Bayes A model described above except for the SNP effects part which changes to $\sum_{j=1}^{n} z_j I_j \alpha_j$ where z_j is the genotype of the jth marker, coded as the number of copies of the minor allele, α_j is the effect of marker j, and I_j is an indicator variable that is equal to 1 if the jth marker has a non-zero effect on the trait and 0 otherwise. A binominal distribution with known probability $\pi = 0.01$ was assumed for I_j.

Estimated variance components were used as prior information and a total of 50,000 MCMC iterations with 10,000 discarded as burn in were implemented. Fixed windows consisting of 20 consecutive SNP markers were used to calculate genetic variance explained by SNPs. However, a sliding SNP window could also be used. The genetic variance of SNP windows was calculated as the sum of each SNP variance where the variance was calculated as $2p_i(1-p_i)u_i^2$, where p_i is the minor allele frequency and u_i is the ith estimated SNP marker effect. The analysis was conducted using GenSel software package (Fernando and Garrick, 2009).

Convergence testing was performed for all parameters following Geweke's (1991) and Heidelberger and Welch's (1983), and visual analysis of trace plots was also performed using Bayesian Output Analysis program in R software 3.1 (R Core Team, 2014).

2.4. Single-step GWAS

The second genome wide association approach was carried out through a single step (single step GBLUP GWAS) (Wang et al., 2012). A single trait model was implemented for the three traits studied similar to the animal model used to estimate the variance components. The analysis was conducted using BLUPF90 software package (Misztal et al.,

Table 5
Results of genome wide association of intra muscular fat (IMF) in a composite beef cattle breed using different methods.

Chr[a]	Position[b]	Genes[c]	% (σ_a^2)[d]
Single step GBLUP **GWAS**			
BTA14	24,326,513	*ATP6V1H, CHCHD7, LYN, MRPL15, OPRK1, PENK, RGS20, RP1*	1.05
BTA14	24,524,205	*RPS20, SDR16C5, SDR16C6, SOX17, TCEA1, TGS1, TMEM68*	1.03
BTA14	24,437,778	*LYN, LYPLA1, MRPL15, NPBWR1*	1.02
BTA14	24,153,510	*OPRK1, RB1CC1, RGS20, RP1, RPS20, SDR16C5*	1.02
BTA14	24,182,406	*LYPLA1, MRPL15, NPBWR1, OPRK1, RB1CC1, RGS20, RP1, SOX1*	1.01
Bayes A			
BTA27	14,591,550	*ACSL1, ANKRD37, C27H4orf47, CASP3, CYP4V2, F11, FAM149A, FAT1, IRF2*	1.82
BTA14	16,387,114	*FAM84B, MTSS1, NDUFB9, NSMCE2, SQLE, TATDN1, TMEM65, TRIB1*	1.46
BTA6	32,548,500	*ATOH1, PDLIM5, SMARCAD1*	1.11
BTA16	24,323,587	*BPNT1,C16H1orf115,EPRS,HLX,IARS2,LYPLAL1,MARK1,MIR194-1,MIR215,MIR664B,MOSC2,RAB3GAP2,SLC30A10*	1.09
BTA16	78,157,760	*ASPM, CD34, CD46, CRB1, F13B, LHX9, MIR2284N*	1.08
Bayes B			
BTA27	14,591,550	*ACSL1, ANKRD37, C27H4orf47, CASP3, CYP4V2, F11, FAM149A, FAT1, IRF2, KIAA1430, KLKB1, PDLIM3, PRIMPOL, SLC25A4, SNX25, SORBS2, TLR3*	1.21
BTA14	16,387,114	*FAM84B, MTSS1, NDUFB9, NSMCE2, SQLE, TATDN1, TMEM65, TRIB1*	1.17
BTA16	24,323,587	*BPNT1, C16H1orf115, EPRS, HLX, IARS2, LYPLAL1, MARK1, MIR194-1, MIR215, MIR664B, MOSC2, RAB3GAP2, SLC30A10*	1.02
BTA6	32,548,500	*ATOH1, PDLIM5, SMARCAD1*	1.01
BTA17	32,818,172	*ANKRD50*	0.06

[a] Bovine chromosome.
[b] Position in base pair based on UMD3.1 bovine genome assembly.
[c] Genes identified according to genome assembly UMD_3.1.
[d] Percentage of genetic variance explained by 20 SNP windows.

2002).

After the estimation of SNP marker effects, the percentage of the genetic variance accounted by 20 SNP markers fixed windows was also estimated in order to detect relevant chromosome regions related to carcass traits. The following equation was used to estimate this percentage per SNP:

$$v_i = 100 \times \left(2p_i q_i \alpha_i^2 / \sum_{i=1}^{nsnp} 2p_i q_i \alpha_i^2 \right),$$

where p_i and q_i are the allele frequencies for the ith SNP calculated based on the dataset, α_i^2 is the SNP marker estimated from the genomic breeding values.

3. Results and discussion

Estimates of variance components and heritabilities of the three ultrasound carcass traits are presented in Table 2. The resulting heritability of FAT was 0.34. Several studies have reported genetic parameter estimates for ultrasound fat thickness in beef cattle (Minick et al., 2002; Hassen et al., 2004). For REA, the heritability was 0.32, which is on the lower end of the spectrum of reported heritabilities (Perkins et al., 1992; Schalles et al., 1993). The heritability of IMF was 0.46 which falls within the reported estimates in the literature (Crews et al., 2003).

The results of the different GWAS methods are shown in Figs. 1–3. The top five windows of 20 SNPs explaining the highest percentage of the genetic variance for each trait using different methods are presented in Tables 3–5. In addition, the genes falling in these top regions of the genome are also reported in Tables 3–5. For FAT, the window with the highest percentage of genetic variance (1.10%) using single step GBLUP GWAS was located on chromosome 14 in position 24 Mb. This same window explained approximately similar percentage of genetic variance using Bayes A and Bayes B (Table 3, Fig. 1).The window located on BTA14 in position 24 Mb also accounted for 1.05% of genetic variance for IMF using single step GBLUP GWAS (Fig. 3). On the other hand, the SNP window explaining the highest percentage of genetic variance for REA was located on BTA6 in position 64 Mb (Fig. 4). Comparing the top 5 SNP windows for each trait across the GWAS methods, we found that only a small number of these windows overlap. This small overlap between the methods could be due to the prior assumptions about the effects of the SNP markers. Single step GBLUP GWAS approach assumes a normal distribution for SNP effects, and calculates their effect from the phenotypes and the genomic relationship matrix (Aguilar et al., 2010; Wang et al., 2012). Further, it assumes equal weights for all SNP markers (Meuwissen et al., 2001; VanRaden, 2008) which is biologically not accurate (Meuwissen et al., 2001). On the other hand, methods Bayes A and Bayes B are nonlinear and assume heterogeneous variances of SNP effects (Meuwissen et al., 2001; Kizilkaya et al., 2010). Furthermore, this inconsistency across the GWAS methods could be due to the genetic architecture of the traits considered. A simulation study by Daetwyler et al. (2010) showed that when the number of QTLs is large, GBLUP is the appropriate model.

The overlapping top SNP windows across the three different GWAS methods associated with FAT and IMF were located in BTA14 (Tables 3 and 5). This region of the genome contains several genes involved in different biological processes. The overlapping significant SNP window across the different GWAS methods for FAT and IMF was located on BTA14 in position 24 Mb. This region included several QTLs previously reported in the literature. Kneeland et al. (2004) mapped three QTLs associated with birth weight in a composite beef cattle breed. Further, Lee et al. (2013) conducted a genome wide association study of carcass traits in Hanwoo beef cattle and detected a significant QTL on BTA14 at 24.3–25.4 Mb associated with carcass weight. Recently, Silva et al. (2017) reported several significant regions on BTA14 associated with backfat thickness and other carcass traits in a *Bos Indicus* cattle breed.

Surveying the genes on BTA14 in the 24 Mb region, several genes were detected: *LYN, LYPLA1, MRPL15, PENK, RGS20, RP1, RPS20, CHCHD7, IMPAD1, FAM110B, IMPAD1, UBXN2B, SDR16C5*. Some of these genes have been reported to have an effect on carcass and weight traits in beef cattle. The *PLAG1–CHCHD7* have been reported to be associated with bovine stature, residual feed intake and fat deposition in both *Bos taurus* and *Bos indicus* breeds (Lee et al., 2013; de Oliveira Silva et al., 2017). Karim et al. (2011) reported the same region of the genome detected in this study on bovine chromosome 14 which mapped two QTLs to the *PLAG1–CHCHD7*. Moreover, Utsunomiya et al. (2017) showed a strong selection signature of *PLAG1* gene in BTA14 and its patterns of introgression into non-European breeds supporting the role of PLAG1 in the change of cattle stature. The *LYPLA*, and *LYN* genes have been associated with feed intake and

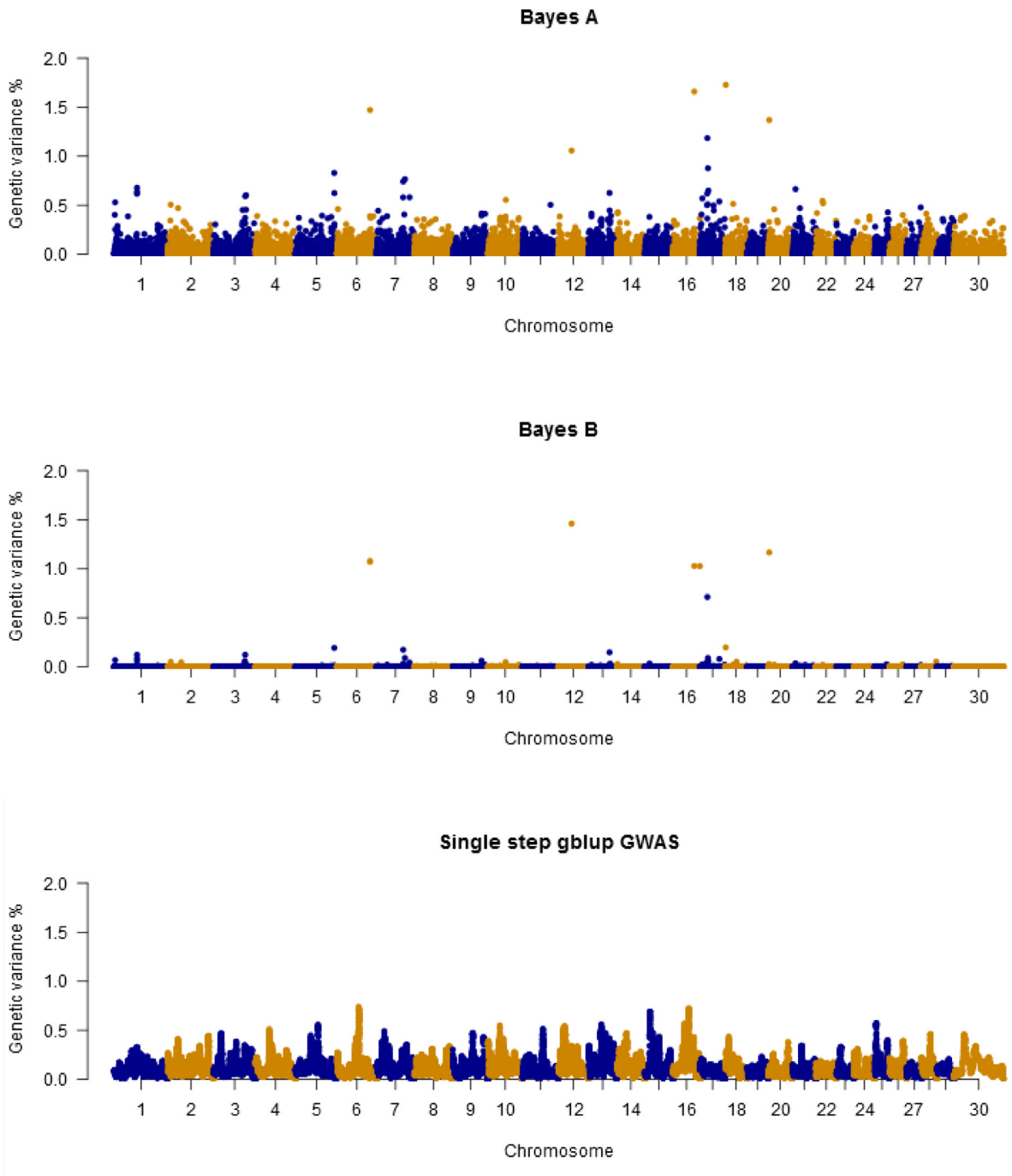

Fig. 4. Manhattan plots of the percentage of additive genetic variance explained by windows of 20 adjacent SNPs for rib-eye area (REA) using three different methods.

growth in beef cattle (Lindholm-Perry et al., 2012; Magalhães et al., 2016) and the *PENK* gene is involved in numerous physiologic functions. The BTA14 is a hot spot for several causative variants. This chromosome has been one of the most widely studied chromosomes for quantitative trait loci (QTL) related to many economically important traits in cattle (Marques et al., 2007). Most QTLs discovered in beef cattle fall into a region of 30 Mb, from 15 Mb to 45 Mb. For instance, QTLs discovered included two QTLs for post weaning average daily gain, four for pre-weaning average daily gain, three for birth weight (Kneeland et al., 2004), three for carcass weight (Mizoshita et al., 2004) and one for marbling score (Casas et al., 2003).

Other regions in chromosomes BTA13, BTA10, BTA22, BTA6, BTA0, and BTA17 have been found associated with FAT and IMF from the three GWAS approaches as shown in Tables 3 and 5.

The GWAS of REA using the three different methods resulted in several SNP windows located mainly in BTA6 and BTA16 (Table 4). These regions explained a relatively high percentage of genetic variance for REA. Some of the regions detected have been reported to be associated with growth and carcass traits (McClure et al., 2010; Saatchi et al., 2014). Genes located in these regions included *GNPDA2, GUF1, KCTD8, YIPF7, ACOT7, DNAJC11, HES2, KCNAB2, KLHL21, ACBD6, CACNA1E, LHX4, MR1, QSOX1, STX6, XPR1*. The gene *GNPDA2* catalyzes the reversible reaction converting D-glucosamine-6-phosphate into D-fructose-6-phosphate and ammonium. This gene in humans has been associated with body mass index, susceptibility to obesity and diabetes (Böttcher et al., 2012; Graff et al., 2013).

By examining the results of the three GWAS approaches for FAT, REA and IMF, it is noticeable that the genetic variance explained by the top 5 SNP windows is relatively small which supports the polygenic nature of these traits. Furthermore, the differences in the associated genomic regions across the three GWAS approaches (Tables 3–5) could be due to the difference in the assumptions in the statistical models used as discussed earlier. For FAT and IMF, the single step GBLUP GWAS approach identified several SNP windows in one region of BTA14. This could be due to SNPs being in strong linkage disequilibrium and therefore the signal being dispersed across the neighboring SNP markers. On the other hand, Bayes A and Bayes B identified the same region in chromosome 14 in addition to other regions on the genome and minimized the noise.

4. Conclusions

Improving carcass traits is an important objective in beef cattle breeding programs. This study revealed additional regions in the bovine genome associated with carcass traits and supported results from previous studies. Further, the results of this genome wide association study showed some differences among the different statistical methods adopted. This could be due to the several limitations genome wide association studies still suffer from as discussed and shown in the literature. Furthermore, the relatively small percentage of genetic variance explained by the top SNP windows supports the polygenic genetic nature of carcass traits in beef cattle.

5. Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We understand that the corresponding author is the sole contact for the editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from (elhamidi.hay@ars.usda.gov).

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