Genome-wide association mapping including phenotypes from relatives without genotypes in a single-step (ssGWAS) for 6-week body weight in broiler chickens

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The purpose of this study was to compare results obtained from various methodologies for genome-wide association studies, when applied to real data, in terms of number and commonality of regions identified and their genetic variance explained, computational speed, and possible pitfalls in interpretations of results. Methodologies include: two iteratively reweighted single-step genomic BLUP procedures (ssGWAS1 and ssGWAS2), a single-marker model (CGWAS), and BayesB. The ssGWAS methods utilize genomic breeding values (GEBVs) based on combined pedigree, genomic and phenotypic information, while CGWAS and BayesB only utilize phenotypes from genotyped animals or pseudo-phenotypes. In this study, ssGWAS was performed by converting GEBVs to SNP marker effects. Unequal variances for markers were incorporated for calculating weights into a new genomic relationship matrix. SNP weights were refined iteratively. The data was body weight at 6 weeks on 27,476 broiler chickens, of which 4,553 were genotyped using a 60k SNP chip. Comparison of genomic regions was based on genetic variances explained by local SNP regions (20 SNPs). After 3 iterations, the noise was greatly reduced for ssGWAS1 and results are similar to that of CGWAS, with 4 out of the top 10 regions in common. In contrast, for BayesB, the plot was dominated by a single region explaining 23.1% of the genetic variance. This same region was found by ssGWAS1 with the same rank, but the amount of genetic variation attributed to the region was only 3%. These findings emphasize the need for caution when comparing and interpreting results from various methods, and highlight that detected associations, and strength of association, strongly depends on methodologies and details of implementations. BayesB appears to overly shrink regions to zero, while overestimating the amount of genetic variation attributed to the remaining SNP effects. The real world is most likely a compromise between methods and remains to be determined.

Keywords: body weight, broiler chicken, genome-wide association, ssGWAS, BayesB, association mapping
the genomic relationships with traditional pedigree relationships (Aguilar et al., 2010). With this approach GWAS is accomplished by converting the estimated breeding values (GEBVs) obtained from ssGBLUP to marker effects and marker weights, which are then used in an iterative approach to update solutions. The theoretical advantage of this method is that it uses all phenotypic information for which either the pedigree or marker effects are known, and can be used for any model for which BLUP estimates of breeding values can be obtained (Wang et al., 2012). GWAS by ssGBLUP can be called ssGWAS. Dikmen et al. (2013) applied ssGWAS for identification of QTLs for body weight at 6 weeks old, among 5 generations (G1, G2, G3, G4, and G5). The latter method was used in this study. Briefly, SNP effects and weights for GWAS were derived as follows (Wang et al., 2012):

1. Let \( D = I \) in the first step.
2. Calculate \( G = ZDZ'q \).
3. Calculate GEBVs for entire data set using ssGBLUP.
4. Convert GEBVs to SNP effects (\( \hat{u} \)): \( \hat{u} = qDZ'(ZDZ'q)^{-1} \hat{a} \), where \( \hat{a} \) is the GEBVs of animals which were also genotyped.
5. Calculate weight for each SNP: \( w_i = \hat{a}_i^2 2 p_i (1-p_i) \), where \( i \) is the i-th SNP.
6. Normalize SNP weights to remain the total genetic variance constant.
7. Loop to 2. (ssGWAS1) or 4. (ssGWAS2).

SNP weights were calculated iteratively either looping through steps 4–6 (ssGWAS1) or through steps 2–6 (ssGWAS2). Iterations with both scenarios increase weights of SNP with large effects and decrease those with small effects, essentially regressing them to the mean. Experiences with simulated data using ssGBLUP (Wang et al., 2012) and of a similar method based on GBLUP (Sun et al., 2012; Zhang et al., 2012) indicated that ssGWAS1 was more suitable for identification of SNPs with the largest effects while ssGWAS2 was superior for more accurate GEBVs.

Percentage of genetic variance explained by i-th region has been calculated as below:

\[
\frac{\text{Var}(a_i)}{\sigma_a^2} \times 100\% = \frac{\text{Var}\left(\sum_{j=1}^{20} Z_{ij} \hat{u}_j\right)}{\sigma_a^2} \times 100\%
\]

Where \( a_i \) is genetic value of the i-th region that consists of contiguous 20 SNPs, \( \sigma_a^2\) is the total genetic variance, \( Z_{ij} \) is vector of genotypes of the j-th SNP for all individuals, and \( \hat{u}_j \) is marker effect of the j-th SNP within the i-th region.

**MODELS AND COMPUTATION**

**Single-step genomic association study**

The ssGWAS method is a modification of BLUP with the numerator relationship matrix \( A^{-1} \) matrix replaced by \( H^{-1} \) (Aguilar et al., 2010):

\[
H^{-1} = A^{-1} + \begin{bmatrix}
0 & 0 \\
0 & G^{-1} - A_{22}^{-1}
\end{bmatrix}
\]

where \( A_{22} \) is a numerator relationship matrix for genotyped animals and \( G \) is a genomic relationship matrix. The genomic matrix can be created following VanRaden (2008) as:

\[
G = ZDZ'q
\]

where \( Z \) is a matrix of gene content adjusted for allele frequencies, \( D \) is a weight matrix for SNP (initially \( D = I \)), and \( q \) is a weighting factor. The weighting factor can be derived either based on SNP frequencies (VanRaden, 2008), or by ensuring that the average diagonal in \( G \) is close to that of \( A_{22} \) (Vitezica et al., 2011).

Experiences with simulated data using ssGBLUP and a similar method based on GBLUP (Sun et al., 2012; Zhang et al., 2012) indicated that ssGWAS1 was more suitable for identification of SNPs with the largest effects while ssGWAS2 was superior for more accurate GEBVs.

For analyses, we applied an animal model with fixed effects of sex and contemporary group and random effects for additive animal and maternal permanent environment. Variance components were estimated by REML based on all the individuals in the pedigree. All analyses for REML, BLUP and ssGWAS were run using the BLUPF90 software (Misztal et al., 2002; Aguilar et al., 2011). For BayesB and CGWAS methods, DP were created from BLUP estimates of EBVs as pseudo-observations, following Garrick et al. (2009) assuming that 0.1 of the genetic variance was not accounted for by SNPs, as in Ostersen et al. (2011). GWAS was then performed using three alternative methods. The first...
method of ssGWAS was run for five iterations with both ssGWAS1 and ssGWAS2 options. The second method of CGWAS was implemented in WOMBAT (Meyer and Tier, 2012). The third method, BayesB was implemented in GenSel (Habier et al., 2011) with $\pi = 0.9$. Estimates of genotypic and residual variances from REML were used as priors in BayesB, which followed a scaled inverse chi-squared distribution with default parameters used in GenSel. The use of the default parameter $\pi = 0.9$ was due to failure for convergence of $\pi$ estimation based on BayesC$\pi$ after 100,000 iterations. A Monte Carlo Markov Chain was completed for 51,000 rounds with Gibbs sampling, of which the first 1000 rounds were discarded as burn-in. Within each Gibbs sample cycle, Metropolis-Hastings samples were run for 10 iterations. Because WOMBAT and GenSel are not able to incorporate missing genotypes, missing SNPs were replaced by their average value for that locus.

Results were compared based on the proportion of total variance explained by the SNP. However, such estimates based on single-SNP were found to be noisy from all the methods due to the high ratio between the number of SNPs and the number of genotyped individuals. Therefore, non-overlapping windows of 20 consecutive SNPs were used to present results in Manhattan plots, instead of single locus. The methods were also compared based on the top 10 ranking windows for genetic variance explained by that window.

The methods were additionally compared in terms of predicted GEBVs. For ssGWAS, GEBVs were obtained directly, whereas for CGWAS and BayesB, GEBVs were calculated as the sum of estimated SNP effects for each genotyped individual. For comparison of accuracy of phenotypic BLUP and ssGWAS, realized accuracies were computed for ssGWAS1 and ssGWAS2, as the ratio of predictive ability over the square root of heritability according to Legarra et al. (2008).

RESULTS AND DISCUSSION

GENETIC ESTIMATIONS

Variance components, calculated from regular phenotypic BLUP based on all individuals in the data set, for maternal permanent environmental, additive and residual variances, were respectively 0.20, 1.14, and 3.88. The estimated heritability of BW6 was 0.22, which was similar to earlier estimates using the same trait (Chen et al., 2011).

Table 1 includes correlations between EBV (obtained from regular BLUP) and GEBVs for genotyped individuals; solutions for GEBVs in ssGWAS1 do not change between iterations, and they are the same as the first iteration in ssGWAS2 (ssGWAS2/1). The correlations between EBV and GEBVs for ssGWAS2 in the first and second iterations, and BayesB, were all $\leq 0.9$; while for CGWAS, and ssGWAS with 3 or more iterations, the correlations were $< 0.9$. As SNP effects are calculated in CGWAS individually, estimates for closely linked SNP were similar, which results in correlated residual and is likely to cause problems due to double counting. The decline in correlations after 2 iterations implies the estimates were over-regressing, resulting in lower accuracy.

Table 2 gives realized accuracies of EBV estimated using phenotypic BLUP and GEBVs estimated using ssGWAS. For these data, the accuracy was maximized by the second iteration (ssGWAS2/2), then declined after the third iteration (ssGWAS2/3). For GBLUP, Sun et al. (2012) added a constant in the equation to calculate SNP variance, mimicking the structure of such formulas in REML. Subsequently, the accuracy reached plateau but did not decline with iterations. In our studies, involving such a constant (results not shown), the accuracy did not improve over ssGWAS2/2 with the original formula. Further, adding a constant makes identification of top QTL more difficult (Sun et al., 2011).

QTL MAPPING

Figures 1–4 show plots of genetic variances accounted for by windows of 20 contiguous SNPs within a chromosome, based on different methods. Windows were neither overlapping nor repetitive. Chromosomes were differentiated by different shades. In total, there were 2031 regions, with an average chromosomal length of 0.45 Mbp.

Figure 1 shows plots by ssGWAS1/1, ssGWAS1/3, and ssGWAS1/5, which indicate iteration 1, 3, and 5 using ssGWAS1, which derives weights and solely iterates on SNPs. On one hand, as the iterations progress, the plots became less noisy, and the peaks associated with the largest regions become more

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**Table 1 | Correlations of EBV obtained from regular BLUP and GEBVs**

| Correlation | EBV |
|-------------|-----|
| ssGWAS2/1c  | 0.91 |
| ssGWAS2/2   | 0.90 |
| ssGWAS2/3   | 0.88 |
| ssGWAS2/4   | 0.87 |
| ssGWAS2/5   | 0.85 |
| BayesBd     | 0.90 |
| CGWAS       | 0.71 |

*GEBVs = Genomic breeding values.

*a Single-step genomic analyses (ssGWAS), BayesB, and classical genome wide association (CGWAS).

*b ssGWAS2/1 = the first iteration of Scenario 1 (ssGWAS2) in ssGWAS, which is equivalent to ssGWAS1.

*c ssGWAS2/2 = the first iteration of Scenario 1 (ssGWAS2) in ssGBLUP, which is equivalent to ssGWAS2/1.

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**Table 2 | Comparison of accuracies of EBV obtained from regular BLUP and GEBVs**

| Methods | Accuracy |
|---------|----------|
| EBV     | 0.34     |
| ssGWAS2/1c | 0.44 |
| ssGWAS2/2 | 0.52     |
| ssGWAS2/3 | 0.52     |
| ssGWAS2/4 | 0.51     |
| ssGWAS2/5 | 0.50     |

*a GEBVs = Genomic breeding values.

*b ssGWAS = Single-step genomic association analyses.

*c ssGWAS2/1 = the first iteration of Scenario 1 (ssGWAS1) in ssGBLUP, which is equivalent to ssGWAS2/1.

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**Table 1 | Correlations of EBV obtained from three approaches**

| Correlation | EBV |
|-------------|-----|
| ssGWAS2/1c  | 0.91 |
| ssGWAS2/2   | 0.90 |
| ssGWAS2/3   | 0.88 |
| ssGWAS2/4   | 0.87 |
| ssGWAS2/5   | 0.85 |
| BayesBd     | 0.90 |
| CGWAS       | 0.71 |

*a GEBVs = Genomic breeding values.

*b Single-step genomic analyses (ssGWAS), BayesB, and classical genome wide association (CGWAS).

*c ssGWAS2/1 = the first iteration of Scenario 1 (ssGWAS2) in ssGWAS, which is equivalent to ssGWAS1.

*d BayesB with $\pi = 0.9$.
distinct. On the other hand, the iterations caused some re-rankings of the top regions (Table 3). The simulation studied by Sun et al. (2011) indicated that a few iterations similar to ssGWAS1 provided the most accurate identification of the top QTLs.

Figure 2 shows plots by ssGWAS2/1, ssGWAS2/3, and ssGWAS2/5 iterating on both SNPs and GEBVs. Please note that the plots for ssGWAS1/1 and ssGWAS2/1 are identical. Compared with ssGWAS1, “thinning” in ssGWAS2 is more rapid. The plot of ssGWAS2/3 clearly points to many distinct regions, while the plot in ssGWAS1/3 seems less so. Note that the accuracy of GEBVs peaked at ssGWAS2/2 to ssGWAS2/3 and declined thereafter, suggesting that plots of ssGWAS2/2 and ssGWAS2/3 are also the most accurate depictions of where the most important regions are.

Figure 3 gives results from the CGWAS method. With this method, more peaks were found than from ssGWAS. However, the two largest regions remained the same as ssGWAS1/1-ssGWAS1/5. The presence of many more peaks in CGWAS than the other methods is most likely a result of strongly linked regions resulting in false positive (Shen et al., 2013).

Figure 4 gives results for the BayesB method. The plot is dominated by a few large regions, with all the other regions representing much smaller variances ≤2.5%. Methods like BayesB are strongly influenced by priors (Van Hulzen et al., 2012), and particularly by the percentage of SNPs assumed to have null effect (π). Studies on the number of genes influencing a quantitative effect estimate the number of <500 (Otto and Jones, 2000; Hayes and Goddard, 2001). Here, we assume that 10% of all SNPs

**FIGURE 1** Proportion of genetic variance of 20-SNP region under the Senarios 1 (ssGWAS1) of extended single-step genomic BLUP (ssGBLUP). (A) The first iteration (ssGWAS1/1). (B) The third iteration (ssGWAS1/3). (C) The fifth iteration (ssGWAS1/5). The x-axis represents region location of 20 SNPs. The y-axis represents the proportion of genetic variance of each region.
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**FIGURE 2** | Proportion of genetic variance of 20-SNP region under the Scenarios 2 (ssGWAS2) of extended single-step genomic BLUP (ssGBLUP). (A) The first iteration (ssGWAS2/1). (B) The third iteration (ssGWAS2/3). (C) The fifth iteration (ssGWAS2/5). The x-axis represents region location of 20 SNPs. The y-axis represents the proportion of genetic variance of each region.

(>4000) have effects. However, some of the alleles are rare variants that are not fully captured by medium or even high density SNP panels (Vinkhuyzen et al., 2012). For populations with small effective size, gains in GEBVs over EBVs in genomic selection are partly due to accounting for major genes, and partly for superior genetic relationships among animals. Fitting single chromosome in a genomic evaluation resulted in 86% of accuracy of GEBVs from using SNPs on all 26 chromosomes (Daetwyler et al., 2012). As the relationship information is replicated on all chromosomes but the QTL effects are not, the majority of large SNP effects may be due to specific population structure and not due to QTLs.

Table 4 shows chromosomal positions and fraction of variances explained by the top 10 regions of the 4 methods: CGWAS, BayesB, ssGWAS1 and ssGWAS2. For ssGWAS1/3 and BayesB, the regions that accounted for the largest genetic variance were on chromosome 27 and identical, but accounted for vastly different amounts of genetic variance: 2.53 and 23.06%, respectively. The order of magnitude difference in genetic variance accounted for by the methods, even though the regions were the same, is due to how total genetic variance is accounted for by the methods. BayesB partitioned all the genetic variance to 10% of the SNPs while ssGWAS partitions the genetic variance among all SNPs. Thus, ssGWAS has more SNPs to distribute the same amount of genetic variance.

Among the top 10 regions in ssGWAS1/3, there were 2, 4, and 6 regions respectively in common with ssGWAS2/3, CGWAS, and BayesB. In contrast, for the top 10 regions in BayesB, there were 6, 1, and 3 in common, respectively, with ssGWAS1/3, ssGWAS2/3
FIGURE 3 | Proportion of genetic variance of 20-SNP region using classical genome wide association studies (CGWAS) implemented by WOMBAT. The x-axis represents region location of 20 SNPs. The y-axis represents the proportion of genetic variance of each region.

FIGURE 4 | Proportion of genetic variance of 20-SNP region using BayesB with $\pi = 0.9$ implemented by GenSel. The x-axis represents region location of 20 SNPs. The y-axis represents the proportion of genetic variance of each region.

Table 3 | Rankings of top 10 regions$^a$ for 5 iterations in ssGWAS$^b$.

| ssGWAS1/1 (ssGWAS2/1)$^c$ | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |
|---------------------------|---|---|---|---|---|---|---|---|---|---|
| ssGWAS1/2                 | 1 | 3 | 2 | 12 | 4 | 9 | 7 | 10 | 5 | 6 |
| ssGWAS1/3                 | 1 | 3 | 2 | 21 | 4 | 11 | 7 | 15 | 8 | 6 |
| ssGWAS1/4                 | 1 | 3 | 2 | 32 | 4 | 14 | 10 | 21 | 9 | 6 |
| ssGWAS1/5                 | 1 | 2 | 4 | 36 | 3 | 14 | 19 | 18 | 10 | 6 |
| ssGWAS2/2                 | 1 | 9 | 6 | 2 | 16 | 20 | 19 | 8 | 7 | 5 |
| ssGWAS2/3                 | 1 | 110 | 62 | 29 | 8 | 233 | 57 | 31 | 21 | 16 |
| ssGWAS2/4                 | 1 | 351 | 256 | 72 | 3 | 575 | 126 | 58 | 22 | 35 |
| ssGWAS2/5                 | 1 | 479 | 472 | 100 | 2 | 766 | 179 | 86 | 25 | 50 |

$^a$Each region consists of 20 SNPs, and in totally there are 2031 regions on whole genome.

$^b$ssGWAS = single-step genomic association analyses.

$^c$ssGWAS1/1 = the first iteration of Scenario 1 (ssGWAS1) in ssGBLUP, which is equivalent to ssGWAS2/1.
Table 4 | Rankings top 10 regions among different methods.

| CGWAS | chr | gVar (%) | ssGWAS1/3 gVar (%) | ssGWAS2/3 gVar (%) | BayesB gVar (%) |
|-------|-----|----------|---------------------|---------------------|-----------------|
|       | 1<sup>1</sup> | 6 | 3.07 | 2 | 1.29 | 62 | 0.38 | 2 | 2.35 |
|       | 2   | 6 | 2.9  | 3 | 0.91 | 110 | 0.26 | 3 | 1.89 |
|       | 3   | 6 | 1.3  | 4 | 0.78 | 8 | 0.84 | 40 | 0.25 |
|       | 4   | 6 | 0.98 | 360 | 0.09 | 810 | 0.01 | 322 | 0.06 |
|       | 5   | 6 | 0.79 | 278 | 0.11 | 565 | 0.02 | 27 | 0.32 |
|       | 6   | 27 | 0.79 | 1 | 2.53 | 1 | 5.65 | 1 | 23.06 |
|       | 7   | 6 | 0.6  | 668 | 0.04 | 1216 | < 0.01 | 1646 | 0 |
|       | 8   | 7 | 0.48 | 314 | 0.1 | 927 | < 0.01 | 99 | 0.14 |
|       | 9   | 12 | 0.48 | 855 | 0.03 | 925 | < 0.01 | 387 | 0.05 |
|       | 10  | 4 | 0.45 | 274 | 0.11 | 903 | < 0.01 | 173 | 0.09 |
| Total |     |   |       |       |       |       |       |       |       |
|       |     |   | 11.84 |       |       |       |       |       |       |

| ssGWAS1/3 | chr | gVar (%) | ssGWAS2/3 gVar (%) | CGWAS gVar (%) |
|-----------|-----|----------|---------------------|-----------------|
|           | 1   | 27 | 23.06 | 1 | 5.65 | 6 | 0.79 | 2 | 23.06 |
|           | 2   | 6 | 2.35 | 2 | 1.29 | 62 | 0.38 | 1 | 3.07 |
|           | 3   | 6 | 1.89 | 3 | 0.91 | 110 | 0.26 | 2 | 2.9 |
|           | 4   | 11 | 1.39 | 15 | 0.43 | 31 | 0.55 | 279 | 0.08 |
|           | 5   | 2 | 1.03 | 42 | 0.28 | 63 | 0.38 | 656 | 0.04 |
|           | 6   | 3 | 1 | 144 | 0.16 | 166 | 0.18 | 11 | 0.43 |
|           | 7   | 4 | 0.73 | 9 | 0.53 | 105 | 0.27 | 450 | 0.06 |
|           | 8   | 5 | 0.68 | 6 | 0.59 | 16 | 0.72 | 423 | 0.06 |
|           | 9   | 2 | 0.59 | 7 | 0.56 | 57 | 0.39 | 32 | 0.29 |
|           | 10  | 2 | 0.54 | 264 | 0.11 | 119 | 0.24 | 53 | 0.22 |
| Total    |     |   | 33.26 |       |       |       |       |       |       |
|          |     |   | 7.39  |       |       |       |       | 9.02 | 7.94 |

(Continued)
Many studies have looked at QTLs or chromosomal regions in chicken for body weight. For example, Rowe et al. (2006) looked for QTLs for 40-day body weight in Cobb-Vantress chickens. They found that chromosomal segments could explain up to 4% phenotypic variation (PV) on chromosomes 1, 4, and 5. Podisi et al. (2013) looked at body weight and gains at different ages for broiler-layer crosses. For body weight at 6 weeks, they identified several QTLs on chromosomes 1–4, 6, 8, 11, and 13 explaining >1.4% PV; the largest QTL was on chromosome 4 and explained 6.0% PV. Neither study found an important QTL on chromosome 27. The large proportion of explained variance could be due to simple models of analyses.

CONSIDERATIONS
Windows were defined with fixed numbers of SNPs (i.e., 20), which might not match every pattern of haplotype blocks. Thus, over- or under-estimation of window variances were possible. Moreover, window variances were calculated based on SNP effects at each locus, which probably contains estimation errors, and translates into more variation in results for ssGWAS2. The noise due to the estimation process could be reduced by using sliding average values for SNP windows rather than point estimates.

Results showed that interpretation of GWAS using BayesB can be misleading. BayesB is based on a mixture model of those SNPs that explain genetic variance and those that do not (π). While the proportion of SNPs that explains genetic variance may become small, the total genetic variance remains constant, and is thus distributed among fewer SNPs resulting in what appears to be an inflated estimate of genetic variation accounted for by a SNP.

Every methodology for GWAS has a weakness. The ssGWAS1 method seems a more useful methodology compared with CGWAS and BayesB when a large number of phenotyped subjects are not genotyped, and obtaining deregressed proofs is difficult or impossible. A limitation of ssGWAS is that the number of iterations is dictated by heuristics at this time. Additional studies (unpublished) indicate that GWAS accuracy with ssGWAS1 is maximized at 2–4 iterations, with a single iteration creating noisy plots, and with more iterations suppressing signals from smaller QTLs. Another weakness of ssGWAS1 is the inability to determine the significance level. Possibilities to address this issue are the permutation test (Churchill and Doerge, 1994), or normalizing each SNP solution to a t-like statistic (McClure et al., 2012). The latter could be difficult to apply to a region including multiple SNPs. Future research may determine the level of significance in ssGWAS1 or ssGWAS2, e.g., following ideas by Garcia-Cortes and Sorensen (2001), where the estimation variances are obtained by sampling.

COMPUTING TIME
In this study, BayesB and CGWAS required DP which included running a regular BLUP, computing accuracies, and creating deregressed proofs. Omitting those procedures, GenSel required 17 h 13 min and WOMBAT required ~6 min. The very fast computing
time in WOMBAT is due to precomputing matrices for prediction, so that computation for an additional marker takes very little time. Traditional algorithms were about 100 times slower for a population with about 1000 animals and 4000 SNP (Meyer and Tier, 2012). The ssGWAS methods were applied directly to the phenotypes without DP, and took about 15 min per iteration.

CONCLUSION
This study compares genomic evaluation and association results between different methods: ssGWAS1, ssGWAS2, CGWAS, and BayesB. Because this was real data and the true values are unknown, it is not possible to conclude which method was most accurate for GWAS, but similarity between BayesB and ssGWAS1 was shown in various aspects. CGWAS was the most different but also found the greatest number of signals. The latter could be due to false positives. Advantages of using ssGWAS includes: (1) no pseudo values are required, (2) complex modeling and multi-trait analysis is possible, and (3) computing is fast and implementation is simple.

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