Genome wide association study of body weight and feed efficiency traits in a commercial broiler chicken population, a re-visitation

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Genome wide association study was conducted using a mixed linear model (MLM) approach that accounted for family structure to identify single nucleotide polymorphisms (SNPs) and candidate genes associated with body weight (BW) and feed efficiency (FE) traits in a broiler chicken population. The results of the MLM approach were compared with the results of a general linear model approach that does not take family structure in to account. In total, 11 quantitative trait loci (QTL) and 21 SNPs, were identified to be significantly associated with BW traits and 5 QTL and 5 SNPs were found associated with FE traits using MLM approach. Besides some overlaps between the results of the two GWAS approaches, there are considerable differences in the detected QTL. Even though the genomic inflation factor ($\lambda$) values indicate that there is no strong family structure in this population, using models that account for the existing family structure may reduce bias and increase accuracy of the estimated SNP effects in the association analysis. The SNPs and candidate genes identified in this study provide information on the genetic background of BW and FE traits in broiler chickens and might be used as prior information for genomic selection.

Genome wide association studies (GWAS) are commonly used to identify single nucleotide polymorphisms (SNPs) and candidate genes associated with quantitative traits. GWAS have revealed important regions associated with production, reproduction and disease resistance traits in chickens1-7.

One of the essential elements in GWAS is a powerful statistical method that can be employed to identify genetic associations in unbiased fashion8. Methods that model population structure by estimating the covariance due to genetic relatedness between individuals has been reported to perform better in terms of detecting true associations than models that ignore population structure9-12.

Kennedy et al.13 reported that using general linear model (GLM) analysis when relations between animals exist, results in an inflated F-test. Consequently it is likely to find an excess of spurious genotype effects when actually no genotype effect exists14. In this situation, the use of mixed-model procedures under an animal model treating single-gene effects as fixed effect and accounting for family relations can provide an exact F-test of associated hypotheses and unbiased estimates of genotype effects13,14.

Population structure mainly refers to population stratification and cryptic relatedness15. Population stratification is the presence of systematic differences in allele frequencies between subpopulations in a population due to different ancestry between study subjects16. Unrecognized population stratification can lead to both false positive and falsenegative findings and can obscure the true association signals if not appropriately corrected17,18.

Cryptic relatedness refers to the phenomenon that some members of a study sample (population) might be related beyond what can be inferred from the pedigree, in which case their genotypes are not independent of the population frequencies19. Because population based association studies assume individual independence of study samples, cryptic relatedness may make these statistical tests less reliable and reduce the robustness and efficiencies of the studies15,17.

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Methods modeling population structure, family structure and cryptic relatedness are expected to perform better than models that ignore these complexities\(^{13,15}\). Mixed models offer a practical and comprehensive approach for simultaneously addressing confounding due to population stratification, family structure and cryptic relatedness\(^{8,16,19}\).

This study aims to identify potential loci and candidate genes associated with body weight (BW) and feed efficiency (FE) traits in a commercial broiler line genotyped with 60 k SNP chip using mixed linear model (MLM) approach that accounts for family structure and compare the results with general linear model (GLM) approach that does not take family structure into account.

**Materials and Methods**

**Ethical statement.** Samples were collected from a commercial flock under the guidance of the local committees for the care and use of animals following the Cobb-Vantress Inc. Animal Welfare Policy. In addition, the experimental protocol was carried out in accordance with the approved guidelines for safeguarding good scientific practice at the institutions in the Leibniz Association.

**Birds and phenotypic data.** Phenotypic data were obtained from Cobb-Vantress broiler breeding company. A total of 5000 male broilers fed standard commercial broiler chicken diet based mainly on maize were raised from hatch to five weeks of age. Only males were studied in this experiment since selection intensity in males is considerably higher than that of females in broiler breeding programs. At the age of 36 days, birds were weighed (BW36) and the heaviest 1000 birds were selected for feed efficiency (FE) experiment and put in individual cages. At the age of 39 days (BW39), birds entered to a 7 day FE experiment and final body weight (BW46) was recorded at the end of the experiment. Total feed intake (FI) was recorded from individually caged birds during the experiment. Body weight gain (Gain) was calculated as the difference between final body weight (BW49) and start weight (BW39). Feed conversion ratio (FCR) was calculated as the ratio of feed intake to body weight gain. After data cleaning, 848 BW and FE records were used for further analysis.

**Genotyping and quality control.** Blood samples from the branchial vein were collected in anticoagulant tubes for DNA extraction. Extraction of genomic DNA was performed using Qiagen 96-well extraction kit (Qiagen, Hilden, Germany). DNA from a total of 864 samples were genotyped using the Illumina 60 K SNP chip (Illumina, San Diego, CA, USA). The Illumina 60 K SNP chip contains 57636 SNPs that are distributed across 29 autosomes (chromosome 1 to 28 and chromosome 32), two linkage groups (LGE 64 and LGE 22 C19 W28_E50C23), and two sex chromosomes (Z and W) using chicken genome assembly Galgal4. In this study only male broilers were examined so only genotypes from the Z sex chromosome were included. Plink software was used for quality control of the genotypic data\(^{20}\). SNPs with low call rates (<95%), minor allele frequency (MAF) < 0.03 and Hardy Weinberg equilibrium (HWE) P-value (<0.0001) were excluded. After quality control a total of 43914 SNPs were retained for GWAS analysis.

**Genome wide association analysis.** Genome wide association analysis was performed for BW traits (BW36, BW39, BW46) and for traits from the FE test (FI, Gain and FCR) using GCTA\(^{21}\). The genomic relationship matrix (GRM) was constructed using methods from Yang et al.\(^{23}\). The effective number of independent SNPs of autosomes were defined by the independent pairwise option in plink\(^{20}\). A total of 19416 independent SNPs were identified and the 5% genome wide significance threshold was adjusted to -log10 (P-value) = 5.60. The threshold P-value for suggestive significant association that allows one false positive association per GWAS was adjusted to -log10 (P-value) = 4.3. Genomic inflation factor (λ), was calculated using the R package GenABEL with “median” option\(^{24}\).

Manhattan plots of genome wide association analysis and quantile-quantile (QQ) plots were created using the qqman package in R software\(^{25}\). The annotated genes that were closest to the top SNPs were identified using Ensembl and NCBI. The reported top SNPs or “lead SNPs” are SNPs which have the highest -log 10 (P-value) among the significant SNPs which are in linkage disequilibrium (LD) with each other in 1 Mb windows. Base pair positions of SNP markers were updated to the latest version of the chicken genome assembly Gallus-gallus-5 (Galgal5).

For FI and Gain, the following linear mixed model was used:

\[
y = \mu + \beta m_i + \beta x + Zu + e
\]

For FCR and BW traits the following linear mixed model was used:

\[
y = \mu + \beta m_i + Zu + e
\]

Where, y is a vector of BW or FE observations, μ is the mean term, β is the SNP effect for marker i, m is a vector of SNPs for the ith SNP genotype indicator variable coded as 0, 1 or 2, βi is the regression coefficient (the effect of start weight on FI and Gain that accounts for differences in start weight), x is a vector of start weights (BW39), u is a vector of random polygenic effects i.e. The effect of all QTL except those on the chromosome where the candidate SNP is located, e is a vector of random residuals. The variance of u was re-estimated each time when a candidate SNP is located, e is a vector of random residuals. The variance of u was re-estimated each time when a candidate SNP is located, e is a vector of random residuals. The variance of u was re-estimated each time when a candidate SNP is located, e is a vector of random residuals. The variance of u was re-estimated each time when a candidate SNP is located, e is a vector of random residuals.
Table 1. Top SNPs associated with body weight and feed efficiency traits using mixed linear model approach.

| Trait                        | Chromosome | Number of significant SNPs | Top SNP in 1 MB window | Galgal5 position (bp) | -log10 (P-value) | SNP effect (SE) | Proximal gene | Distance from gene |
|------------------------------|------------|-----------------------------|------------------------|-----------------------|------------------|----------------|---------------|-------------------|
| Body weight (36days)         | 12         | 8                           | rs13612706             | 12867052              | 5.03             | -0.039 (0.009) | PTPRG         | Within            |
| Body weight (36 days)        | 14         | 1                           | rs14073523             | 5337950               | 4.89             | 0.036 (0.008)  | CACNA1H       | Within            |
| Body weight (36 days)        | 8          | 1                           | rs16617885             | 1883743               | 4.79             | -0.051 (0.012) | LOC107053920  | Within            |
| Body weight (39 days)        | 12         | 1                           | rs10723005             | 11570033              | 4.89             | -0.054 (0.012) | CACNA1H       | Within            |
| Body weight (39 days)        | 12         | 3                           | rs316610173            | 12931647              | 4.74             | -0.047 (0.011) | PTPRG         | Within            |
| Body weight (39 days)        | 12         | 2                           | rs15652523             | 10278318              | 4.72             | -0.043 (0.010) | LOC107054392  | 2.52 Kb upstream |
| Body weight (39 days)        | 14         | 1                           | rs14073523             | 5337950               | 4.67             | 0.041 (0.010)  | CACNA1H       | Within            |
| Body weight (39 days)        | 1           | rs13880135                  | 66076666               | 4.44                  | 0.040 (0.010)    | SOX5          | Within        |
| Body weight (39 days)        | 23         | 1                           | rs16190017             | 3741996               | 4.38             | 0.043 (0.011)  | RSPO1         | Within            |
| Body weight (46 days)        | 6          | 1                           | rs315083186            | 7305184               | 4.78             | 0.043 (0.010)  | LOC107054392  | Within            |
| Feed intake                  | 1          | rs1584287                   | 110928416              | 4.96                  | -0.111 (0.021)   | KDM6A         | Within        |
| Body weight gain             | 8          | 1                           | rs16617885             | 1883743               | 4.43             | -0.058 (0.013) | LOC107053920  | Within            |
| Body weight gain             | 17         | 1                           | rs14098962             | 7902999               | 4.32             | -0.054 (0.012) | LOC107052218  | Within            |
| Feed conversion ratio        | 17         | 1                           | rs14098962             | 7902999               | 4.78             | 0.082 (0.019)  | LOC107052218  | Within            |

Comparison of MLM and GLM approaches. The results of the present study were compared with the results of the GLM analysis by Reyer et al., which does not take family structure into account, using the following model.

\[ y = \mu + \beta m_i + \beta_1 x + e \]  

Where, y is the BW or FE observations, \( \mu \) is the mean term, \( \beta \) is the SNP effect for marker i, m is vector of markers for the ith SNP genotype indicator variable coded as 0, 1 or 2, \( \beta_1 \) is the regression coefficient (the effect of start weight on FI and Gain) that accounts for differences in start weight, x is a vector of start weights (BW39) for FI and Gain, and, e is the random residual. The variance of e is \( \sigma_e^2 \). Base pair positions of SNP markers in Reyer et al. were updated to the latest version of the chicken genome assembly, Gallus-gallus-5 (Galgal5) for easy comparison of the results with the current study.

Results GWAS results for body weight traits. Using MLM approach, the present study revealed 3 QTL which have suggestive significance association with BW36, 6 QTL with BW39 and 2 QTL with BW46 (Table 1). A total of 11 QTL and 21 SNPs reached the suggestive significance level with BW traits. The top SNPs and candidate genes associated with BW36 are located on chromosome 12, 14, and 8 while the top SNPs associated with BW39 are located on chromosome 12, 14, 1 and 23. The top SNPs associated with BW46 are located on chromosome 6 and 1 (Table 1). Manhattan plots and QQ plots of body weight traits are shown in Figs 1 and 2, respectively. All the reported QTL in Table 1 are suggestive, no SNP reached the genome-wide significance level for BW and FE traits in the present study, which suggests that BW and FE traits are controlled by many genes, each with small effects. All of the reported top SNPs were found inside the candidate genes in an intronic region except SNP rs15652523 associated with BW39, which is, located 2.52 kb upstream of the candidate gene LOC107053920 (Table 1).

SNP, rs14042911, is one of the top SNPs (\(-\log 10 \text{ (P-value)} = 5.15\)) associated with BW36 which is located on chromosome 12. Due to the proximal position to the top SNP, PTPRG (protein tyrosine phosphatase, receptor type G) is proposed as candidate gene associated with BW36 (Table 1). PTPRG is a protein coding gene which is a member of the protein tyrosine phosphatase (PTP) family. In humans members of the PTP gene family are known to be signaling molecules that regulate a variety of cellular processes including cell growth and differentiation, mitotic cycle, and oncogenic transformation27. Moreover, SNPs, rs14073523 and rs16617885, located on chromosome 12 and 14 are top SNPs associated with BW36. CACNA1H (calcium voltage-gated channel subunit alpha 1 H); a protein coding gene and LOC107053920 (uncharacterized gene) are the proximal genes to the top SNPs, rs14073523, and rs16617885, respectively. Wang et al. identified QTL located on chromosome 14 associated with abdominal fat weight in chickens. The detected QTL for BW traits in the current study suggest that the genetic variance of BW was not exhausted after the pre-selection rather there are many genes with small effects left in the population. This is a remarkable finding relative to our expectation given the fact that this broiler line has been pre-selected for BW. Mebratie et al. also noted an increase in genetic variance of BW after several generations of selection in a commercial broiler chicken population.

This study also revealed 6 QTL located on chromosome 12, 14, 1 and 23, to have suggestive significance association with BW39, 3 of them located on chromosome 12. SNP, rs10723005, is one of the top SNPs, associated with BW39 found on chromosome 12 which is located in an intron region of CCDC71 (coiled-coil domain containing 71 recombinant protein). SNPs, rs316610173, and, rs15652523, which are located on chromosome 12 and SNPs,
rs14073523, rs13880135, and rs16190017, located on chromosome 14, 1 and 23, respectively, are also found associated with BW39 (Table 1). PTPRG, LOC107054435 (uncharacterized gene), CACNA1H, SOX5 (transcription factor SOX-5) and RSPO1 (R-spondin 1) are candidate genes, with putative contribution to the variation in BW39. They are found proximal to SNPs, rs316610173, rs14073523, rs13880135 and rs16190017, respectively (Table 1). In humans, SOX5 is involved in the regulation of embryonic development and in the determination of the cell fate while RSPO1 gene in mice is involved in the rapid onset of crypt cell proliferation.

Two QTL located on chromosome 6 and 1, showing suggestive significance association with BW46 were identified with MLM approach. SNP, rs315083186, located on chromosome 6 and, SNP, rs314956606, located on chromosome 1 were found significantly associated with BW46 (Table 1). The candidate genes associated with
BW46 are LOC101748440 (uncharacterized gene) and ADIPOR2 (adiponectin receptor 2), which is a protein coding gene involved in fatty acid oxidation and glucose uptake in humans27. In the chicken QTL database48, 3 QTL located on chromosome 12 and 2 QTL located on chromosome 12 are reported to be associated with BW36 and BW46, respectively. One of the 3 QTL reported in the chicken QTL database for BW36 by Reyer et al.5 is overlapping with the identified QTL in the current study. However, the others are different QTL on the same chromosome, suggesting that chromosome 12 is potential chromosome for QTL associated with BW traits.

**GWAS results for feed efficiency traits.** In this study a total of 5 QTL and 5 SNPs that showed suggestive significance association with FE traits were identified using MLM approach. QTL located on chromosome 1 is found associated with feed intake while 2 QTL located on chromosome 8 and 17 are found associated with body weight gain. Two QTL located on chromosome 17 and 6 are found significantly associated with FCR. The top SNPs and proximal genes associated with FI, Gain and FCR are reported in Table 1. Manhattan plots and QQ plots of feed efficiency traits are shown in Figs 3 and 4, respectively.

QTL that contain SNP, rs15384287, located on chromosome 1 is found to be associated with feed intake (Table 1). The candidate gene associated with feed intake is KDM6A (lysine demethylase 6A). In line with this study, Yuan et al.7 reported a region on chromosome 1 that contains 8 SNPs which are significantly associated with feed intake in laying hens. Similarly, Mignon-Graesteau et al.29 reported four QTL associated with feed intake on chromosome 1 in chickens. Gao et al.30 and Tran et al.31 also reported QTL located on chromosome 1 to be associated with Gizzard weight in chickens which might have positive correlation with feed intake.

Two QTL located on chromosome 8 and 17 reached the suggestive significance level with body weight gain. SNP rs16617885, located on chromosome 8 and SNP, rs14098962, located on chromosome 17 are found associated with body weight gain (Table 2). LOC107053920 and LOC107053920 are uncharacterized candidate genes associated with the trait which are found proximal to the significant SNPs, rs16617885 and rs14098962, respectively. SNP, rs16617885, located on chromosome 8 is also found associated with BW36 with overlapping uncharacterized candidate gene, LOC107053920 (Table 1).

Two QTL located on chromosome 17 and 6 showed suggestive significance association with FCR. SNPs, rs14098962 and rs14568465, located on chromosome 17 and 6, respectively are found associated with FCR (Table 1). CTNNA3 (catenin alpha 3) and LOC107052218 (uncharacterized gene) are the candidate genes found proximal to SNPs, rs14098962 and rs14568465, respectively. SNP, rs14098962, located on chromosome 17 candidate gene LOC107052218 are also found associated with body weight gain (Table 1). Yuan et al.7 reported a region on chromosome 17 which is significantly associated with residual feed intake (RFI) in laying hens. In the chicken QTL database28 different authors have reported different QTL on chromosome 1 to be associated with FI. Similarly QTL on chromosomes 6 and 17 were reported to be associated with FCR in the QTL database. The reported chromosomies are overlapping with the chromosomies associated with FI and FCR in the current study. However, the QTL regions are different except the QTL reported by Reyer et al.5 for FCR located on chromosome 6 which were identified using Bayesian method of analysis for the same broiler line.

**Comparison of results in MLM and GLM approach.** The results of the MLM analysis in this study were compared with the results of the GLM analysis by Reyer et al.5 (Table 2). There are some overlaps between the identified QTL and candidate genes for BW36 and FCR. However, for most of the BW and FE traits, the identified QTL and candidate genes were different suggesting that the two methods do not necessarily give similar results. Table 3 shows comparison of genomic inflation factor (λ) between MLM and GLM analysis. The λ values in both approaches are “benign” and not significantly different from each other suggesting that population stratification is not a strong concern in our data.

Reyer et al.5 have reported GWAS results for two BW traits (BW36 and BW46) and three FE traits (FI, Gain and FCR) using the same data. Table 2 shows the reported GWAS results of the BW and FE traits derived from a general linear model (GLM) by Reyer et al.5. The GLM analysis was replicated in this study to compute the λ values reported in Table 3 and we found similar significant SNPs and -log10 (P-values) for all of the BW and FE traits as reported by Reyer et al.5 (Table 2). BW39 was not considered in the analysis of Reyer et al.5 but considered both in the replicated GLM analysis and the MLM analysis in this study.

Using the GLM approach, Reyer et al.5 reported 2 QTL and 9 SNPs that reached the suggestive significance level, located on chromosome 12 and 14 which were associated with BW36. The reported top SNPs, rs13612706, rs14073523, and proximal candidate genes associated with BW36 are consistent with the present study (Tables 1 and 2). SNP, rs13612706, showed higher -log10 (P-value) in the GLM analysis compared to the MLM approach, whereas SNP, rs14073523, was indicated by a higher -log10 (P-value) in the MLM approach than the GLM approach (Tables 1 and 2). In both MLM and GLM approaches 8 SNPs located on chromosome 12 were found significantly associated with BW36 with overlapping candidate gene, PTPRG (Tables 1 and 2). All the reported QTL in Reyer et al.5 are those that reached the suggestive significance level (-log10 (P-value) ≥ 4.3). No QTL reached genome wide significance level (-log10 (P-value) ≥ 5.6) except QTL on chromosome 17 which is associated with FCR (Table 2).

Reyer et al.5 also reported 2 QTL and 2 SNPs associated with BW46 located on chromosome 8 and Z, 1 QTL and a single SNP associated with body weight gain located on chromosome 17, 1 QTL and 3 SNPs associated with feed intake located on chromosome 5, 19 and 26, which are not consistent with the present study (Table 2). Moreover, Reyer et al.5 reported 4 QTL and 5 SNPs which have significant association with FCR, 2 of them located on chromosome 17, 1 located on chromosome 4 and the remaining QTL located on chromosome 22 (Table 2). Among the reported top SNPs associated with FCR, SNP rs4098962, located on chromosome 17 was found overlapping with the present study with higher -log10 (P-value) in the GLM approach compared to the MLM approach. However, the reported candidate genes are different (Tables 1 and 2).
For most of the BW and FE traits, the reported QTL in this study are not consistent with the reported QTL by Reyer et al. A total of 12 QTL and 20 SNPs associated with two BW (4 QTL and 11 SNPs) and three FE traits (8 QTL and 9 SNPs), were reported by Reyer et al. using the GLM approach (Table 2). By applying the MLM approach, the present study identified a total of 10 QTL and 17 SNPs, associated with the same BW (5 QTL and 12 SNPs) and FE (5 QTL and 5 SNPs) traits (Table 1). Among the identified 10 QTL in the MLM approach only 2 QTL associated with BW36 and FCR were found overlapping with the GLM approach while the 8 identified QTL were different from the ones reported in Reyer et al. for the same BW and FE traits.

**Discussion**

Body weight and feed efficiency traits are the most important economic traits in the poultry industry. Body weight is the live weight of birds at a given age and feed efficiency (FE) is the ability of birds to convert a certain input to a certain output (e.g. Kg of feed into Kg of meat). Among the number of ways to assess feed efficiency, the most widely used are feed conversion ratio (FCR) and residual feed intake.

We have performed GWAS for body weight and feed efficiency traits in a commercial broiler chicken population using a MLM approach, taking family structure into account. The results were compared with the results of a GLM approach, which does not take family structure into account.

Royer et al. also used Bayesian (Multi-marker) approach, which is more robust to population stratification and reported more significant SNPs associated with BW and FE traits than the GLM approach. However, in this paper the results were only compared with the single marker GLM approach which is comparable to the single marker MLM approach in the present study.

Xu et al. reported that chromosome 1 and 4 are the two critical chromosomes influencing growth traits particularly body weight in chickens. In this study, SNPs on chromosome 1 were found to be associated with BW39 and BW46 while no significant SNP were found on chromosome 4 for any of the BW traits under study. Podisi et al. 34
also reported two significant QTL for body weight at 12 weeks of age on chromosome 1 in broiler-layer cross female chickens.

Some of the BW and FE traits share consistent QTL and candidate genes. BW36 and BW39 share consistent region on chromosome 14 and candidate genes, PTPRG and CACNA1H, while BW46 does not share those candidate genes with BW36 and BW39. The effects of the lead SNPs with standard errors in parenthesis for BW36 and BW39 were found to be 0.036 (0.008) and 0.041 (0.010), respectively. This might indicate that the effects of these genes are smaller on BW46 due to increasing importance of other genes for the trait, suggesting that the identified genes are age dependent and the two traits (BW36 and BW39) might be genetically correlated. Mebratie et al.26 have reported that the genetic correlation between BW at different ages increased as the distance between BW measurements decreased. This might be due to changes in the physiological system of the chickens with age. Indeed, Schaeffer35 states that there might be genes that “switch on” and “off” at a certain age of an animal which could lead to changes in physiology and performance. Similarly, Carlborg et al.36 concluded that there are different genes and gene actions involved in growth at different developmental stages.

BW36 and body weight gain share an overlapping region on chromosome 8 and consistent candidate gene, LOC107053920, with SNP effects – 0.051 (0.012) and –0.058 (0.013), respectively (Table 1). This might suggest high positive genetic correlation between BW36 and body weight gain which is not surprising since body weight gain is a component of body weight. Furthermore, body weight gain and FCR share consistent QTL on chromosome 17 and candidate gene LOC107052218 (Table 1) with SNP effects – 0.054 (0.012) and 0.082 (0.019), respectively.

**Figure 4.** Quantile-quantile plots of feed efficiency traits using mixed linear model approach.

| Trait                  | Chromosome | Number of significant SNPs | Top SNP in 1MB window | Galgal5 position (bp) | -log10(P-value) | Candidate gene          |
|-----------------------|------------|----------------------------|-----------------------|-----------------------|------------------|-------------------------|
| Body weight (36 days) | 12         | 8                          | rs13612706            | 12867052              | 5.31             | PTPRG                   |
| Body weight (36 days) | 14         | 1                          | rs14073523            | 5337950               | 4.42             | CACNA1H                 |
| Body weight (36 days) | 8          | 1                          | rs16617885            | 1883740               | 5.21             | PTPRG, NR5A1, NR6A1    |
| Body weight (46 days) | Z          | 1                          | rs14753816            | 19805476              | 4.89             | HTR1A                   |
| Feed intake           | 5          | 1                          | rs16266739            | 6549732               | 4.38             | SPON1                   |
| Feed intake           | 19         | 1                          | GGaluGA001282 (rs313913143) | 1878406       | 4.61             | ENSGALG0000002830      |
| Feed intake           | 26         | 1                          | rs15467595            | 539371                | 4.50             | KDM5b                   |
| Body weight gain      | 17         | 1                          | GGaluGA117403 (rs312843163) | 9904101       | 5.29             | GPR144, NR5A1, NR6A1  |
| Feed conversion ratio | 4          | 2                          | rs14445503            | 31108872              | 5.04             | HHIP                    |
| Feed conversion ratio | 17         | 1                          | GGaluGA117403 (rs312843163) | 9904101       | 6.57             | NR5A1, NR6A1           |
| Feed conversion ratio | 17         | 1                          | rs14098962            | 7902999               | 4.86             | RXRA                    |
| Feed conversion ratio | 22         | 1                          | GGaluGA186837 (rs312757200) | 4533949       | 5.07             | ADRA1A                  |

**Table 2.** Top SNPs associated with body weight and feed efficiency traits using general linear model approach by Reyer et al. (2015). The position of SNPs is updated to the latest chicken genome assembly (Galgal5).
This broiler line is pre-selected for BW (only heaviest birds were entered to the FE experiment) and undergone several generations of selection for feed efficiency. This phenomenon might affect the detection power of our GWAS and estimated SNP effect sizes since the genetic variance of BW and FE traits might be reduced due to the pre-selection and several generations of intense selection. Mebratie et al. have reported that the SNP based estimated genetic variance of BW (0–0.006 kg²) and FE traits (0.001–0.006 kg²) in this broiler population is very small with high standard error and among others, one of the reasons for the reported very small estimates of genetic variance was pre-selection of the broiler line for BW.

We have conducted GWAS using MLM approach, which takes into account family structure and compared the results with the GLM approach by Reyer et al. which does not take family structure into account. A number of studies have shown that methods that model family structure perform better than models that ignore family structure. A widely used approach to evaluate whether confounding due to population stratification, family structure and cryptic relatedness exists is to compute the genomic inflation factor ($\lambda$), which is defined as the median $\chi^2$ (1 degree of freedom) association statistic across SNPs divided by its theoretical median under the null distribution. Values of genomic inflation factor ($\lambda$) > 1 generally indicate population stratification or other confounders, such as family structure or cryptic relatedness. Values of $\lambda$ < 1.05 are considered “benign” regarding power and type I error, although inflation in $\lambda$ is proportional to sample size.

Table 3 shows the $\lambda$ values of BW and FE traits using the MLM approach in the present study and the GLM approach by Reyer et al. The genomic inflation factor values suggest that population structure is not a strong concern in our data and the values are not significantly different from each other in the two methods. Moreover, the genomic relationship matrix of individuals shows that there are only few half sib and full sib relations in the current data suggesting that family structure is not a strong concern. However, there is a slightly higher inflation of $\lambda$ values in the results of Reyer et al. compared to the results of the present study. This may suggest that although there is no strong family structure in the population, using MLM analysis that takes into account existing family structure may increase power to detect true associations than ignoring this kind of sample structure which may result spurious associations.

In a simulation study, Thornton et al. have noted that in the absence of markers with unusual allele frequency differences and population structure, a widely used approach is to use mixed models. For the commercial broiler chicken data used in this study, family structure is not a strong concern. However, as suggested by different authors, we have used a MLM approach that takes into account the existing family structure and revealed differences in the identified top SNPs and candidate genes associated with BW and FE traits compared with the GLM approach by Reyer et al. This strengthens the suggestion that, even though there is no strong family structure in the data, MLM approach that uses the genomic relationship matrix to account for the existing family structure may decrease bias and improve accuracy of the association analysis.

The results of this study might provide insight about the genetic background of body weight and feed efficiency traits. Furthermore, the study emphasizes that GWAS using the two approaches (GLM and MLM) does not necessarily give similar results even with the absence of strong family structure in the data.

### Table 3. Comparison of lambda values with standard errors in general linear model (GLM) and mixed linear model (MLM) analysis.

| Trait                  | Lambda GLM | SE   | Lambda MLM | SE   |
|------------------------|------------|------|------------|------|
| Body weight (36 days)  | 1.0812     | 9.921616e-05 | 1.0532     | 0.0001531706 |
| Body weight (39 days)  | 1.0925     | 8.277596e-05 | 1.0423     | 8.860991e-05 |
| Body weight (46 days)  | 1.0592     | 0.002739952 | 1.0057     | 0.0001031297 |
| Feed intake            | 1.0429     | 8.538701e-05 | 1.0242     | 0.0001203372 |
| Body weight gain       | 1.0022     | 6.283618e-05 | 0.9955     | 9.700197e-05 |
| Feed conversion ratio  | 1.0513     | 0.0002345801 | 1.0258     | 0.0001170539 |

**Conclusions**

GWAS for BW and FE traits was performed in a commercial broiler chicken population. The present study has identified 11 QTL and 21 SNPs associated with BW traits and 5 QTL and 5 SNPs associated with FE traits. The results of this study provide insight on QTL and genes that are involved in the genetics of BW and FE traits in broiler chickens and can be used as fundamental information for genomic selection. Moreover, the MLM approach, which takes into account the existing family structure by using the genomic relationship matrix, resulted in different QTL for most of the analyzed BW and FE traits compared to the GLM approach that ignored the existing family structure. Although, there is no strong family structure in this population, the use of MLM approach may increase power to detect true associations compared to the GLM approach that does not take family structure into account as suggested by previous studies.
Data Availability

Data supporting this paper were obtained from Cobb-Vantress chicken breeding company. The phenotype and genotype data are available only upon agreement with Cobb-Vantress and should be requested directly from the breeding company.

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**Author Contributions**
W. Mebratie conceived the study, analyzed the data and wrote the manuscript. J. Jensen and H. Bovenhuis conceived and supervised the study, made substantial contributions to the interpretation of results and revised the manuscript. H. Reyer and K. Wimmers revised the manuscript.

**Additional Information**
Competing Interests: The authors declare no competing interests.

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