Genetic Architecture of Abdominal Fat Deposition Revealed by a Genome-Wide Association Study in the Laying Chicken

Jun GUO , Dan SHAO , Qiang WANG , Yongfeng LI , Taocun DOU , Xingguo WANG , Yuping HU , Liang QU [*] , Haibing TONG [*]

Article

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Jun Guo, Dan Shao, Qiang Wang, Yongfeng Li, Taocun Dou, Xingguo Wang, Yuping Hu, Liang Qu * and Haibing Tong *

Jiangsu Institute of Poultry Science, Yangzhou 225125, China
* Correspondence: liangquyz@126.com (L.Q.); tonghb@163.com (H.T.)

Abstract: Fat has a high energy density and excessive fatness has been recognized as a problem for egg production and the welfare of chickens. The identification of a genetic polymorphism controlling fat deposition would be helpful to select against excessive fatness in the laying hen. This study aimed to estimate genomic heritability and identify the genetic architecture of abdominal fat deposition in a population of chickens from a Dongxiang blue-shelled local breed crossbred with the White Leghorn. A genome-wide association study wase conducted on abdominal fat percentage; egg production and body weights using a sample of 1534 hens genotyped with a 600K Chicken Genotyping Array. The analysis yielded a heritability estimate of 0.19 ± 0.04 for abdominal fat percentage; 0.56 ± 0.04 for body weight at 72 weeks; 0.11 ± 0.03 for egg production and 0.24 ± 0.04 for body weight gain. The genetic correlation of abdominal fat percentage with egg production between 60 and 72 weeks of age was 0.35 ± 0.18. This implies a potential trade-off between these two traits related to allocation of resource. Strong positive genetic correlations were found between fat deposition and weight traits. A promising locus close to *COL12A1* on chromosome 3, associated with abdominal fat percent, was found in the present study. The other region located around *HTR2A* on chromosome 1, where allele substitution was predicted to be associated with body weight gain, accounted for 2.9% of phenotypic variance. Another region located on chromosome 1, but close to *SOX5*, was associated with egg production. These results may be used to influence the balanced genetic selection for laying hens.

Keywords: abdominal fat deposition; genomic heritability; genetic correlation; body weight gain; egg production

1. Introduction

Excessive fat deposition is undesirable for egg production in laying hens because it is associated with poorer feed efficiency, increases the cost of egg production and affects the health of the hens. As a consequence, interest in controlling abdominal fat pad deposition is growing in the field of layer breeding, though sufficient abdominal fat is required to safeguard egg production, especially in the later laying period. The solution requires balancing the fat pad weight with the impact on other production traits. In a suitable breeding plan, a genetic model of the selected traits should be correctly evaluated and parameters should be exactly described. Understanding the genetic parameters and architecture of abdominal fat deposition would be useful for layer breeding.

Selecting for a healthier abdominal fat pad would have a faster rate of genetic gain due to its moderate heritability and large genetic variance within populations. Some studies suggested that abdominal fat was a polygenic trait with moderate to high heritability. However, most previously reported genetic parameters were estimated using juvenile meat-type chickens [1–3]. There are considerable differences in the gene expression profiles and metabolic patterns between the young and adult stages. Longitudinal analysis also revealed that heritability changed over time [4,5], but

this study is unaware of any prior efforts to assess heritability changes in laying hens and few genetic correlation coefficients have been reported, especially with egg production.

Advances in the development of genotyping technology have enabled genetic evaluation and selection in poultry production using a genomic relationship matrix. High-throughput association studies have revealed significant associations for economically important traits such as egg size [6], breast muscle weight [7], ascites syndrome [8], body weight [9], feed efficiency traits [10], egg production [11] and feather pecking behavior [12]. Genome-wide association studies (GWASs) have also been performed in meat-type chickens for fat deposition [13–15] and body weight gain [16,17]. To our knowledge, no GWASs are available for other major traits in laying chickens such as fat deposition and body weight gain during laying period.

The aim of this study was to estimate the genetic parameters between abdominal fat deposition and performance traits using a genomic relationship matrix and to evaluate the genetic architecture of fat deposition and performance traits using GWAS. The genetic knowledge of abdominal fat deposition and performance traits will show how selection can affect fat deposition and anticipate and prevent the undesirable side effects of selection.

2. Materials and methods

2.1. Animals

Phenotypic data were obtained from an experimental farm at the Jiangsu Institute of Poultry Science. This population was developed by reciprocal crosses between Dongxiang blue shelled chickens and White leghorn. More details about the F2 population are described by Yi et al. [6]. 0-day-old chicks were vent sexed, wing-banded and vaccinated for Marek's disease on day zero. All chicks were reared in the battery brooders and transferred to group cages at six weeks of age. Brooders and cages were maintained at 20 to 25 °C with artificial illumination. Pullets were then transferred to single-hen cages at 17 weeks of age. The light treatment gradually increased by 1 h per week until 16 h of light was provided. The laying mash contained 16.5% crude protein and provided 2750 kcal of metabolizable energy/kg.

2.2. Phenotypic measurements

Five traits were measured including abdominal fat weight (AFW), body weight at 28 weeks (BW28) and 72 weeks of age (BW72) and egg production (EN) between 60 weeks and 72 weeks. The body weight was measured on live birds after overnight fasting. Egg number produced from 60 weeks to 72 weeks was recorded daily for each bird and then summated as a total. After slaughter, abdominal fat weight were measured.

For each trait, the most extreme values that varied by +3 and –3 standard deviations from the mean were removed prior to the analysis. All experiments were approved by the Institution of Animal Care and Use Committee in Jiangsu Institute of Poultry Science (permit number: JPIAE 2011–0005).

2.3. DNA extraction, genotyping and quality control

Blood samples were obtained from the brachial vein by standard venipuncture. Genomic DNA was extracted from the samples by overnight proteinase K digestion of lysed whole blood followed by phenol/chloroform extraction. A total of 1534 hens from a F2 resource population were genotyped with an Affymetrix® Axiom® 600K Chicken Genotyping Array (GeneSeek Inc., Lincoln, NE, USA) [18]. After the quality control procedure, 22 samples with the dish quality control less than 0.82 or single nucleotide polymorphism (SNP) call rate less than 97% were excluded from the sample pool. Markers with a missing SNP call rate exceeding 5%, minor allele frequency less than 0.01 and Hardy-Weinberg equilibrium test P-value less than 1×10^{-6} were eliminated. All SNPs located in the sex chromosomes and those not mapped in the chicken assembly were excluded from the analysis. All autosomal SNPs from 1,512 qualified samples were used for imputation implemented by the Beagle

Version 4 software package based on localized haplotype clustering [19]. A total of 435,243 SNPs and 1,512 birds were obtained for the further analyses after filtering for imputation results using PLINK (v1.90b6.21, Shaun Purcell, https://www.cog-genomics.org/plink/#recent) [20].

2.4. Genome-wide association analysis

Before the association test, an independent SNP set was identified using the PLINK command --indep-pairwise 25 5 0.2 for principal component analysis (PCA) analysis. The top five principle components were assigned as covariates in the association analyses. In the present study, the effective number of independent tests was set to 59,308, estimated by sample [21], so the genome-wide suggestive and significant P-values were 1.69×10^{-5} and 8.43×10^{-7}, respectively. The candidate genes closest to the associated SNPs in GeneCards (http://www.genecards.org/), Ensembl (http://asia.ensembl.org, version 107), and NCBI (http://www.ncbi.nlm.nih.gov) database were searched. The positions of interesting SNPs were obtained from Ensembl version 107 and NCBI bGalGal1.mat.broiler.GRCg7b.

Conditional analysis was also performed to identify the potential associated SNPs that might be masked in the putative region by a strong signal. Briefly, the top SNP was selected into covariates. Association analysis conditioning was then implemented based on selected SNPs to iteratively search for the top SNPs one by one using a stepwise model selection procedure until no SNP had a conditional P-value that passed the significance level.

GEMMA (v0.98.5, Xiang Zhou and Matthew Stephens, https://github.com/genetics-statistics/GEMMA) was used to implement a standard linear mixed model in which a single environmental variable was explained by SNP genotype and where relatedness was introduced by a random effect [22]. The statistical model used for the association test could be represented by the matrix expression:

$$y = W\alpha + X\beta + Zu + \varepsilon$$

where y is the vector of phenotypic values for all hens, W is the incidence matrix of fixed effects including a column of ones, α is the vector of the corresponding coefficients including the intercept, X is the vector of marker genotypes, β is the corresponding effect size of the marker, Z is design matrices relating observations to u, and u is the vector of random effects with u~(0,GRMσ_a^2), where σ_a^2 is the genetic variance and GRM represented the genomic relationship matrix as calculated with the GCTA package and used to replace pedigree relationship matrices [23] and ε is the vector of the residual variances with ε~N (0, Iσ_e^2), where σ_e^2 is the residual variance component. The statistical model was used as described above for single-marker GWAS. The Wald test statistic was used as a standard to select SNPs associated with abdominal fat and growth traits. To evaluate systematic bias of the linear regression model, the genomic inflation factor was calculated by the ratio of the median of the observed P-values to that of the expected test statistics. Manhattan plots were generated with R as described by Yi et al. [6]. Quantile–quantile (Q-Q) plots were obtained with the gap package in R [24].

2.5. Heritability

In order to investigate the roles of additive genetic contributions to phenotypic variations, a restricted maximum likelihood method was applied to estimate the heritability and genetic correlation coefficients based on the genomic relationship matrix in the BLUPF90 software [25]. The genetic variance explained by chromosomes and individual SNPs with the additive genetic effect was estimated and the mixed linear model used to estimate the genetic variance for each chromosome. The chicken genome was partitioned into 28 autosomes and two linkage groups. The use of the GCTA software package allowed the pedigree relationships matrix to be replaced by the genomic relationships matrix and the eigenvectors obtained by PCA were embedded as covariates [23]. A regression analysis was used to evaluate the relationship between the variance explained by each chromosome and its length.

2.6. Linkage disequilibrium analysis and genes identification

Linkage disequilibrium (LD) in target regions was visualized with Haploview 4.2 [26]. The LD blocks were defined according to the Gabriel criteria [27]. Genes that overlap in target regions were identified with the Ensembl BioMart webtool (http://www.ensembl.org/biomart/).

2.7. Shared loci analyses

Because significant correlations were found between fat deposition and production traits with a genomic relationship matrix, the following step was aimed at revealing the overlap loci that influence both fat deposition and production traits. The GWAS-PW software was applied to identify shared loci associated with a pair of traits [28]. This program used a Bayesian method to assess the posterior probabilities of association (PPA) for four models where one block harbored a genetic variant only associated with one trait in model 1 and model 2, another block harbored a genetic variant that was associated with both traits in model 3 and the last block harbored different genetic variants that were separately associated with each trait in model 4. In model 3, a value of PPA exceeding 0.9 is considered to jointly influence both traits at the significance level, whereas a value of PPA exceeding 0.6 is considered influential at the suggestive level.

3. Results

3.1. Description of traits and genetic parameters

The statistical description of abdominal fat, egg production and growth traits is summarized in Table 1. The sample size differs among traits because of missing observations. The coefficient of variation for BW72 at 13.9% and EN at 27.9% was lower than for AFP and BWG at 44.5% and 62.7%, respectively, showing that the relative internal variability for AFP and BWG was high. The highest heritability was obtained for body weight at 72 weeks. The moderate to high heritability estimated for weight traits suggests that these traits could be significantly improved.

The phenotypic and genetic coefficients parameters are presented in Table 2. In this study, AFP showed strong genetic correlations with weight traits and moderate negative genetic correlations with egg production between 60 and 72 weeks of age. Egg production shows low genetic correlation with weight traits. Strong and positive genetic correlations among weight traits were found.

Table 1. Statistical description of abdominal fat, body weight and egg production.

Trait *	Sample size	Average ± SD	Genetic variance	Residual variance	Phenotypic variance	Heritability
AFP (%)	1329	4.45±1.98	0.48±0.10	2.05±0.10	2.53±0.10	0.19±0.04
BW72 (kg)	1493	1.44±0.20	0.023±0.003	0.018±0.001	0.042±0.002	0.56±0.04
BWG (g)	1472	212.31±133.12	4028.52±747.42	13028.02±664.73	17053.01±700.71	0.24±0.04
EN	1489	53.29±14.89	25.53±7.13	211.89±9.51	237.38±9.00	0.11±0.03

Table 2. Estimates of genetic (above the diagonal) and phenotypic (below the diagonal) correlations with their approximate standard errors (in parenthesis).

Traits	AFP	BW72	BWG	EN
AFP	--	0.55±0.09	0.79±0.08	-0.35±0.18
BW72	0.39±0.03	--	0.88±0.03	0.15±0.14
BWG	0.42±0.02	0.79±0.01	--	0.14±0.17
EN	-0.10 ± 0.03	0.04±0.03	-0.06±0.03	--

3.2. SNP association and candidate genes

The quantile–quantile (QQ) plot for each trait showed that that most of the observed P-values fit well to the expected P-values, except in the tail region of distribution that corresponded to associated SNPs, shown in Figure 1. The genomic inflation factors were 0.997 for abdominal fat percent, 1.021 for egg production and 1.026 for body weight gain, indicating that inflation effects due to population

5

stratification or undetected genotyping error were small to negligible in each trait of interest. For each trait, only the most significant regions were selected for further downstream analysis.

One significant SNP located on chromosome 3 was rs13695700, found in the downstream region of collagen type XII alpha 1 gene *COL12A1.* It had supporting SNPs spanning a region between 80.76M and 81.06M as the chicken reference genome GRCg7b and identified for abdominal fat percentage based on Bonferroni correction for multiple testing, shown in Table 3 and Figure 2.

Eleven SNP effects that mapped to the long arm of the chromosome 1 were identified for body weight gain in Figure 2 and 110 SNP effects reached suggestive significance. Three chromosomes *GGA1, 2 and 4* harbored these suggestive significant SNPs. A strong association was identified for a region on GGA1 that spanned 2 Mb between 168.27 and 170.25 Mb and contained all genome-wide and 81 suggestive significant SNPs associated with body weight gain between 28 and 72 weeks. The SNP rs317160250 was the lead SNP associated with body weight gain, seen in Table 3. The *5-Hydroxytryptamine Receptor 2A (HTR2A)* was the proximal genetic locus to the lead SNP and encoded one of the receptors as a neurotransmitter with many roles.

The GWAS for egg production revealed a significantly associated genomic region on GGA1. The SNP rs313399567 was the lead SNP associated with egg number produced between 60 and 72 weeks, which was located in this region. Due to the proximal position to the top SNP, *SRY-Box Transcription Factor 5 (SOX5)* was proposed as a candidate gene associated with egg production. The genotype and allele frequencies for this most associated SNP are given in Table 3.

Conditional analysis.

To check for any potential markers that might be masked by single-step analysis, conditional association analyses based on significantly associated SNPs were carried out. First, any markers detected as retained after the top SNP associated with body weight gain were treated as covariates and all significant SNPs dropped out after stepwise conditional analysis. Second, the conditional analysis for egg production was repeated and no significant association was found on chromosome 1.

Overlap genetic locus analyses.

To explore any shared loci that jointly affect fat deposition and production traits, pairwise GWAS were applied to identify which pleiotropic regions were shared between paired traits. We found that one locus reached a suggestive level for a shared association between abdominal fat deposition and body weight gain with a PPA3 of 0.85 and body weight at 72 weeks with a PPA3 of 0.81. This genomic region harbored the above-mentioned SNP mapped on chromosome 3. No region was uncovered between fat deposition and egg production.
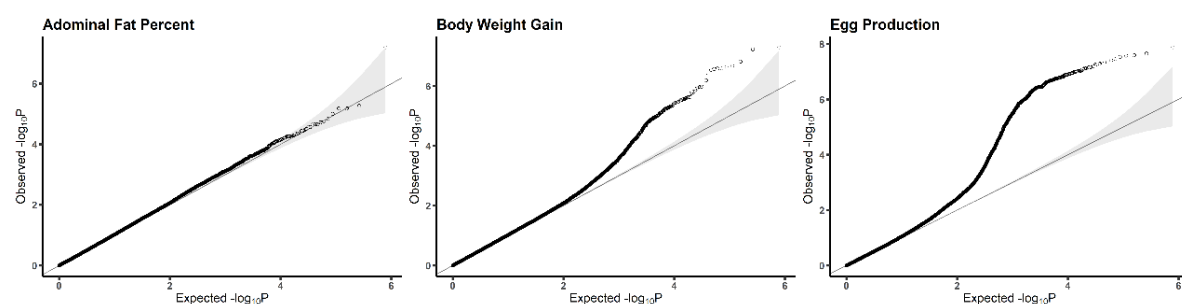

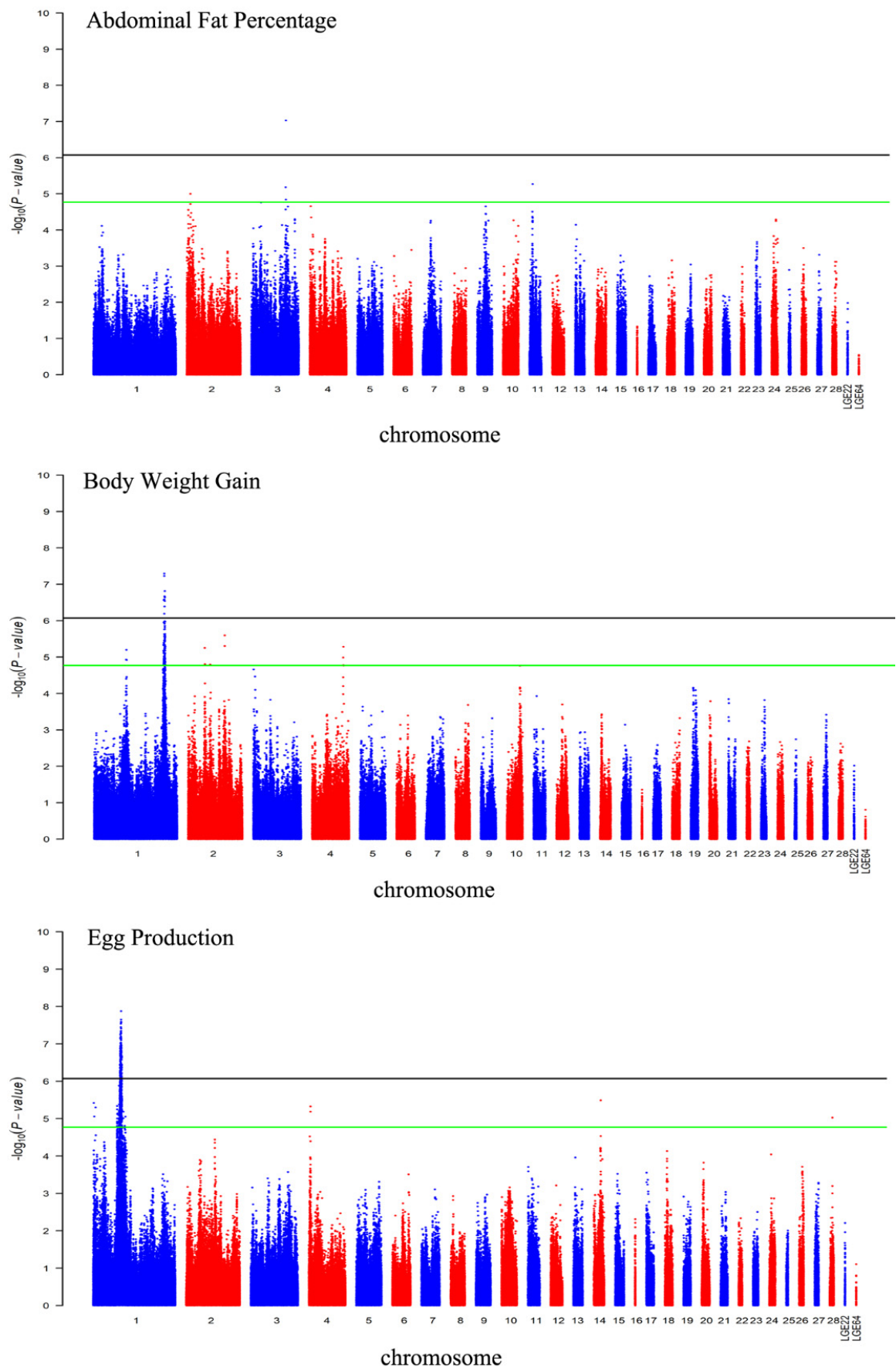**Figure 1.** Quantile–quantile plot on the negative logarithmic scale.

Figure 2. Manhattan plot of the P-value in the chicken GWAS.

4. Discussion

Genetic evaluation

The estimates of genetic correlations from this study provided unequivocal proof that abdominal fat deposition interacts with egg production and body weight gain. Unlike previous studies, the relationship matrix was constructed from genomic information, rather than pedigree data. The advantage of this method lies in more accurate description of the relative relationship and offers some ability to adjust for Mendelian sampling terms [29,30]. It has been observed that excessively fat hens tend to lay fewer eggs [31,32] but to our knowledge there was no known genetic correlation with reproductive performance. Yet adequate fatness is necessary to maintain good egg production. Using the quantitative genetics method, this study found that abdominal fat deposition has a negative genetic correlation with egg production, providing a rationale to balance fat deposition and egg production during the laying hen breeding program.

The positive genetic correlation between abdominal fat percentage and body weight gain was 0.79 ± 0.08. These results are consistent with a previous study by Garwood et al. [33], who reported that abdominal fat weight is positively correlated (0.84) with body weight gain. Conversely, Chen et al. [34] reported the genetic correlation of abdominal fat proportion was weakly negative with body weight gain in young broiler and unknown for the laying hen. It is generally considered that estimates of genetic correlations are less precise than those of heritability, especially for a highly variable trait such as fat deposition.

Genetic architecture

Table 3. Lead SNP from the significantly associated region for abdominal fat percent, body weight gain and egg production in chickens.

SNP	Chr	position	candidate gene	Distance (kb)	MAF	substitute	Proportion phenotypic variance	LRT p-value	Length of block (kb)	Trait
rs13695700	3	81057418	COL12A1	downstream 457kb	0.275	G/A	0.025	9.31×10^{-8}	6.9	Abdominal fat percent
rs317160250	1	168419997	HTR2A	upstream 164kb	0.362	A/G	0.029	5.08×10^{-8}	11.2	Body weight gain
rs313399567	1	65972279	SOX5	upstream 0.68kb	0.499	A/G	0.024	7.44×10^{-8}	299	Egg production

In this phenotypic study of fat deposition, a large variation in abdominal fat percentage was obtained. Given the observed small genetic effect relative to the large variation in phenotype, it was not surprising that few loci were identified during the association study. The GWAS showed that one SNP located near *COL12A1* gene was significantly associated with abdominal fat deposition.

Adipose tissue is not merely composed of simple adipocytes and each cell is closely associated with the extracellular matrix (ECM). The *COL12A1* is a key component of ECM, which took part in the remodeling of adipose tissue in fatness and metabolic disease [35]. In another GWAS of a broiler population, *COL12A1* was regarded as one of candidate genes for abdominal fat deposition and validated with quantitative PCR gene expression analyzed between high- and low-fat weight groups [36]. There is also evidence the differential expression level of *COL12A1* was related to abdominal fat deposition using RNA-seq [37] and proteomics profiles [38]. The results from this study are therefore consistent with a genetic contribution where *COL12A1* makes ECM changes leading to abdominal fat deposition.

Knowledge of the genetic architecture of fat deposition must also be supported by the study of the interaction of related traits. These results also provided essential data for a better understanding of genetic correlations related to fat deposition. The implicated region linked with *COL12A1* and its effects on the extracellular matrix remodel were also associated with phenotypic variations such as fat deposition and body weight at 72 weeks and body weight gain.

This study also identified through GWAS that *HTR2A* was associated with body weight gain. The *HTR2A* gene encodes a serotonin receptor and the neuroactive ligand–receptor pathway plays

an important role in chicken growth by regulating food appetite [39]. Molecular genetic studies show that serotonin receptors affect the appetite and body weight through the melanocortin 4 receptor [40]. Previous studies have confirmed the importance of the genomic region that included *HTR2A* as exercising genetic control over body weight and fatness [41,42] and this study provided further evidence of an association between serotonin receptor polymorphism and body weight gain.

Another important finding was that polymorphism in the upstream of the *SOX5* gene was significantly associated with egg production. It belongs to a member of the SOX family, which have SRY-related conserved HMG boxes in their structure. Earlier studies noted the importance of the expression of *SOX5* in influencing egg production. Through a transcriptome profile analysis, Ma et al. identified *SOX5* as one of the core genes affecting laying-rate difference [43]. Through association analyses in cattle, *SOX5* is regarded as one of candidate genes for female fertility [44,45] and through whole genome scanning analyses in dairy goats, it is related to litter size [46]. The consistency of the findings with those of other studies is encouraging, however further validation research may be required in order to understand the genetic architecture on egg production more comprehensively.

5. Conclusions

These results confirmed hypotheses of a potential trade-off between fatness and egg production. However, pairwise GWAS analysis did not provide significant results for colocalization between the paired traits. There is substantial evidence that fat deposition, egg production and body weight gain are heritable and polygenic and that the majority of the genetic variance of both traits is captured by the genomic relationship matrix. A promising candidate gene involved in fat deposition was mapped to chromosome 3. A pleiotropy effect from this region appears to be identified with pairwise GWAS. The identification of the pleiotropic region is helpful for understanding the genetic background of fatness in chickens. These results also provided additional evidence of an important role for *HTR2A* in body weight gain, a potential role for chrome 1, 2 and 4 in the growth traits and a key role for *SOX5* in persistence of lay. Taken together, these results advance the understanding of the genomic architectures of fatness, body weight and egg production.

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