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Abstract

Two functional and positional candidate genes were selected in a region of chicken chromosome 1 (GGA1), based on their biological roles, and also where several quantitative trait loci (QTL) have been mapped and associated with performance, fatness and carcass traits in chickens. The insulin-like growth factor 1 (IGF1) gene has been associated with several physiological functions related to growth. The lysine (K)-specific demethylase 5A (KDM5A) gene participates in the epigenetic regulation of genes involved with the cell cycle. Our objective was to find associations of selected single-nucleotide polymorphisms (SNPs) in these genes with performance, fatness and carcass traits in 165 F2 chickens from a resource population. In the IGF1 gene, 17 SNPs were detected, and in the KDM5A gene, nine SNPs were detected. IGF1 SNP c.47673G>A was associated with body weight and haematocrit percentage, and also with feed intake and percentages of abdominal fat and gizzard genotype × sex interactions. KDM5A SNP c.34208C>T genotype × sex interaction affected body weight, feed intake, percentages of abdominal fat ($p=0.0001$), carcass, gizzard and haematocrit. A strong association of the diplotype × sex interaction ($p<0.0001$) with abdominal fat was observed, and also associations with body weight, feed intake, percentages of carcass, drums and thighs, gizzard and haematocrit. Our findings suggest that the KDM5A gene might play an important role in the abdominal fat deposition in chickens. The IGF1 and KDM5A genes are strong candidates to explain the QTL mapped in this region of GGA1.

Introduction

In a Brazilian F2 chicken resource population, Nones et al. (2006) mapped quantitative trait loci (QTL) for growth, carcass and fatness traits in a specific region (between markers ADL0234 and LEI0071) of chicken chromosome 1 (GGA1). QTL for growth and fat deposition were also mapped in other populations in the same region (Abasht et al. 2006; Liu et al. 2007). Unpublished mapping results from our group narrowed one QTL for body weight at 41 days to a region between LEI0146 and LEI0174, where the centromere of GGA1 is located (Galkina et al. 2006), and 107 genes were predicted in the previous version of the chicken sequence (Gallus_gallus-2.1, http://www.ncbi.nlm. nih.gov/mapview/) or 134 genes in the latest version (Gallus_gallus-4.0). Two positional and functional candidate genes were selected among those 107 genes based on their biological role summarised below, with the objective of detecting polymorphisms present in our F2 population and conducting association studies with performance, fatness and carcass traits: the insulin-like growth factor 1 (IGF1) gene and the lysine (K)-specific demethylase 5A (KDM5A) gene.

The IGF1 gene was mapped at 166 cM on GGA1 in the male genetic map of the East Lansing population (http:// www.thearkdb.org/arkdb/), between markers LEI0146 and LEI0174 (Klein et al. 1996). IGF1 is among the bestcharacterised muscle growth-promoting factors. In addition to circulating IGF1, mainly synthesised by the liver under growth hormone (GH) control, there is also local production by skeletal muscle of distinct IGF1 splicing products (Sandri 2008). IGF1 has been associated with several physiological functions in mammals and birds, such as growth, cellular proliferation, differentiation of muscle, cartilage and bones (Schmid 1995; Duclos et al. 1999; Fisher et al. 2005), stimulation of erythropoiesis (Schmid 1995) and

proliferation of satellite cells (Machida and Booth 2004). The IGF system seems to exhibit the same general characteristics in birds as in mammals, including the stimulatory effects on cultured muscle cells (Duclos et al. 1999). Variability in the IGF1 gene structure exists in the chicken, as shown by the 70 singlenucleotide polymorphisms (SNPs) described in the dbSNP database in 2012 (http://www.ncbi.nlm.nih.gov/snp/).

The KDM5A gene (also known as RBP2 or JARID1A) is also located between markers LEI0146 and LEI0174 on GGA1, 5.02 Mb from IGF1. KDM5A is a histone demethylase, which participates in the epigenetic regulation of the expression of genes involved with the withdrawal from the cell cycle and subsequent induction of differentiation, including in myogenic, adipocyte, osteogenic, haematopoietic, among other cell types (Benevolenskaya et al. 2005; Christensen et al. 2007; Lopez-Bigas et al. 2008). A total of 275 SNPs were described in the KDM5A gene in chickens in 2012 (dbSNP database).

Our aim was to evaluate the associations of two polymorphisms selected in the IGF1 and KDM5A genes, located in a QTL region on GGA1, with performance, fatness and carcass traits in a chicken F2 resource population.

Materials and methods

Population and traits

The Embrapa F2 chicken resource population originated from a cross between a broiler male line (TT) and a layer line (CC). Both pure lines were developed by the Embrapa Swine and Poultry Research Centre and were under multitrait selection for

six (TT) and eight (CC) generations when the F2 population was created (details are in Nones et al. 2006). A total of 2,063 F2 chickens were obtained from 17 hatches during 8 months, from 21 full-sib families. Seven of these families (652 F2 chickens) were used in the QTL mapping study (Nones et al. 2006).

Performance, fatness and carcass traitswere evaluated in F2 chickens. Performance traits were as follows: body weight at 35 and 41 days, feed intake and feed conversion from 35 to 41 days. Weight at 42 days was measured after 6 h of fasting and transportation to the slaughterhouse. Slaughtering took place on day 42, when carcasses were eviscerated and stored at −4 °C. The following carcass traits were recorded: weights of abdominal fat, eviscerated carcass (no organs, head, neck or shank), breast (with skin and bones), shank, drums and thighs, heart, lungs, liver and gizzard. Percentages of carcass traits were calculated relative to body weight at 42 days. Blood samples were collected at slaughter to obtain haematocrit values (by the micro-haematocrit method) as percentages.

Sequencing and selection of SNPs for genotyping

Six pairs of primers were designed to amplify 650–800 bp of exonic and intronic regions from chicken IGF1 and KDM5A genes (Online Resource 1). Amplified fragments from six TT males, six CC females (parental generation) and ten F1 chickens were individually sequenced for polymorphisms identification. Genomic sequences for both genes are available in the GenBank database (from the reference assembly accession no. NC 006088.2 based on Gallus_gallus-2.1): IGF1 gene (gene ID 418090, region 57,327,749 to 57,376,177 bp) and KDM5A gene (gene ID 418148, region 62,358,974 to 62,407,496 bp).

Polymerase chain reaction (PCR) assays were performed in a total volume of 50 μL containing 20 ng of genomic DNA, 2 pmol of each primer, 10× PCR buffer (50 mM KCl, 10 mM Tris–HCl, pH 8.5), 50 mM of MgCl2, 10 mM of dNTPs and 1 U of Platinum® Taq DNA polymerase (Life Technologies™). The PCR conditions were: 95 °C for 1 min, followed by 30 cycles of 95 °C for 1 min, 50–62 °C (depending on the primer, Online Resource 1) for1min, 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR products, purified with the GFX 96 PCR Purification Kit® (GE Healthcare), were sequenced on both strands using the BigDye Terminator Cycle Sequencing Ready Reaction® kit (Life Technologies™), in the ABI PRISM 3100 Genetic Analyzer ® sequencer (Life Technologies™). The nucleotide sequences were analysed using Phred, Phrap and Consed (Ewing et al. 1998; Gordon et al. 1998).

Among the 26 SNPs detected in the IGF1 and KDM5A genes, two (c.47673G>A from IGF1 and c.34208C>T from KDM5A) were selected for further investigation and association studies. Two families (165 F2 chickens) were selected from seven full-sib families, according to QTL fine mapping (unpublished results), taking into consideration the linecross and half-sib analyses. One QTL for body weight at 41 days was narrowed down to a small interval on GGA1 (LEI0146–LEI0174) containing 28.8 cM in the line-cross analysis, with 1 % genome-wide significance (F-ratio=21.8, confidence interval=97 cM and 3.2 % of phenotypic variance explained by the QTL). The results from the half-sib analyses indicated that this QTL for body weight at 41 days was segregating in the progeny of these two families.

Besides that, the two SNPs were selected based on five criteria: (1) segregation analysis along the parental and F1 generations; (2) SNPs in the exonic region; (3) informativeness in the two families in which the QTL were segregating; (4) linkage

with the other SNP identified in the same gene; and (5) proximity with the other SNP chosen.

The genotyping of F2 chickens with the two SNPs was performed using TaqMan® Universal PCR Master Mix (Life Technologies™), Custom TaqMan® SNP Genotyping Assay (Life Technologies™) and the LightCycler® 480 System (Roche), using the endpoint genotyping method. Genotyping conditions included a pre-incubation at 95 °C for 10 min, with a ramp rate of 4.4 °C.s^{-1}. The amplification consisted of 45 cycles: 95 °C for 15 s with ramp rate 4.4 °C.s^{-1}, 60 °C for 1 min with ramp rate 2.2 °C.s^{-1}, final extension at 72 °C for 1 s with ramp rate 4.4 °C.s^{-1} and cooling at 40 °C for 10 s with ramp rate 1.5 °C.s^{-1}.

Statistical analyses

Genotypic frequencies of polymorphisms, haplotypes and diplotypes (combination of genotypes from the two candidate genes polymorphisms) were obtained using the FREQ procedure of SAS (2003). Association analyses of genotypes and diplotypes of the polymorphisms with phenotypic traits were conducted using analysis of variance in the GLMprocedure of SAS (2003). Models included the fixed effects of hatch, sex, family, genotype or diplotype of candidate genes polymorphisms and the genotype (or diplotype) × sex interaction, as well as the random error. Due to the data structure, it was not possible to test the interactions involving hatch and family effects. Body weight at 35 days was included as a covariate in the models for feed intake and feed conversion from 35 to 41 days in preliminary analyses. Found to be non-significant in any case, the covariate was eliminated from the final models. p values<0.05 were considered to be significant. Additive effects were estimated using

linear contrasts and dominance effects using quadratic contrasts, only for the loci with three putative genotypic classes.

Results

SNP discovery in the IGF1 and KDM5A genes

In the IGF1 gene, 17 SNPswere detected, fromwhich 14were new. All the SNPs were in intron 3 (mainly at the intron 3′end), although we sequenced several different regions of this gene (Online Resource 1). From the 17 SNPs identified, 13 were not segregating in our F2 population, including three SNPs previously described in the dbSNP database (rs14827900, rs14827901 and rs15274895), with the same mutations (T/G, A/C and T/A, respectively). Four SNPs were segregating in our population (Table 1) and the c.47673G>A SNP was selected for the trait association studies shown below, but it was genotyped only in one family (74 chickens), because it was fixed in the other. The other SNPs identified in the IGF1 gene were not informative in the other five F1 families analysed.

In the KDM5A gene, nine new polymorphisms were detected in exonic and intronic regions (Table 1). The SNPs in exonic regions were synonymous mutations and the c.45957C>T SNP was located in the 3′ UTR region. In the dbSNP database, 275 SNPs were described in this gene in chickens, but none of them was identified in our population. All nine SNPs discovered were segregating in our population (Table 1). The c.34208C>T (KDM5A) SNP was selected for the trait association studies.

Allele and genotype frequency of SNPs in the IGF1 and KDM5A genes, and their diplotypes

Two genotypes (A/A and A/G) were found for the c.47673G>A (IGF1) SNP and all three possible genotypes (C/C, C/T and T/T) for the c.34208C>T (KDM5A) SNP. For the IGF1 SNP, allele A was predominant in the two families (allele frequencies: 0.75 and 1.00), whereas for the KDM5A SNP, allele C was more frequent in family 1 (0.76) and allele T was more frequent in family 2 (0.53). The genotypic frequencies of the IGF1 SNP were 0.50 for A/A and A/G in family 1, and 1.0 for A/A in family 2, and of the KDM5A SNP, they were 0.51 (C/C) and 0.49 (C/T) in family 1, and 0.30 (C/C), 0.45 (C/T) and 0.25 (T/T) in family 2.

The reconstruction of haplotypes for all 165 F2 chickens was performed manually (Table 2). Five diplotypes were identified, but one was excluded from the analysis (H1H4) due to its low frequency in the population (3 %). Therefore, the frequency of the minor diplotype considered in the analyses was 13 % (Table 2).

Association analyses

The means, standard deviations, minimum and maximum values for the 15 phenotypic traits evaluated in 165 F2 chickens are listed in Table 3. There were 15 hatch classes in this dataset, with the number of chicks ranging from 4 to 20 per subclass. Family subclasses included 91 and 74 individuals, and sex subclasses 84 males and 81 females. Hatch effects were detected on all traits for both SNPs and the diplotype analyses, whereas family and sex effects were detected on most, but not on all, traits (Tables 4, 5 and 6).

The c.47673G>A IGF1 SNP was associated with body weight at 41 days and haematocrit % (Table 4). The A/A individuals had higher body weight (1,039±13 g) and haematocrit value (29.24±0.32 %) than those with the A/G genotype (977±24 g

and 27.83±0.58 %, respectively). There were also significant effects of the SNP × sex interaction on feed intake from 35 to 41 days and percentages of abdominal fat and gizzard, indicating that the genotypes of this SNP had different effects on those traits in females and males (Fig. 1). No differences in feed intake and percentages of abdominal fat and gizzard were detected between males with the A/A and A/G genotypes, whereas females with the A/A genotype had higher feed intake than those with the A/G genotype (p=0.0013). On the other hand, females with the A/G genotype had higher gizzard percentage than those with the A/A genotype (p=0.0002) (Fig. 1). Although an SNP × sex interaction effect was detected on the percentage of abdominal fat, no difference was detected between females of the two genotypes for this trait.

For the c.34208C>T KDM5A SNP, genotype × sex interaction effects affected body weight at 41 days, feed intake, percentages of abdominal fat, carcass, gizzard and haematocrit, indicating that the genotypes of this polymorphism had different effects on these traits in females and males (Table 5). The KDM5A SNP showed additive effects on body weight at 41 days, feed intake and gizzard percentage only in females. The T allele increased body weight and feed intake and decreased gizzard percentage in females (Fig. 2). In males, the KDM5A SNP showed dominance effects on body weight at 41 days, feed intake and carcass percentage. Therefore, males with C/C or C/T genotypes had higher body weight at 41 days, feed intake and carcass percentage than those with T/T. This SNP also had an additive effect on abdominal fat percentage and haematocrit percentage only in males, with the C allele increasing their percentages.

The diplotype × sex interaction affected body weight at 41 days, feed intake and the percentages of abdominal fat, carcass, drums and thighs, gizzard and haematocrit,

indicating different expression of the diplotypes depending on the sex of the chicken (Table 6). For all the diplotypes, males were heavier and ingested more feed than females, as expected, except for the H2H2 diplotype (Fig. 3). On the other hand, females with the H2H2 (ATAT) diplotype had higher abdominal fat and haematocrit percentages than males with the same diplotype. Females with the H1H1 (ACAC) and H2H2 diplotypes had higher carcass percentages than males with the same diplotype. Females with the H1H3 (ACGC) diplotype had higher gizzard percentages than males with the same diplotype. Males with the H1H2 and H1H3 diplotypes had higher drums and thighs percentages than females with the same diplotypes.

Discussion

Knowing the function of genes is essential for a better understanding of the chicken genetic architecture, especially regarding those genes that control traits of economic importance, such as performance and fatness traits. In the current study, 23 new SNPs were identified in two positional candidate genes to explore their role in controlling such quantitative traits.

We have identified IGF1 and KDM5A as positional candidate genes for QTL related to growth, carcass and fatness traits that were mapped in a specific region of GGA1 (ADL0234 and LEI0071) byNones et al. (2006). Besides that, these genes have important physiological functions, strongly associated with these traits. The IGF1 gene has been intensely studied because it is associated with growth, proliferation and differentiation of muscle, cartilage and bones in chickens and mammals (Schmid 1995; Duclos et al. 1999; Fisher et al. 2005). KDM5A is a promising candidate gene

for growth and fatness traits in chickens; however, no studies are available regarding its association with performance traits in chickens, until now.

In our population, 17 SNPs were detected in the IGF1 gene (14 new) and 16 were located in a region of 903 bp at the end of intron 3, which can be considered an SNP cluster. According to the dbSNP database (http://www.ncbi.nlm. nih.gov/snp/), there are nine SNPs in this region. Clark et al. (2003) defined SNP clusters as a collection of SNPs into a cluster such that no gap therein exceeds 50 kb in humans. Amos (2010) reported that SNPs are non-randomly distributed in the genome and are clustered in association with recombination hotspots.

Different studies identified polymorphisms in the chicken IGF1 gene (Zhou et al. 2005; Bennett et al. 2006; Bian et al. 2008; Sato et al. 2012), mainly in the promoter region, which was not analysed in this study. None of the SNPs described in these studies were identified in our population, probably due to differences in the genetic background of populations.

In the current study, the c.47673G>A IGF1 SNP was associated to body weight at 41 days. Associations of body weight with an IGF1 polymorphism were also reported by Zhou et al. (2005), Bennett et al. (2006) and Bian et al. (2008). Zhou et al. (2005) identified an additive effect of an IGF1 genotype on body weight. In the present study, the SNP associated to body weight could be in linkage disequilibrium with the SNPs described in the previous studies, as well as with the causal site.

Associations of the c.47673G>A IGF1 SNP with feed intake, percentages of abdominal fat and gizzard, and haematocrit were also identified in this study. Feed intake and abdominal fat are traits of great importance in poultry breeding programs, and gizzard is related with digestion and nutrient absorption. Amills et al.

(2003) found a suggestive association ($p \leq 0.05$) of an SNP in the promoter region of chicken IGF1 gene with feed efficiency, and they suggested that it might have been produced by linkage disequilibrium with another mutation located in the IGF1 locus or another linked gene.

IGF1 is an important growth hormone, mediating the anabolic and linear growth-promoting effect of the pituitary GH protein. Most IGF1 is secreted by the liver and is transported to other tissues (Laron 2001). Tomas et al. (1998) showed that exogenous IGF1 infusion in chickens, with diverse genetic backgrounds, enhanced growth rates and feed efficiency, and decreased the carcass fat content. Increased circulating IGF1 concentrations decrease insulin levels and acts in the lipogenic activity, thereby, reducing fatness. The role of IGF1 in the regulation of erythropoiesis is not completely understood, but Miyagawa et al. (2000) reported that IGF1 stimulated wide stages of erythroid development and that IGF1 plays an important role in the regulation of human erythropoiesis. Therefore, there is evidence that the IGF1 gene is related to the reduction in fat deposition and enhancement of feed efficiency in chickens, and also to erythropoiesis stimulation in mammals.

Nine novel SNPs were identified in the KDM5A gene in different regions (Table 1). The effect of the c.34208C>T KDM5A SNP on performance and carcass traits was influenced by the sex of the chicken in the present study. The KDM5A protein is a histone demethylase, therefore, this gene is also related to cellular proliferation and differentiation in mammals. Methylation of histones is a modification that regulates chromatin structure and transcription activation, involving epigenetic mechanisms (Christensen et al. 2007). Recently, Stratmann and Haendler (2011) reported that KDM5A regulates the expression of the progesterone receptor in humans.

Progesterone participates in the regulation of several functions in chickens, such as ovulation, gonadal differentiation and sexual behaviour (Camacho-Arroyo et al. 2007). DiTacchio et al. (2011) also reported that this gene has an important role in circadian clock function. The effects of mutations in this gene in birds could be similar to those already observed in mammals, explaining, at least partially, why the genotypes of this polymorphism had different effects on performance and carcass traits in females and males.

Peng et al. (2009) studied the expression of 12 genes involved in histone modifications during the proliferation and differentiation of skeletal muscles in pig embryos, including the KDM5A gene. This gene was differentially expressed in the musculature of embryos at different developmental stages, indicating that it could be a good candidate gene for growth traits.

The KDM5A SNP showed an additive effect on body weight at 41 days, feed intake and gizzard percentage in females, and a dominance effect on body weight, feed intake, and carcass percentage in males. Due to its distinct gene action in males and females, the KDM5A gene could be involved in differences in performance traits between sexes.

There were remarkable differences between C/C and T/T chickens for the c.34208C>T KDM5A SNP. In the two families, the T allele was present only in the broiler parent (the sire), whereas the C allele was found in both the broiler and the layer parent. The T/T females had similar weight and ingested the same quantity of feed as T/T males, but they had higher abdominal fat and lower gizzard percentage than T/T males. On the other hand, C/C females had lower weight and feed intake but higher gizzard percentage than T/T males. These results are supported by the

findings of Gaya et al. (2006), who estimated the genetic correlations between performance and carcass traits in a broiler line. Gizzard weight did not seem to be related to feed intake or abdominal fat content based on the estimates of genetic correlation between each one of these traits and gizzard weight (0.03 and 0.09, respectively).

A pair of haplotypes is called a diplotype, and the diplotype approach helps the dissection of SNP effects, especially if the SNPs are linked. A strong association of the KDM5A SNP × sex interaction ($p=0.0001$) and the diplotype × sex interaction ($p<0.0001$) with abdominal fat percentage was observed. In a broiler line, the heritability estimate of abdominal fat content was 0.53±0.04, suggesting that this trait would respond to selection (Gaya et al. 2006). The c.47673G>A IGF1 and c.34208C>T KDM5A SNPs could be included in SNP genotyping assays for genome-wide association studies of fat deposition in chickens.

In the current study, A/A (c.47673G>A IGF1) and T/T females (c.34208C>T KDM5A) had higher abdominal fat percentages than those with other genotypes; accordingly, ATAT (H2H2) females had higher abdominal fat percentages than those with other diplotypes. An opposite result was observed for A/A (c.47673G>A IGF1) and T/T males (c.34208C>T KDM5A), and the respective diplotype H2H2, with low abdominal fat percentages. Further, the diplotype results for feed intake (H1H3 females) and percentage of gizzard (H2H2 females) were also in accordance with the single SNP results.

The H2H2 diplotype helped to explain the difference in abdominal fat and haematocrit percentages between males and females. However, for body weight, feed intake and percentage of drums and thighs, similar values were observed for males

and females with this diplotype. Moreover, H2H2 females had low gizzard %. The H1H1 (ACAC) and H2H2 diplotypes helped to explain the difference in carcass percentages between males and females. The H2H2 diplotype could be potentially used in SNP panels to select male chickens with low fat percentages in breeding programs.

The percentage of drums and thighs was associated with the diplotype, but not with either one of the genotypes. This is an advantage of the haplotype-based approach, as reported by Morris and Kaplan (2002). Only H1H2 (ACAT) and H1H3 (ACGC) males had higher drums and thighs percentages than females.

The physical distance between IGF1 and KDM5A genes is 4.9 Mb (NCBI Map Viewer, Gallus_gallus-4.0 reference assembly), and the genetic distance between the c.47673G>A IGF1 and c.34208C>T KDM5A SNPs was estimated to be 14.3 cM, according to the QTL fine mapping results (Online Resource 2). Therefore, the SNPs studied here may not be the causal mutations, but they could be in linkage disequilibrium with the causal mutations. We selected these two genes, among the 107 genes in the LEI0146–LEI0174 interval, because they have important functions related to the traits evaluated in this study, whereas the functions of many of the other genes were not well known or, apparently, not related to the QTL mapped in this region.

Our findings, together with the biological functions and positions of the IGF1 and KDM5A genes in a QTL region in GGA1, suggest that these genes and their diplotypes could, at least partially, explain the QTL previously mapped in the region. However, validation of these associations in commercial populations is needed before their practical application. They might be used in the future as markers in assisted

selection to increase production efficiency in chickens, particularly for abdominal fat, because of the strong association between this trait and the KDM5A SNP and the diplotype. The KDM5A gene should be further investigated in the chicken, because it is involved with cellular proliferation and differentiation, and embryonic development in other species, and it may be involved in differences in performance traits between males and females.

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Tab.1

SNPs identified in the chicken IGF1 and KDM5A genes and their allele frequencies in the parental generation (n=12 P individuals)

SNP[a]	Location	NCBI identifier (ss number)	Allele frequency (allele)	
IGF1 gene				
c.38915C>G	Intron 3	475875648	0.29 (C)	0.71 (G)
c.47044T>C	Intron 3	475875649	0.79 (T)	0.21 (C)
c.47545A>G	Intron 3	475875657	0.25 (A)	0.75 (G)
c.47673G>A[b]	Intron 3	475875658	0.21 (G)	0.79 (A)
KDM5A gene				
c.24441A>G	Exon 14	475875662	0.62 (A)	0.38 (G)
c.24471G>A	Exon 14	475875663	0.67 (G)	0.33 (A)
c.24508A>G	Intron 14	475875664	0.88 (A)	0.12 (G)
c.24528C>G	Intron 14	475875665	0.88 (C)	0.12 (G)
c.34208C>T[b]	Exon 21	475875666	0.79 (C)	0.21 (T)
c.34347G>T	Intron 21	475875667	0.58 (G)	0.42 (T)
c.34444C>T	Intron 21	475875668	0.54 (C)	0.46 (T)
c.40411T>G	Intron 25	475875669	0.50 (T)	0.50 (G)
c.45957C>T	Exon 28	475875670	0.54 (C)	0.46 (T)

[a] GenBank accession no. NC_006088.3 based on Gallus_gallus-4.0, from the ATG translation initiation site

[b] SNPs selected for the genotyping of F2 chickens

Tab.2

Frequency of haplotypes and diplotypes inferred based on the SNPs in the chicken IGF1 and KDM5A genes (n=165 F_2 individuals)

Haplotype	Diplotype	c.47673G > A (*IGF1*)	c.34208C > T (*KDM5A*)	Frequency (*n*)
H1 (AC)		A	C	0.53 (176)
H2 (AT)		A	T	0.36 (117)
H3 (GC)		G	C	0.10 (33)
H4 (GT)		G	T	0.01 (4)
	H1H1 (ACAC)	A/A	C/C	0.20 (33)
	H1H2 (ACAT)	A/A	C/T	0.44 (73)
	H1H3 (ACGC)	A/G	C/C	0.20 (33)
	H2H2 (ATAT)	A/A	T/T	0.13 (22)
	H1H4 (ACGT)[a]	A/G	C/T	0.03 (4)

[a] The diplotype H1H4 was excluded from the association analysis

Tab.3

Descriptive statistics for the traits evaluated in this study (n=165 F2 individuals)

Traits	Mean	SD	Min	Max
Body weight at 35 days (g)	795	119	536	1,071
Body weight at 41 days (g)	1,014	168	578	1,398
Body weight at 42 days (g)	973	165	549	1,374
Feed intake from 35 to 41 days (g)	619	142	318	1,176
Feed conversion from 35 to 41 days (g feed/g gain)	2.91	0.80	2.07	7.55
Abdominal fat %	1.37	0.58	0.11	3.49
Carcass %	64.73	2.02	57.72	75.34
Breast %	16.36	1.04	13.92	20.00
Shank %	4.11	0.40	3.13	4.98
Drums and thighs %	21.30	1.33	18.31	31.40
Heart %	0.68	0.14	0.32	1.20
Lungs %	0.85	0.17	0.44	1.36
Liver %	2.71	0.32	1.97	3.60
Gizzard %	2.46	0.39	1.74	3.55
Haematocrit %	28.70	3.16	20.00	42.00

Tab.4

p-values for hatch, family and sex effects, and the associations between c.47673G>A IGF1 SNP and performance, fatness and carcass traits

Traits	Hatch	Family	Sex	Genotype	G × S
Body weight at 41 days (g)	0.0192	0.0204	<0.0001	0.0325	NS
Feed intake from 35 to 41 days (g)	<0.0001	NS	<0.0001	0.0482	0.0113
Abdominal fat %	0.0121	<0.0001	NS	NS	0.0032
Gizzard %	<0.0001	0.0002	0.0133	0.0044	0.0192
Haematocrit %	0.0008	NS	NS	0.0493	NS

G×S = genotype by sex interaction

Tab.5

p-values for hatch, family and sex effects, and the associations between c.34208C>T KDM5A SNP and performance, fatness and carcass traits

Traits	Hatch	Family	Sex	Genotype	G × S
Body weight at 41 days (g)	0.0196	0.0001	<0.0001	0.0193	0.0034
Feed intake from 35 to 41 days (g)	<0.0001	0.0003	<0.0001	0.0087	0.0021
Abdominal fat %	0.0117	<0.0001	<0.0001	0.0480	0.0001
Carcass %	0.0002	NS	NS	NS	0.0451
Gizzard %	0.0008	0.0047	NS	NS	0.0399
Haematocrit %	0.0007	NS	NS	NS	0.0169

G×S=genotype by sex interaction

Tab.6

p-values for hatch, family and sex effects, and the associations between IGF1 and KDM5A diplotypes and performance, fatness and carcass traits

Traits	Hatch	Family	Sex	Diplotype	D × S
Body weight at 41 days (g)	0.0291	0.0094	<0.0001	0.0356	0.0095
Feed intake from 35 to 41 days (g)	<0.0001	0.0110	<0.0001	0.0136	0.0020
Abdominal fat %	0.0072	<0.0001	0.0001	0.0305	<0.0001
Carcass %	<0.0001	NS	NS	NS	0.0021
Drums and thighs %	<0.0001	NS	0.0071	NS	0.0450
Gizzard %	0.0002	<0.0001	NS	0.0241	0.0124
Haematocrit %	0.0003	NS	NS	NS	0.0378

D×S=diplotype by sex interaction

Fig.1

Effects of the c.47673G>A IGF1 SNP genotype × sex interaction on feed intake from 35 to 41 days, percentages of abdominal fat and gizzard

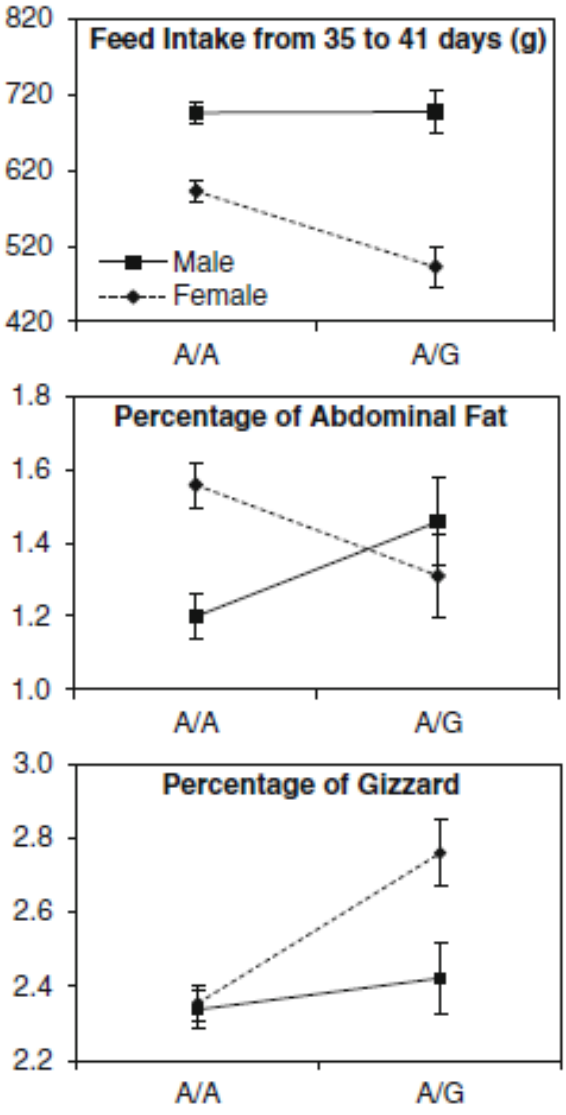

Fig.2

Effects of the c.34208C>T KDM5A SNP genotype × sex interaction on body weight at 41 days, feed intake from 35 to 41 days, percentages of abdominal fat, carcass, gizzard and haematocrit in males and females. Additive (a) and dominance (d) effects at *p<0.05, **p<0.01 and ***p<0.001

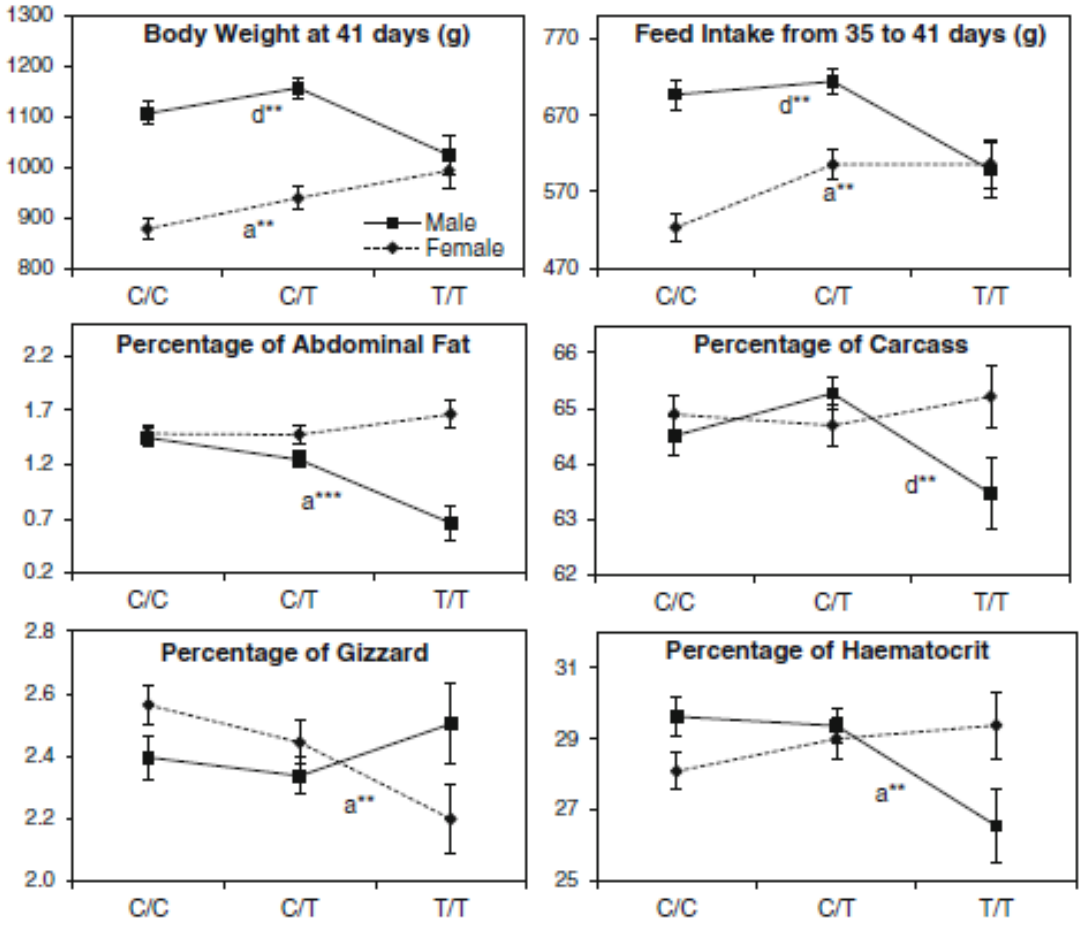

Fig.3

Effects of the diplotype × sex interaction on body weight at 41 days, feed intake from 35 to 41 days, percentages of abdominal fat, carcass, gizzard, drums and thighs, and haematocrit in males and females. Differences between sexes at *p<0.05, **p<0.01 and ***p<0.001