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Crossbreeding analyses and polymorphic associations of gallinacin genes with growth traits in chickens

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ABSTRACT

A simple crossbreeding experiment between Fayoumi (F) and Rhode Island Red (R) and their F_1 of ½R½F and ½F½R crosses was conducted. A total number of 480 chicks produced from four genetic groups was used to estimate direct additive genetic effects (G^I), maternal effects (G^M) and direct heterosis (H^I) for growth traits by using generalized least squares procedure. The studied traits were body weights at hatch, 2, 4, 6, 8 and 10 weeks of age and daily gains during the intervals of 0-2, 2-4, 4-6, 6-8 and 8-10 weeks of age. Candidate gallinacin genes of *GAL 2, GAL 3, GAL 4* and *GAL 5* were genotyped using PCR-RFLP, associating the SNP with body weights and gains.

Direct additive effects were mostly significantly ($P \leq 0.01$) in favour of R breed by 7.4 to 57.9 g for body weights and by 0.8 to 1.8 g for daily gains. The estimates of maternal effect were mostly significantly ($P^{<}0.01$) in favour of R breed and ranging from 0.8 to 25.1 g for body weights and 0.5 to 2.3 g for daily gains. All the estimates for direct heterosis were positive and significant ($P^{<}0.01$) and ranged from 0.15 to 35.2 g for body weights and 0.3 to 1.5 g for daily gains.

The *GAL 2* gene was one homozygous genotype in the four genetic groups, while in *GAL 3, GAL 4* and *GAL* 5 genes only one homozygous genotype in Fayoumi breed was observed. The genotypes of *Gal* 3 gene had significant associations with most body weights and gains ($p < 0.05$) in R, ½R½F and ½F½R genetic groups. The genotypes of *GAL 4* and *GAL* 5 genes were associated significantly with most body weights and daily gains during the intervals of 0-2 and 2-4 weeks of age in½R½F and ½F½R genetic groups. In practice, the molecular associations obtained for *GAL 3, GAL 4* and *GAL* 5 genes could be used in marker assisted selection programs to improve growth traits in chickens.

1. Introduction

Crossbreeding is one of the most approaches that could be used to improve growth traits in chickens. In Egypt, some studies (e.g Iraqi et al., 2011; 2013; Amin et al., 2017; Radwan and Mahrous, 2018) showed significant heterotic and direct and maternal additive effects on body weights and gains of chicks at different ages. To attain more genetic gains in crossbreeding program, the molecular technologies are used as a new horizon for identification of molecular markers to be used in marker-assisted selection programs (Wakchaure et al., 2015). One of the molecular approaches that are useful in this concept is the detection of associations between candidate genes and growth traits in poultry. In this concept, several studies have reported that there were significant associations between *IFNG*, iNOS, *IL-2* and *IFN-*γ candidate genes and body weights and gains in poultry (Ye et al., 2006; Ahmed, 2010; Cahyadi et al., 2013; Lim et al., 2013; Zhao et al., 2015; Molee et al., 2016; Liu et al., 2018; Yi et al., 2018). Seo et al. (2013) reported that chicks of CC genotype of *TSH-β* gene were significantly heavier than chicks of GG genotype in Cornish chickens ($p < 0.05$). Anh et al. (2015) found that chicks of AG and GG genotypes in *GH* gene had higher body weights and daily gains ($p < 0.01$) than chicks of AA genotype. Zhao et al. (2015) with *IGFBP-2* gene reported that chicks of AA genotype had significantly heavier body weights at hatch and 12 weeks of age than that of AB genotype ($p < 0.05$). Horinouchi et al. (2018) with Cholecystokinin type A receptor gene found that daily gain of AA genotype was significantly higher than that of AC and CC genotypes in Miyazaki Jitokko chickens. Jin et al. (2018) showed that chicks of TT genotype of *Pit-1* gene had significant heavier body weight at 70 day

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than chicks of CT and CC genotypes, while AA genotype recorded the heaviest body weight than AT and TT genotypes. Jin et al. (2018) with SNPs of *Pit-1* gene reported that GG genotype had significantly higher body gain than that of AG genotype and TT genotype had also significantly higher gain than those of CT and CC genotypes. Thinh et al. (2019) reported that chicks of GG genotype of *GH* gene had higher body weights and daily gains than other genotypes. For studying the immune response in terms of gallinacin candidate genes and their associations with growth traits in chickens, gallinacin genes of 1 to 13 have been mapped and these gallinacins located on chromosome 3 are involved in the innate immune system response against microbial infections (Zhou and Lamont, 2003; Ganz, 2003; Xiao et al., 2004). Unfortunately, investigations concerning associations of gallinacin genes with growth in poultry are scarce. In an attempt to investigate the previous concepts, a crossbreeding experiment between Fayoumi (F) and Rhode Island Red (R) was performed to estimate the crossbreeding effects in terms of direct, maternal and heterotic effects on body weights and gains and to detect the SNP associations of four immunity-related gallinacin genes with body weights and gains in chickens. Some studies have reported that there were polymorphic associations of immune-related genes with growth performance in some crosses and commercial chicken lines (e.g. Ahmed, 2010; Cahyadi et al., 2013; Molee et al., 2016).

2. Materials and methods

2.1. Crossbreeding plan performed

Simple crossbreeding experiment was performed between Fayoumi (F) and Rhode Island Red (R) to get ½R½F cross and the reciprocal ½F½R cross. The experiment was started in November 2016 in the Poultry Farm, Department of Animal Production, Faculty of Agriculture, Benha University, Egypt. Pullets of F and R breeds used in the experiment were chosen randomly and came from El-Takamoly chicken project, Alazab, Fayoum governorate, Egypt and housed in battery cages. The F breed is a very old breed of chicken originating in Fayoum governorate, south west of Cairo and west of the Nile, Egypt. The R breed is an American dual purpose, developed in Rhode Island and Massachusetts in the mid 18th century. Rhode Island Red chickens are good egg layers but can be raised for both meat and egg production. Birds of this breed are highly popular mainly for their hardiness and egg laying abilities.

Pullets of F and R breeds were randomly divided into two groups (60 hens/breed). The first group was mated with 10 cocks from the same breed while the second group was mated with 10 cocks from the other breed using artificial insemination. Consequently, the pedigreed eggs from each individual breeding pen of the four mating groups were collected daily for seven days and were hatched in the hatchery. On hatching day, 120 chicks (12 chicks from every sire) were randomly chosen from each genetic group (Table 1) and the chicks were wing banded and then transferred immediately to the incubating rooms in January 2017. Chicks from each genetic group were reared in battery cages under continuous fluorescent lighting (10 watt/m^2), in an isolation room until 10 weeks of age. The chicks were kept under similar hygienic and environmental conditions, vaccinated against Newcastle and Gumboro disease and provided un-medicated corn soybean-based meal diet (not containing antibiotics, coccidiostats, or growth promoters). The chicks were provided water ad *libitum*. Chicks produced from all genetic groups were fed ad *libitum* during growing period (from hatch up to 10 weeks of age) on diet containing 21% protein and 2700 kcal/kg. Minerals and vitamins were adequately supplied to cover the chicks' requirements according to NRC (1994).

Table 1
Number of chicks produced and their sires and dams in different genetic groups.

Genetic group of chicks[†]	No. of chicks	Genetic group of sires	No. of sires	Genetic group of dams	No. of dams
F	120	F	10	F	60
R	120	R	10	R	60
½R½F	120	R	10	F	60
½F½R	120	F	10	R	60
Total	480		40		240

[†] F = Fayoumi breed; R = Rhode Island Red breed.

2.2. Model for estimating the crossbreeding genetic effects

Data set of body weights at hatch, 2, 4, 6, 8 and 10 weeks of age and daily weight gains during the intervals from hatch to 2, 2 to 4, 4 to 6, 6 to 8 and 8 to 10 weeks of age were analyzed across all genetic groups using the following animal model (Groeneveld et al., 2010):

$$y = Xb + Z_a u_a + e$$

Where y = the vector of observations of body weights and daily gains; b = the vector of fixed effects of genetic groups (four levels), sex (males and females); X and Z_a = incidence matrices corresponding to fixed and additive random effects of the birds ($\mathbf{u_a}$), respectively; e = the residual error. According to the theory of Dickerson (1992), the solutions of the crossbreeding genetic group effects were obtained using the procedure of generalized least squares (GLS) and applying the PEST software (Groeneveld, 2006).

The parameters representing differences between the breeds in terms of direct additive genetic effects (G^I), maternal effects (G^M) and direct heterosis (H^I) were estimated. The coefficients relating genetic crossbreeding parameters to the means of the genetic groups (Table 2) were estimated according to Dickerson (1992) and Wolf (1996). Thus, we have three parameters to be estimated (the vector of estimable crossbreeding genetic effects called **b**-vector):

$$\mathbf{b}[(G_F^I - G_R^I)G^M H^I]$$

The solutions of **b** were calculated by the method of generalized least squares (GLS) using the following equation:

$$\hat{\mathbf{b}} = (\mathbf{X}'\mathbf{V}^{-}\mathbf{X})^{-1}\mathbf{X}'\mathbf{V}^{-}\mathbf{y}$$

Where **X** was the matrix of coefficients of estimable crossbreeding effects, $\mathbf{V}^{-}$ = the inverse of generalized variance–covariance matrix error, with the variance–covariance matrix of the estimate of b being,

$$Var(\hat{\mathbf{b}}) = (\mathbf{X}'\mathbf{V}^{-}\mathbf{X})^{-1}$$

2.3. Blood sampling and DNA extraction

Chicks belonging to four genetic groups (24 chicks from each group of F, R, ½R½F and ½F½R) were used. The laboratorial analyses for molecular biology were carried out in the Labs of Genetics Department, Faculty of Agriculture, Benha University, Egypt, and Avian Pathology Section, Department of Veterinary Medicine, University of Bari, Italy since April 2017 to September 2017. Approximately 3-5 ml venous

Table 2
Genetic groups of chicks with their sires and dams and coefficients of the matrix relating means of the genetic groups with crossbreeding parameters.

Genetic group			Mean	Coefficients of the matrix				
Chick	Sire	Dam		G_F^P	G_R^P	G_F^M	G_R^M	H^I
F	F	F	1	1	0	1	0	0
R	R	R	1	0	1	0	1	0
½R½F	R	F	1	0.5	0.5	0.5	0.5	1
½F½R	F	R	1	0.5	0.5	0.5	0.5	1

G^PF and G^PR = Direct additive genetic effects for Fayoumi breed and Rhode Island Red breed; G^MF and G^MR = maternal effects for Fayoumi breed and Rhode Island Red breed, respectively; H^I = Direct heterosis.

Table 3
Primer sequence and PCR-RFLP assay conditions for genotyping SNPs of gallinacin genes in chromosome 3 using restriction enzymes.

Gene (GenBank accession no.)	Primer sequences (forward/reverse)	PCR Product size (bp)	Annealing Temp per Time (°C/s)[1]	Restriction Enzyme
GAL 2 (AY621317)	5'-GGCACAAAGGGTAAAGTATGG -3' 5'- GAGGGGTCTTCTTGCTGCTGA -3'	583	55.1/30	*HpyCH4IV*
GAL 3 (AY621318)	5'- GCACCACAAGAAGCCCAGGAA -3' 5'- AACTCCAGCCCTTACCACTCA -3'	664	57.3/30	*AvaI*
GAL 4 (AY621319)	5'- TGGGGATCTTAGAGGTCTTTT -3' 5'- TTTTCCACAGATATTGCTTTT -3'	600	51.0/30	*AluI*
GAL 5 (AY621320)	5'CTCCCAGCAAGAAAGGAACCTG -3' 5'-CACAGTCCTGGGGTAATCCTCG-3'	623	59.0/30	*HinfI*

[1] PCR annealing temperature and time for primer

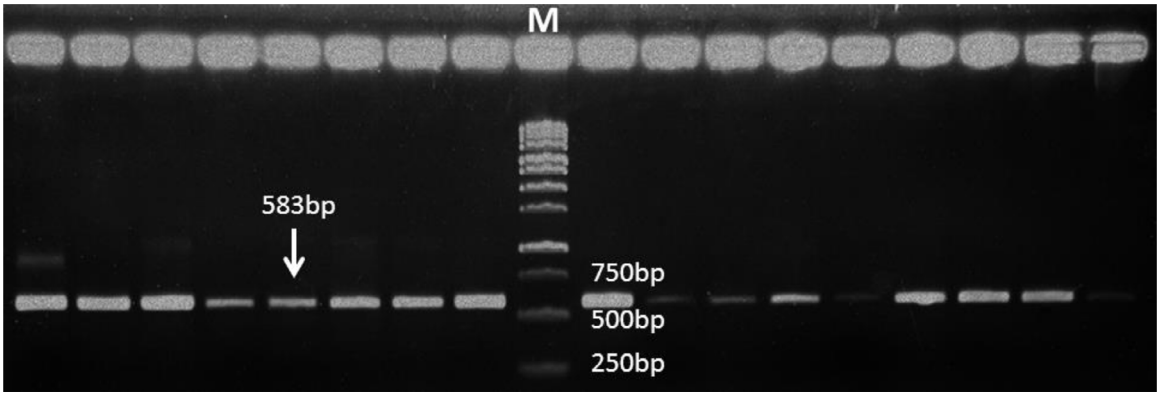

Fig. 1. Images of gel documentation system for amplifying PCR products of *GAL 2, GAL 3, GAL 4* and *GAL 5* genes in the studied genetic group of chickens, Fig. 1(a). A 583 bp PCR products of *GAL 2* gene in F, R, ½R½F and ½F½R chickens. Fig. 1(b). A 664 bp PCR products of *GAL 3* gene in F, R, ½R½F and ½F½R chickens. Figure 1(c). A 600 bp PCR products of *GAL 4* gene in F, R, ½R½F and ½F½R chickens. Fig. 1(d). A 623 bp PCR products of *GAL 5* gene in F, R, ½R½F and ½F½R chickens.

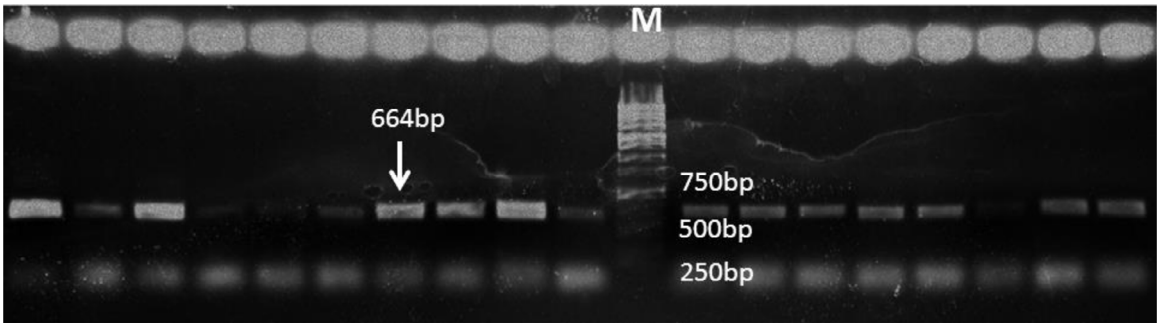

Fig. 1. (*continued*)

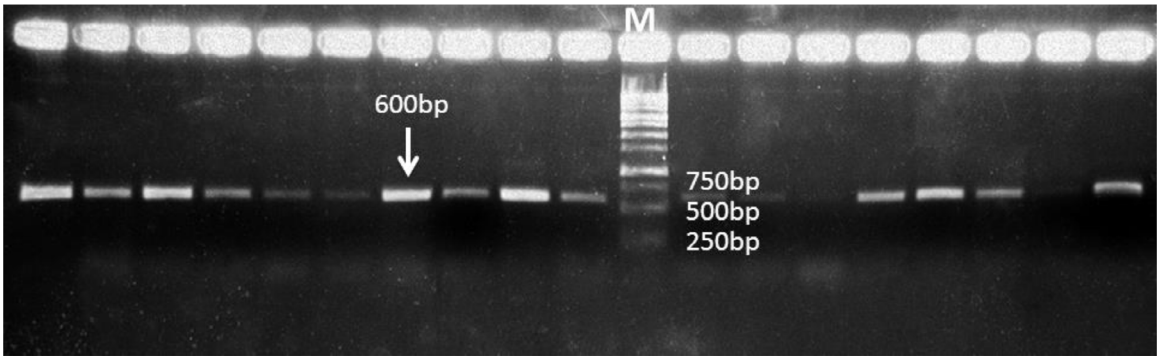

Fig. 1. (*continued*)

blood sample per chick was collected from the wing vein by a 2-gauge 1.5-injection needle into tubes containing EDTA as anticoagulant. Genomic DNA was extracted from Whole Blood Genomic DNA Purification Mini Kit (Cat No. #K0781, Thermo Scientific).

2.4. Polymorphic assessment using PCR-RFLP

Preliminarily, the primer sequences were assessed *insilico* (http://insilico.ehu.es/PCR/) and each gene of *GAL 2, GAL 3, GAL 4* and *GAL 5*

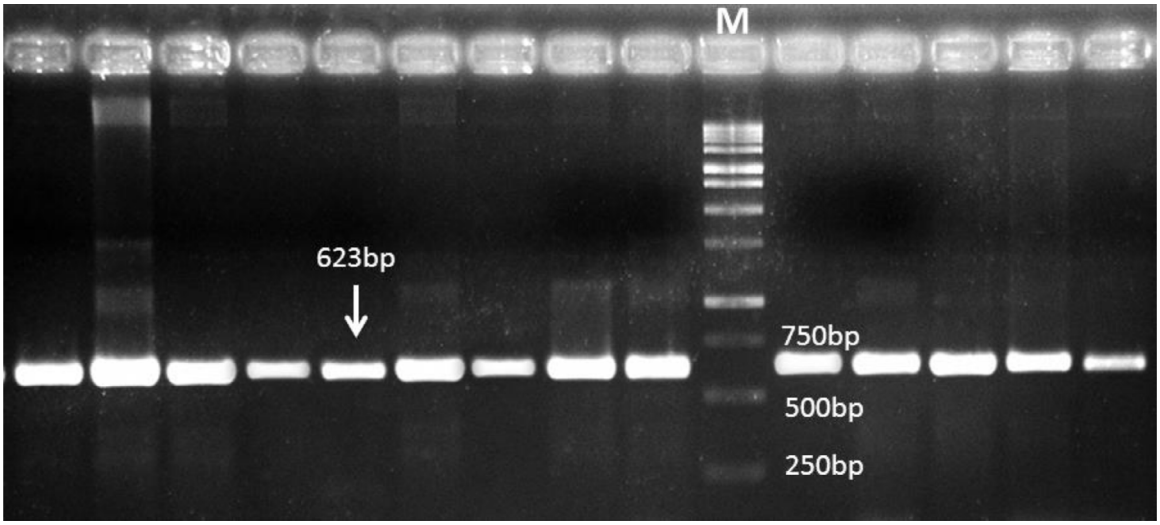

Fig. 1. (*continued*)

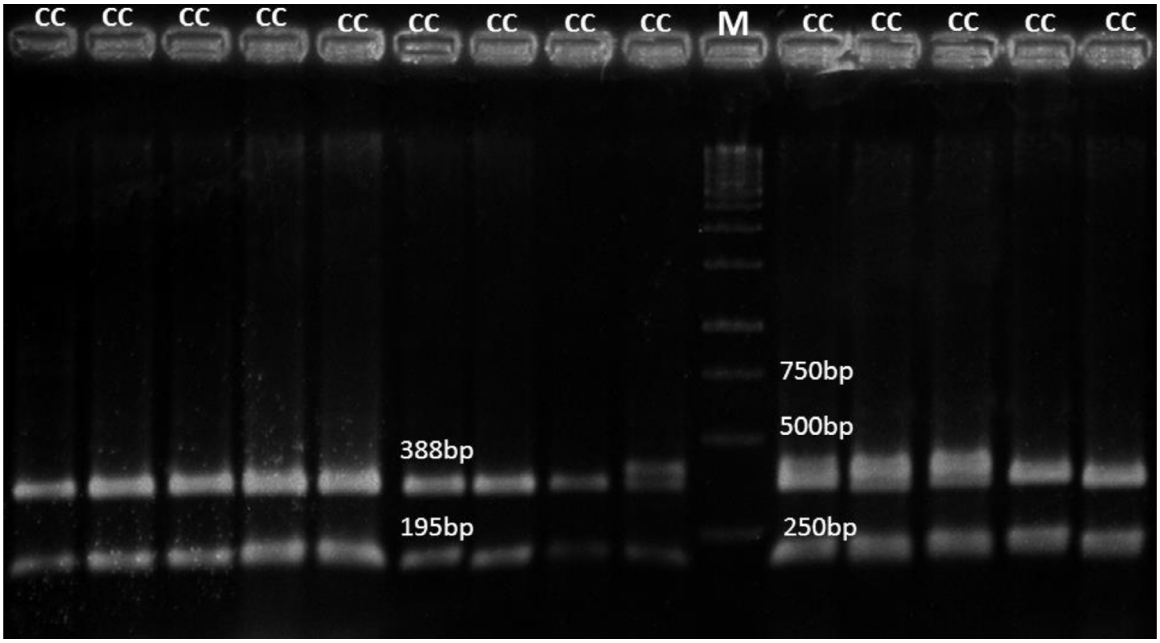

Fig. 2. Images of gel documentation system for PCR fragments of gallinacin genes digested with the proper restriction enzymes in the studied genetic group of chickens, Fig. 2(a). RFLP analysis specific Fragments for *GAL 2* gene digested by *HpyCH4IV* restriction enzyme in F, R, ½R½F and ½F½R chickens., Fig. 2(b). RFLP analysis specific fragments for *GAL 3* gene digested by *Ava1* restriction enzyme in F, R, ½R½F and ½F½R chickens. Fig. 2(c). RFLP analysis specific fragments for *GAL 4* gene digested by *Alu1* restriction enzyme in F, R, ½R½F and ½F½R chickens. Fig. 2(d). RFLP analysis specific fragments for *GAL 5* gene digested by *Hinf1* restriction enzyme in F, R, ½R½F and ½F½R chickens.

(each coding for a respective avian beta-defensin) was characterized by amplifying a portion using the proper primer pair listed in Table 3 (Hasenstein *et al.*, 2006). On chromosome 3, PCR amplifications were carried out in 50 *μl* reaction mixture composed of 4 *μl* genomic DNA (100 ng/*μl*) as a template, 50 pmol of each primer, 2.5 mM dNTP' (dATP, dCTP, dTTP and dGTP; ABgene, Surrey, UK), 10X PCR buffer, 25 mM $MgCl_2$, and 2.5 unit *Taq* DNA polymerase. The PCR-RFLP reactions were carried out in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, California, USA) with the following cycling: an initial denaturation step at 94˚C for 3 min, followed by 39 cycles at 93˚C for 45 s, at the optimum annealing temperature for 30 seconds and final elongation step for 60 seconds (72˚C). After 39 cycles, amplification was followed by 10 min of elongation (72˚C) and cooling down to 4˚C and storage. The PCR products (Fig. 1) were electrophoresed on 1.5 % agarose gel stained with ethidium bromide and visualized by a UV transilluminator. The PCR product of each gene was digested with the proper restriction enzyme (Fig. 2), Each reaction consisted of a 25 *μl* mix including 0.5 μl(10u/*μl*) of restriction enzyme (Fermentas), 2.5*μl* of 10x NE Buffer, 5 *μl* of PCR product, 0.1mg/*ml* acetylated Bovine serum albumin (BSA), and 16.75 *μl* of sterile dH_2O.The digested fragments were visualized by electrophoresis on 2.5 % agarose gel at 120 V in 1x TAE. The 250 bp DNA step ladder (Promega) was included in each run. After electrophoresis, the gel was stained with ethidium bromide 0.5μg/ml. Fragments were visualized by using a UV transilluminator and documented in Gel Doc^{TM}XR^{+}(BIO-RAD).

2.5. Model for detecting the polymorphic associations

For detecting the polymorphic associations between the genotypes

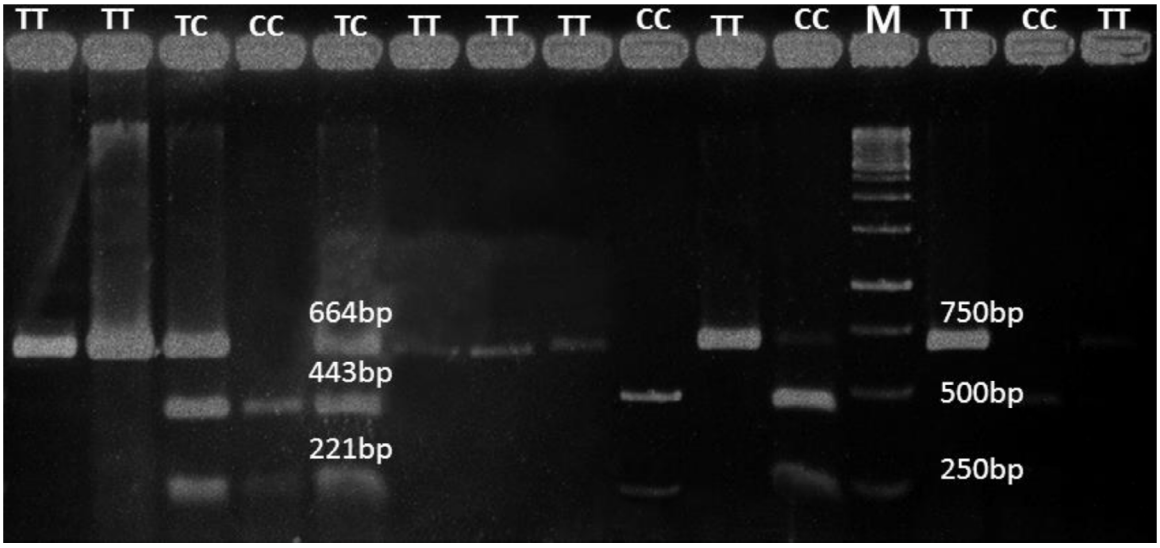

Fig. 2. (*continued*)

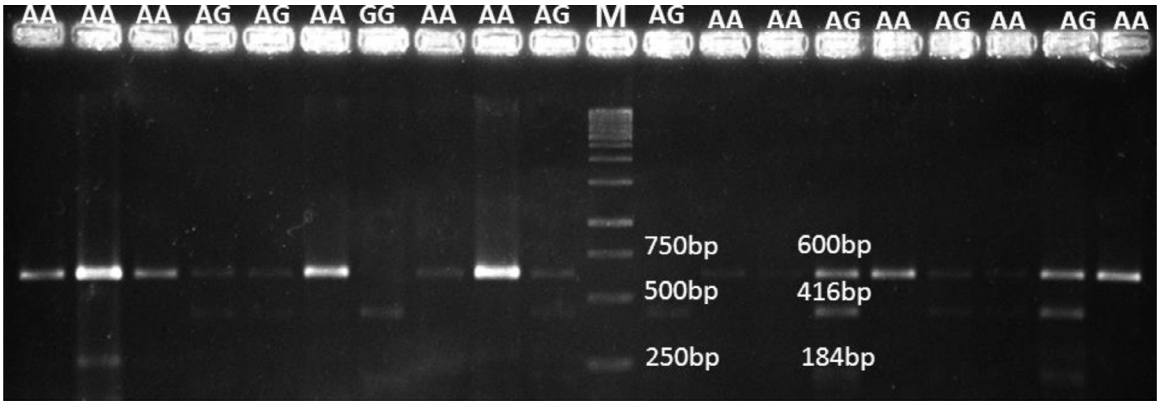

Fig. 2. (*continued*)

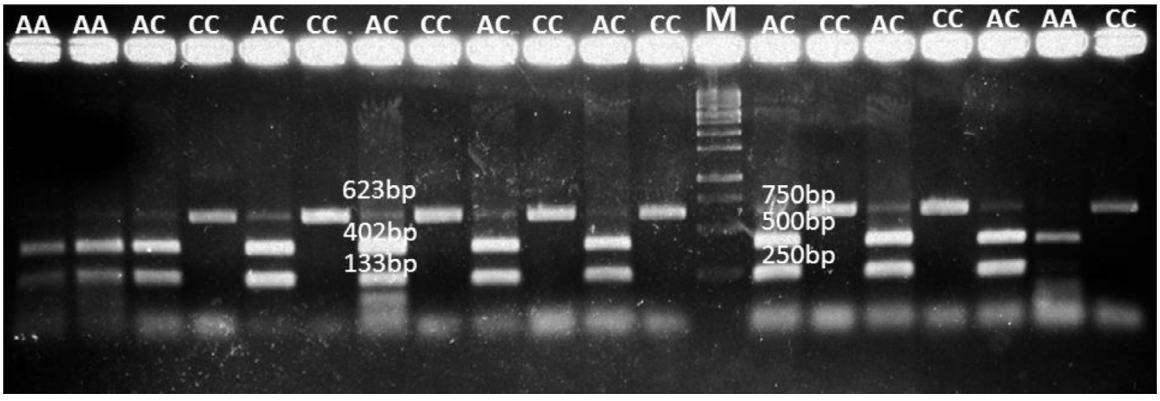

Fig. 2. (*continued*)

of gallinacin genes and body weights and gains in each genetic group separately, the effects of SNPs genotypes of gallinacin genes on these traits were estimated using the PEST software (Groeneveld, 2006) and applying the animal model after adding the i^{th} genotype of gallinacin gene (three genotypes). The solutions of genotypes of gallinacin genes were calculated by the method of generalized least squares (GLS) described previously.

3. Results and discussion

3.1. Genetic groups comparisons

The generalized least square means (GLM) of body weights and gains in each genetic group of the chicks are presented in Table 4. The GLM for body weights and daily gains in R breed were significantly higher than F breed, ½R½F cross were significantly higher than the reciprocal ½F½R cross at 0, 2, 4, 6, 8 and 10 weeks of age and daily gains during the intervals of 0-2 and 2-4 weeks of age. Mahmoud and El-Full (2014) reported that R breed had favourable daily gains during

Table 4
Generalized least-square means (GLM) and their standard errors (SE) for body weights (BW) and daily gains as affected by genetic groups of the chicks.

Trait[+]	F		R		½R½F		½F½R	
	GLM	SE	GLM	SE	GLM	SE	GLM	SE
BW, biweekly (g):								
BW0	32^{b}	0.4	35^{a}	0.4	33^{b}	0.4	35^{a}	0.4
BW2	104^{d}	2.0	139^{a}	2.4	110^{c}	1.8	131^{b}	2.0
BW4	199^{d}	4.6	281^{a}	4.7	214^{c}	4.4	264^{b}	4.5
BW6	363^{d}	7.6	477^{a}	8.9	433^{c}	6.7	462^{b}	7.3
BW8	575^{d}	8.8	725^{a}	10.3	656^{c}	7.7	706^{b}	8.4
BW10	802^{d}	10.14	996^{a}	11.91	884^{c}	8.90	956^{b}	9.70
DG, biweekly interval (g):								
DG0-2	5.0^{c}	0.16	7.7^{a}	0.17	5.3^{c}	0.33	6.9^{b}	0.34
DG2-4	6.6^{c}	0.39	10.5^{a}	0.40	7.4^{b}	0.74	9.8^{a}	0.76
DG4-6	11.7^{b}	0.78	15.5^{a}	0.82	14.2^{ab}	1.42	15.2^{a}	1.37
DG6-8	14.9^{b}	0.70	17.9^{a}	0.71	16.0^{ab}	1.19	16.9^{a}	1.21
DG8-10	16.1^{b}	0.46	19.5^{a}	0.48	16.2^{b}	0.79	17.4^{ab}	0.80

[+] BW= Body weight; DG= Daily body gain. Different letters in the same row indicate significant differences at $P<0.05$.

the intervals of 8-12 and 0-12 weeks of age. Radwan and Mahrous (2018) in Sinai dual purpose chickens (S), Rhode Island Red (R) and Fayoumi breeds and their crosses found that S × R cross and its reciprocal R × S had heavier body weights and daily gains than other genetic groups.

3.2. Direct additive effects (G^I), Maternal effects (G^M) and Direct heterotic effects (H^I)

The estimable generalized least square solutions of G^I were significantly in favour of R breed by 7.4, 14.7, 37.2, 49.9 and 57.9 g for body weights at 2, 4, 6, 8 and 10 weeks of age ($P<0.01$), respectively and ranged from 0.8 to 3.1 g for daily gains (Table 5), i.e. percentages of G^I were in favour of R breed by 1.8 to 9.2% for body weights and from 4.2 to 17.3% for daily gains. Iraqi et al. (2011) found that estimates of G^I were mostly significant in favour of Matrouh dual purpose chickens by 2.2 to 9.3% for body weights and 8.4 to 10.4% for daily gains relative to Inshas dual purpose chickens ($p<0.01$). Iraqi et al. (2013) showed that estimates of G^I were significantly in favor of White Leghorn chickens and ranged from 2.5 to 14.2 % for body weights and from 6.7 to 21.1% for daily gains. Also, Radwan and Mahrous (2018) found that the estimates of G^I for body weights and weight gains at different ages were in favour of Fayoumi breed compared to Sinai and Rhode Island Red.

The estimable G^M were significantly in favour of R breed by 0.8, 10.4, 26.4, 14.3, 25.1 and 22.0 g for body weights at hatch, 2, 4, 6, 8 and 10 weeks of age, and by 0.5, 2.3, 0.7, 0.8 and 1.6 g for daily gains during the intervals of 0-2, 2-4, 4-6, 6-8 and 8-10 weeks of age, respectively (Table 5), i.e. the percentages ranging from 2.4 to 11.0% for body weights and from 2.4 to 13.6% for daily gains. These estimates of G^M indicated that chicks mothered by R breed are preferred for growth traits compared to chicks mothered by F breed. Iraqi et al. (2011) stated that the effects of G^M on body weights were significant and ranged from 0.1 to 5.8% for body weights and from 0.2 to 5.0% for daily gains in favour of Matrouh dams when crossed with Inshas chickens. Taha and Abd El-Ghany (2013) reported that estimates of G^M for body weights at different ages were high and in favour of Mandarah dual purpose chickens at hatch and 16 weeks of age. Radwan and Mahrous (2018) found that the estimates of G^M were significantly in favour of Fayoumi by 8.8 and 10.6 g at 8 and 12 weeks of age, while the estimates were in favour of Rhode Island Red by 0.4 and 4.5 g for body weights at hatch and 4 weeks of age.

The estimates of H^I were non-significant for earlier body weights at hatch, 2 and 4 weeks of age, while the estimates of 32.3, 31.7 and 35.1 g were significantly heavier in chicks at later ages of 6, 8 and 10 weeks of age, respectively (Table 5). However, the H^I percentages were 0.4 to 7.7% for body weights and 1.3 to 6.0% for daily gains, i.e. crossing R breed with F breed gave considerable heterosis in body weights and gains. Iraqi et al. (2011) showed that heterosis estimates were positive and highly significant with percentages ranging from 6.9 to 9.1% for body weights and 0.5 to 11.3% for daily gains. Iraqi et al. (2013) reported that crossing Golden Montazah dual purpose chickens with White Leghorn was associated with the existence of significant and high percentages of heterotic effects on body weights and gains, the estimates averaged 12.6% for body weights, and 16.5% for daily gain traits. In crossing between El-Salam and Mandarah dual purpose chickens, Taha and Abd El-Ghany (2013) reported that the heterosis percentages for body weight were moderately positive at 2, 4, 8, 12, 16 and 20 weeks, being 3.6, 5.2, 4.1, 10.7, 11.5 and 7.4%, respectively. Radwan and Mahrous (2018) reported that the estimates of heterosis were significantly positive for body weight at hatch, 4, 8 and 12 weeks of age and weight gains at 0-4, 8-12 and 0-12 weeks of age.

Table 5
The generalized least square solutions for direct additive effects ($G^I = G^I_F - G^I_R$), maternal effects ($G^M = G^M_F - G^M_R$) and heterotic effects and their standard errors (SE) for body weights and gains in crossing Fayoumi (F) with Rhode Island Red (R)

Trait[+]	N	G^I solution (units)	SE	G^I as %[++]
BW, biweekly (g):				
BW0	480	-0.6^{ns}	0.01	-1.8
BW2	451	-7.4**	0.02	-6.1
BW4	441	-14.7**	0.02	-6.1
BW6	436	-37.2**	0.03	-8.8
BW8	436	-49.9**	0.04	-7.6
BW10	436	-57.9**	0.02	-6.4
DG, biweekly interval (g):				
DG0-2	474	-0.8**	0.005	-17.3
DG2-4	440	-1.2**	0.03	-7.1
DG4-6	437	-3.1**	0.03	-11.4
DG6-8	436	-1.8**	0.02	-5.5
DG8-10	436	-1.5**	0.02	-4.2
	N	**G^M solution (units)**	**SE**	**G^M as %[++]**
BW, biweekly (g):				
BW0	480	-0.8**	0.01	-2.5
BW2	451	-10.4**	0.01	-8.6
BW4	441	-26.4**	0.01	-11.0
BW6	436	-14.3**	0.02	-3.4
BW8	436	-25.1**	0.02	-3.9
BW10	436	-22.0**	0.02	-2.4
DG, biweekly interval (g):				
DG0-2	474	-0.5**	0.03	-10.8
DG2-4	440	-2.3**	0.02	-13.6
DG4-6	437	-0.7**	0.02	-2.6
DG6-8	436	-0.8*	0.02	-2.4
DG8-10	436	-1.6**	0.06	-4.5
	N	**H^I solution (units)**	**SE**	**H^I as %[++]**
BW, biweekly (g)::				
BW0	480	0.15^{ns}	0.01	0.4
BW2	451	0.63^{ns}	0.02	0.5
BW4	441	1.3^{ns}	0.02	0.5
BW6	436	32.3**	0.03	7.7
BW8	436	31.7**	0.04	4.9
BW10	436	35.2**	0.02	3.9
DG, biweekly interval (g):				
DG0-2	474	0.3**	0.03	6.0
DG2-4	440	0.3^{ns}	0.03	1.8
DG4-6	437	1.2**	0.03	4.4
DG6-8	436	0.45^{ns}	0.02	1.3
DG8-10	436	1.5**	0.02	4.2

[+] BW= Body weight; DG= Daily body gain.
[++] Percentages of G^I, G^M and H^I computed as {Estimate of G^I, G^M and H^I in units/[(F+R)/2]x100}; ns = non-significant
** = $P<0.01$.

Table 6
Generalized least square means and their standard errors (GLM ± SE) for body weights and gains as affected by SNPs genotypes of *GAL 3* gene in each genetic group separately.

Trait[+]	Breed or genetic group	Genotypes TT		TC		CC	
		GLM	SE	GLM	SE	GLM	SE
Body weight:							
BW0	R	33[b]	2.3	33[b]	2.6	39[a]	1.1
	½R½F	35	2.5	36	1.1	36	1.3
	½F½R	–	–	28[b]	3.2	34[a]	1.6
BW2	R	137[a]	11.1	121[ab]	12.4	114[b]	5.4
	½R½F	125[a]	8.9	117[a]	5.8	103[b]	5.0
	½F½R	–	–	119[b]	25.9	144[a]	17.4
BW4	R	259	26.0	250	24.1	250	9.8
	½R½F	261[a]	13.5	224[b]	13.7	205[c]	11.7
	½F½R	–		230[b]	39.3	302[a]	17.4
BW6	R	472[a]	24.5	437[b]	36.6	447[b]	16.0
	½R½F	511[a]	42.0	466[b]	22.1	411[c]	19.0
	½F½R	–	–	393[b]	71.1	497[a]	47.9
BW8	R	707[a]	28.9	691[ab]	47.9	672[b]	20.9
	½R½F	750[a]	44.1	679[b]	23.2	644[c]	19.9
	½F½R	–	–	623[b]	83.9	756[a]	56.5
BW10	R	980[a]	28.8	956[b]	55.8	957[b]	24.5
	½R½F	993[a]	45.5	906[b]	24.0	873[c]	20.5
	½F½R	–	–	916[b]	83.5	1038[a]	56.2
Daily gain:							
DG0-2	R	7.4[a]	0.57	6.2[ab]	0.42	5.2[b]	0.57
	½R½F	6.5[a]	0.45	5.8[ab]	0.78	4.8[b]	0.39
	½F½R	–	–	6.5[b]	0.96	7.8[a]	0.40
DG2-4	R	8.7	0.64	9.3	0.86	9.7	0.86
	½R½F	9.8[a]	1.40	7.7[b]	0.80	7.3[b]	0.70
	½F½R	–	–	7.8[b]	1.52	11.2[a]	0.54
DG4-6	R	15.4	1.08	13.3	0.80	13.8	1.08
	½R½F	18[a]	1.03	17.2[a]	1.79	14.7[b]	0.89
	½F½R	–	–	11.4[b]	1.59	14.1[a]	0.67
DG6-8	R	16.5	0.81	16.7	0.81	17.4	0.60
	½R½F	16.9	0.84	15.2	0.97	16.6	1.69
	½F½R	–	–	16.1[b]	1.33	18.6[a]	0.56
DG8-10	R	19.1	0.88	17.8	0.65	18.4	0.88
	½R½F	16.8	0.92	15.7	0.80	15.7	1.60
	½F½R	–	–	21.3	1.39	20.0	0.58

[+] BW and DG = Body weight and daily gain; Fayoumi breed was monomorphic.

Different letters in the same row indicate significant differences at $P < 0.05$.

Table 7
Generalized least square means and their standard errors (GLM ± SE) for body weights and gains as affected by SNPs genotypes of *GAL 4* gene in each genetic group separately.

Trait[+]	Breed or genetic group	Genotypes AA		AG		GG	
		GLM	SE	GLM	SE	GLM	SE
Body weight:							
BW0	R	32[b]	1.3	37[a]	2.1	36[a]	3.7
	½R½F	36[ab]	1.1	37[a]	1.2	34[b]	2.0
	½F½R	–	–	33	1.7	33	1.3
BW2	R	121	6.1	124	9.7	114	21.2
	½R½F	110	5.1	116	5.8	113	7.7
	½F½R	–	–	134[b]	9.6	143[a]	5.0
BW4	R	249	12.4	253	19.6	240	37.6
	½R½F	250[a]	21.4	229[b]	13.2	210[c]	11.6
	½F½R	–	–	257[b]	17.1	303[a]	13.7
BW6	R	442[a]	19.6	449[a]	30.8	419[b]	66.5
	½R½F	435[b]	19.5	466[a]	22.2	434[b]	35.9
	½F½R	–	–	442[b]	30.2	495[a]	24.3
BW8	R	678[a]	25.6	688[a]	40.3	643[b]	18.8
	½R½F	662[b]	19.6	688[a]	22.3	659[b]	36.1
	½F½R	–	–	685[b]	34.5	753[a]	27.7
BW10	R	1035[a]	19.6	1023[a]	46.1	912[b]	76.2
	½R½F	888[b]	29.3	925[a]	22.3	873[b]	27.8
	½F½R	–	–	894[b]	34.7	971[a]	36.1
Daily gain:							
DG0-2	R	5.9	0.57	6.2	0.30	6.3	0.57
	½R½F	5.7	0.72	5.7	0.44	5.3	0.36
	½F½R	–	–	7.8[a]	0.76	6.9[b]	0.44
DG2-4	R	8.9	0.64	9.2	0.86	9.1	0.86
	½R½F	9.9[a]	1.21	8.2[ab]	0.74	7.1[b]	0.60
	½F½R	–	–	8.8	1.14	11.4	0.66
DG4-6	R	12.8	0.80	13.9	1.08	13.8	1.08
	½R½F	13.4	1.65	16.5	1.01	16.5	0.82
	½F½R	–	–	13.1	1.24	13.9	0.71
DG6-8	R	15.9	0.60	17.1	0.81	16.8	0.81
	½R½F	16.3	1.67	16.0	1.02	16.4	0.83
	½F½R	–	–	17.4	1.04	18.5	0.60
DG8-10	R	16.1	0.65	18.3	0.88	18.1	0.88
	½R½F	16.3	1.55	16.3	0.77	15.8	0.95
	½F½R	–	–	22.1	1.05	19.6	0.60

[+] BW and DG = Body weight and daily gain; Fayoumi breed was monomorphic.

Different letters in the same row indicate significant differences at $P < 0.05$.

3.3. Molecular associations of GAL 3 gene genotypes with body weights and gains

The generalized least square means (GLM) of the three genotypes of *GAL 3* gene indicate that this gene was associated significantly ($p < 0.05$) with all body weights and gains in different genetic groups (Table 6). The chicks of genotype TT in R breed had significant heavier body weights than TC and CC genotypes with GLM of 137, 472, 707 and 980 g at 2, 6, 8 and 10 weeks of age, respectively. In chicks of ½R½F crossbred, the homozygous TT genotype had heavier significant body weights than TC and CC genotypes with GLM of 125, 241, 511, 751 and 993 g at 2, 4, 6, 8 and 10 weeks of age, respectively, while chicks of the genotype CC in ½F½R crossbred had heavy significant body weights of 34, 144, 303, 497, 756 and 1038 g compared to TC genotypes at hatch, 2, 4, 6, 8 and 10 week of age, respectively.

The GLM of the three genotypes of *GAL 3* gene reported that the chicks of genotype TT in R breed had significant higher body gains than TC and CC genotypes with GLM of 7.4 g during the interval of 0-2 weeks of age (Table 6). The homozygous TT genotype in chicks of ½R½F crossbred had higher significant body gains than TC and CC genotypes with GLM of 6.5, 9.8 and 18 g during 0-2, 2-4 and 4-6 weeks of age, respectively, while the genotype CC in ½F½R crossbred had higher significant body gains of 7.8, 11.2, 14.1 and 18.6 g than TC genotype at 0-2, 2-4, 4-6 and 6-8 weeks of age, respectively.

3.4. Molecular associations of GAL 4 gene genotypes with body weights and gains

Genotypes SNP of *GAL 4* gene were associated significantly ($p < 0.05$) with most body weights in each genetic group separately (Table 7). The genotype AG in R breed were significantly heavier body weights of 37, 448, 688 and 1023 g than GG genotype at hatch, 6, 8 and 10 weeks of age, respectively. The body weights of 37, 466, 688 and 925 g for chicks of genotype AG in ½R½F crossbred were significantly heavier than chicks of AG and GG genotypes at hatch, 6, 8 and 10 weeks of age. In ½F½R crossbred, chicks of genotype GG had significantly heavier body weights of 143, 303, 495, 753, and 971 g than chicks of AG genotype at 2, 4, 6, 8 and 10 weeks of age, respectively.

Genotypes SNP of *GAL 4* gene showed that this gene was associated significantly ($p < 0.05$) with body gains in ½R½F and ½F½R genetic groups (Table 7). The differences in body gains among genotypes in R breed were non-significant during different weeks of age. The body gains of 9.9 g for chicks of genotype AA in ½R½F crossbred were significantly higher than chicks of GG genotype at 2-4 weeks of age. In ½F½R crossbred, chicks of genotype AA had significantly higher body gains of 7.8 g than chicks of AG genotype at 0-2 weeks of age.

Table 8
Generalized least square means and their standard errors (GLM ± SE) for body weights and gains as affected by SNPs genotypes of *GAL 5* gene in each genetic group separately.

Trait[+]	Breed or genetic group	Genotypes CC		CA		AA	
		GLM	SE	GLM	SE	GLM	SE
Body weight:							
BW0	R	34^{ab}	2.2	32^{b}	1.0	38^{a}	2.8
	½R½F	35	1.7	36	0.9	–	–
	½F½R	33	3.2	34	4.8	33	2.6
BW2	R	125	12.0	119	5.4	125	15.5
	½R½F	113	8.9	110	5.1	–	–
	½F½R	133^{b}	8.6	138^{b}	25.9	170^{a}	17.4
BW4	R	250^{b}	23.6	252^{b}	10.7	268^{a}	30.4
	½R½F	202^{b}	19.4	224^{a}	10.4	–	–
	½F½R	270^{c}	13.5	294^{b}	39.3	326^{a}	26.4
BW6	R	444^{b}	38.8	453^{b}	17.6	471^{a}	49.9
	½R½F	440	33.9	445	18.2	–	–
	½F½R	460^{b}	24.5	498^{a}	71.1	493^{a}	47.9
BW8	R	678^{b}	50.2	684^{b}	22.9	711^{a}	64.7
	½R½F	659	34.6	673	18.6	–	–
	½F½R	714^{b}	28.9	761^{a}	83.9	749^{a}	56.5
BW10	R	912^{b}	54.4	923^{b}	24.7	1035^{a}	70.0
	½R½F	898	36.1	899	19.4	–	–
	½F½R	953	28.8	957	83.5	980	56.2
Daily gain:							
DG0-2	R	6.5	0.40	6.4	1.08	6.2	1.53
	½R½F	5.5	0.39	5.3	0.78	–	–
	½F½R	7.1^{b}	0.77	7.4^{b}	0.37	9.8^{a}	1.10
DG2-4	R	9.0	0.52	9.3	1.39	10.1	1.97
	½R½F	6.3^{b}	1.5	8.1^{a}	0.54	–	–
	½F½R	9.7	1.21	11.1	0.59	11.0	1.72
DG4-6	R	13.8	0.80	14.1	2.13	14.4	3.02
	½R½F	16.9	1.43	15.8	0.73	–	–
	½F½R	11.8^{b}	1.56	13.6^{a}	0.75	14.5^{a}	2.20
DG6-8	R	16.9	0.55	16.8	2.08	17.4	1.47
	½R½F	15.6	1.29	16.2	0.66	–	–
	½F½R	18.1	1.14	17.9	0.55	19.1	1.62
DG8-10	R	17.6	0.24	17.8	0.66	18.1	0.93
	½R½F	15.9	1.21	15.7	0.62	–	–
	½F½R	19.1	1.72	19.5	0.59	21.7	1.21

[+] BW and DG = Body weight and daily gain; Fayoumi breed was monomorphic.

Different letters in the same row indicate significant differences at $P < 0.05$.

3.5. Molecular associations of GAL 5 gene genotypes with body weights and gains

The SNP genotypes of *GAL 5* gene were associated significantly ($p < 0.05$) with different body weights in each genetic group separately (Table 8). The genotype AA in R breed had heavy significant body weights of 38, 268, 471, 711 and 1035 g relative to CC and CA genotypes at 0, 4, 6, 8 and 10 weeks of age. In ½R½F crossbred, CA genotype (224 g) was significantly heavier in body weight than CC genotype at 4 week of age, while chicks of genotype AA in ½F½R crossbred had significant heavier body weights of 170, 326, 493 and 749 g than CC genotype at 2, 4, 6 and 8 weeks of age.

The SNP genotypes of *GAL 5* gene were associated significantly ($p < 0.05$) with body gains at 0-2, 2-4 and 4-6 weeks of age in genetic groups of ½R½F and ½F½R (Table 8). The genotypes of R breed showed insignificant differences among genotypes in body gains at different weeks of age. In ½R½F crossbred, CA genotype was significantly higher body gain of 8.1 g than CC genotype at 2-4 weeks of age, while chicks of the genotype AA had significant higher gains of 9.8 and 14.5 g than CC genotype in ½F½R crossbred during the intervals of 0-2 and 4-6 weeks of age.

Regarding the associations between other candidate genes and body weights and gains in poultry, several studies confirmed significant associations (e.g. Zhou et al., 2005 with *IGF1*gene; Seo *et al.,* 2013 with TSH- β gene; (El Moujahid et al., 2014) with leptin receptor gene; Anh et al., 2015 with *GH* gene; Zhao et al., 2015 with *IGFBP-2* gene; Molee et al., 2016 with *MHC II* gene; Kazemi *et al.,* 2018 with *IL-2* gene; Horinouchi et al., 2018 and Yi et al., 2018 with *CCKAR* gene; Jin et al., 2018 with *Pit-1* gene; Thinh et al., 2019 with *INS* gene).

4. Conclusions

Crossing Fayoumi (F) with Rhode Island Red (R) was associated with beneficial heterotic effects to produce chicks with heavy body weights and gains. Based on direct and maternal effects, Fayoumi breed could be used as a sire and Rhode Island Red as a dam to improve body weights and gains.

The significant polymorphic associations were observed between all gallinacin genes (*GAL 3, GAL 4* and *GAL 5)* and body weights and gains in chicks of R, ½R½F and ½F½R, i.e. the candidate *GAL 3, GAL 4* and *GAL 5* genes could be used as marker-assisted selection in order to improve growth performance of chickens. In particular, the associations between *GAL 4* and *GAL 5* genes and body weights and gains in chicks of ½R½F and ½F½R concluding that considerable improvements could be achieved using these genes as genetic markers in selection program.

Availability of data and materials

The data used in the present study were obtained from the experiment performed in the Poultry Farm, Faculty of Agriculture, Benha University, Egypt. Data used and analyzed are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experimental procedures were approved by Animal Production Department, Faculty of Agriculture, Benha University, Egypt.

Authors' contributions

Medhat Saleh collected and analyzed the data and wrote the manuscript. Maher Khalil and Mahmoud Iraqi conceived, supervised and designed the study, and assisted in interpretations of the results and writing up the manuscript. Antonio Camarda supervised the laboratorial analyses. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that there is no conflict of interest for this study.

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Supplementary materials

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References

Ahmed, A., 2010. Associations of polymorphisms in four immune-related genes with antibody kinetics and body weight in chickens. Asian-Austr. J. Anim. Sci. 23, 1089–1095.

Amin, E.M., Dlebshany, El-, Amira, E., Debes, A.A., 2017. Using general and specific combining abilities to expected breeding values, genetic values and hybrid performance in chickens. Egypt. Poult. Sci. 37 (IV), 1289–1302.

Anh, N.T.L., Kunhareang, S., Duangjinda, M., 2015. Association of chicken growth hormones and insulin-like growth factor gene polymorphisms with growth performance and carcass traits in Thai broilers. Asian-Austr. J. Anim. Sci. 28, 1686–1695.

Cahyadi, M., Seo, D., Jin, S., Choi, N., Park, H.B., Heo, K.N., Kang, B.S., Jo, C., Lee, J.H., 2013. Association of SNPs in ODC and PRDM16 with body weight traits in Korean native chicken. Korean J. Poult. Sci. 40, 157–162.

Dickerson, G.E.T., 1992. Manual for evaluation of breeds and crosses of domestic animals 47 Food and Agriculture Organization of the United Nations, Rome.

El Moujahid, M.E., Chen, S., Jin, S., Lu, Y., Zhang, D., Ji, C., Yang, N., 2014. Association of leptin receptor gene polymorphisms with growth and feed efficiency in meat-type chickens. Poult. Sci. 93, 1910–1915.

Ganz, T., 2003. Defensins: antimicrobial peptides of innate immunity. Nat. Rev. Immunol. 3, 710–720.

Groeneveld, E., 2006. PEST User´s Manual. Institute of Animal Husbandry and Animal Behaviour. FAL, Germany.

Groeneveld, E., Kovač, M., Mielenz, N., 2010. VCE 6, Users guide and reference manual, Ver.6.0.2. Neustadt, Germany.

Hasenstein, J.R., Zhang, G., Lamont, S.J., 2006. Analyses of five gallinacin genes and the Salmonella enterica serovar Enteritidis response in poultry. Infect. Immun. 74, 3375–3380.

Horinouchi, S., Nakayama, H., Takahashi, H., 2018. Effect of a single nucleotide polymorphism in the Cholecystokinin type A receptor gene on growth traits of the Miyazaki Jitokko chicken. J. Poult. Sci. 56, 96–100.

Iraqi, M., Khalil, M., El-Attrouny, M., 2013. Estimation of crossbreeding components for growth traits in crossing Golden Montazah with White Leghorn chickens. In: VIth International Conference: Balnimalcon, 2013 Tekirdag/Turkiye, October 3-5, 2013, pp. 494–504.

Iraqi, M.M., Hanafi, M., El-Moghazy, G.M., El-Kotait, A., Abdel A'al, M.A., 2011. Estimation of crossbreeding effects for growth and immunological traits in a crossbreeding experiment involving two local strains of chickens. Livestock Res. Rural Dev. 23.

Jin, S., He, T., Yang, L., Tong, Y., Chen, X., Geng, Z., 2018. Association of polymorphisms in Pit-1 gene with growth and feed efficiency in meat-type chickens. Asian-Austr. J. Anim. Sci. 31, 1685–1690.

Kazemi, H., Najafi, M., Ghasemian, E., Rahimi-Mianji, G., Pirsaraei, Z.A., 2018. Polymorphism detection of promoter region of IFN-γ and IL-2 and their association with productive traits in Mazandaran native breeder fowls. J. Genet. 97, 843–851.

Lim, H., Han, J., Oh, J., Lee, H., Jeon, G., Lee, J., Seo, D., Cahyadi, M., Song, K., Choi, K., 2013. Association of SNPs from iNOS and TLR-4 genes with economic trait in chicken. Korean J. Poult. Sci. 40, 83–89.

Liu, L., Cui, Z., Xiao, Q., Zhang, H., Zhao, X., Wang, Y., Yin, H., Li, D., Zhu, Q., 2018. Polymorphisms in the chicken growth differentiation factor 9 gene associated with reproductive traits. BioMed. Res. Int 2018 online.

Mahmoud, B.Y., El-Full, E.A., 2014. Crossbreeding components for daily gain and growth rate traits in crossing Rhode Island Red with Gimmizah chickens. Egypt. Poult. Sci. 34, 151–163.

Molee, A., Kongroi, K., Kuadsantia, P., Poompramun, C., Likitdecharote, B., 2016. Association between single nucleotide polymorphisms of the major histocompatibility complex class II gene and Newcastle disease virus titre and body weight in Leung Hang Khao chickens. Asian-Austr. J. Anim. Sci. 29, 29–35.

NRC, 1994. National research council nutrient requirements of poultry. Nineth revised edition (1994). J Appl. Poult. Res. 3, 101.

Radwan, Lamiaa, 2018. Improving growth traits and estimating heterosis, additive and maternal effects a cross diallel mating among three lines selected heaviest body weight under heat condition. Egypt. Poult. Sci. J. 38, 895–907.

Seo, J., Oh, J.D., Choi, E.J., Lim, H.K., Seong, J., Song, K.D., Lee, J.H., Lee, H.K., Kong, H.S., Jeon, G.J., 2013. Effects of SNP in TSH-β Gene of Chicken on Economic Traits. Korean J. Poult. Sci. 40, 115–120.

Taha, A., Abdel-Ghany, F., 2013. Improving production traits for El-salam and Mandarah chicken strains by crossing. I-Estimation of crossbreeding effects for growth production traits. Alex. J. Vet. Sci. 39, 18–30.

Thinh, N.H., Tuan, H.A., Vinh, N.T., Doan, B.H., Giang, N.T.P., Frédéric, F., Nassim, M., Linh, N.V., Dang, P.K., 2019. Association of single nucleotide polymorphisms in the insulin and growth hormone gene with growth traits of Mia Chicken. Indian J. Anim. Res online April 2019.

Wakchaure, R., Ganguly, S., Praveen, P., Kumar, A., Sharma, S., Mahajan, T., 2015. Marker assisted selection (MAS) in animal breeding: a review. J. Drug Metab. Toxicol. 6, 127–130.

Wolf, J., 1996. User's Manual for the Software Package CBE. Research Institute of Animal Production, Prague-Uhrineves, Czech Republic Version 4.0 (A universal program for estimating crossbreeding effects).

Xiao, Y., Hughes, A.L., Ando, J., Matsuda, Y., Cheng, J.F., Skinner-Noble, D., Zhang, G., 2004. A genome-wide screen identifies a single β-defensin gene cluster in the chicken: implications for the origin and evolution of mammalian defensins. BMC Genomic 5, 56–66.

Ye, X., Avendano, S., Dekkers, J., Lamont, S., 2006. Association of twelve immune-related genes with performance of three broiler lines in two different hygiene environments. Poult. Sci. 85, 1555–1569.

Yi, Z., Li, X., Luo, W., Xu, Z., Ji, C., Zhang, Y., Nie, Q., Zhang, D., Zhang, X., 2018. Feed conversion ratio, residual feed intake and cholecystokinin type A receptor gene polymorphisms are associated with feed intake and average daily gain in a Chinese local chicken population. J Anim. Sci. Biotech. 9, 50–58.

Zhao, X., Li, M., Xu, S., Liu, G., 2015. Single nucleotide polymorphisms in IGFBP-2 gene and their associations with body weight traits on Jinghai Yellow chicken. Braz. J. Poultry Sci. 17, 497–502.

Zhou, H., Lamont, S., 2003. Association of transforming growth factor β genes with quantitative trait loci for antibody response kinetics in hens. Anim. Genet. 34, 275–282.

Zhou, H., Mitchell, A., Mcmurtry, J., Ashwell, C., Lamont, S.J., 2005. Insulin-like growth factor-I gene polymorphism associations with growth, body composition, skeleton integrity, and metabolic traits in chickens. Poult. Sci. 84, 212–219.