



USING BIOINFORMATICS SNP TO IMPROVE IMMUNE GENETIC RESPONSE AGAINST SOME PATHOGENS IN POULTRY

By

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5. SUMMARY

Simple crossbreeding experiment was performed between Fayoumi (F) and Rhode Island Red (RIR) chickens to get ½F½RIR cross and its reciprocal ½RIR½F cross. The experiment was started in November 2016 in the Poultry Farm, Department of Animal Production, Faculty of Agriculture, Benha University, Egypt. The laboratorial examinations for bacterial count were carried in the labs of Research Park, Faculty of Agriculture, Benha University. The laboratorial analyses for molecular biology were carried out in the Labs of Genetics Department, Faculty of Agriculture, Benha University, Egypt, and molecular biology labs, Avian Pathology Section, Department of Veterinary Medicine, University of Bari, Italy during short-term period from April 2017 to September 2017. A total number of 480 chicks fathered by 40 sires and mothered by 240 dams were chosen randomly for quantitative analyses and 96 samples for molecular analyses. The main objectives of the present study were: (1) to estimate the crossbreeding effects (i.e. direct additive effect, maternal additive effect and direct heterosis) on body weights (BW) at hatch, 1, 2, 4, 6, 8 and 10 weeks of age, daily gains (DG), feed intake (FI) and feed conversion (FC) during the intervals of 0-2, 2-4, 4-6, 6-8 and 8-10 weeks of age, *Salmonella typhimurium* and *Enterococcus faecium* colonization in the cecum, caecal *pH* and IgA, IgG and IgM antibody titers at the 4^{th} week of age, (2) to characterize the polymorphism of SNPs in the gallinacins 2, 3, 4, and 5 candidate genes across the four genetic groups using the PCR-RFLP technique, (3) to detect the associations between immune gallinacin candidate genes with body weights and feed conversions, *Salmonella* and *Enterococcus* ceacal bacterial count and antibody titers and (4) to identify

SNPs of Gallinacin genes between parental breeds and their crosses using bioinformatics analyses. The method of Generalized Least Squares (GLS) were used. The most relevant results of this study could be summarized as follows:

Quantitative genetic analyses of growth, feeding and immune traits

- The generalized least square solutions of the direct additive effects (G^I) were significantly ($P<0.01$) in favour of RIR breed by 0.6 to 57.9 g for body weights, 0.8 to 3.1 g for daily gains, 0.6 to 2.6 g for feed intakes and 0.08 to 0.7 g feed/ g weight for feed conversions, with percentages ranging from 1.8 to 9.2% for body weights, 4.2 to 17.3% for daily gains, 2.6 to 16.6% for feed intakes and 2.5 to 23.6% for feed conversions. The estimable G^I were significantly ($P<0.01$) in favour of F breed by 0.19 $\log_{10}$ cfu/*ml* for salmonella count, 0.195 $\log_{10}$ cfu/*ml* for *Enterococcus faecium* count, 0.169 OD 450 nm for IgA, 0.038 OD 450 nm for IgG and 0.051 OD 450 nm for IgM antibody titer traits, with percentages of 9.5% for *Salmonella typhimurium* count, 8.9% for *Enterococcus faecium* count, 15.1% for IgA, 3.6% for IgG and 4.9% for IgM antibody titers.
- The generalized least square solutions of the maternal additive effect (G^M) were significantly in favour of RIR breed by 0.8 to 26.4 g for body weights, 0.5 to 2.3 g for daily gains, 0.1 to 1.9 g for feed intakes and 0.02 to 0.9 g feed/ g weight for feed conversions, with percentages ranging from 2.4 to 11.0% for body weights, 2.4 to 13.6% for daily gains, 0.7 to 3.2% for feed intakes and 1.3 to 29.8% for feed conversions. The estimable G^M were significantly in favour of F breed

by 0.089 $\log_{10}$ cfu/*ml* for *Salmonella typhimurium* count, 0.036 OD for IgA, 0.049 OD 450 nm for IgG and 0.041 OD 450 nm for IgM antibody titer traits, with percentages of 4% for *Salmonella typhimurium* count, 6.3% for IgA, 4.6% for IgG and 3.9% for IgM antibody titers.

- The generalized least square solutions of the direct heterosis (H^I) were positive and significant and ranged from 0.15 to 35.2 g for body weights, 0.3 to 1.5 g for daily gains, 0.1 to 2.2 g for feed intakes and 0.005 to 1.0 g feed/ g weight for feed conversions, with percentages ranging from 0.3 to 7.7% for body weights, 1.3 to 6.0% for daily gains, 1.2 to 4.7% for feed intakes and 1.7 to 32.2% for feed conversions. The estimable direct heterosis for *Salmonella typhimurium* count, *Enterococcus faecium* count and IgA antibody titers were significantly associated with improvements in these traits by 0.079 $\log_{10}$ cfu/*ml*, 0.155 $\log_{10}$ cfu/*ml* and 0.051 OD 450 nm, respectively, with percentages of 3.7% for *Salmonella typhimurium* count, 7.2% for *Enterococcus faecium* count, 2.2% for IgA and the heterotic percentages were negilable and amountly 0.4% for caecal *pH*, 0.2% for IgG and 0.9% for IgM antibody titers.

Bioinformatics analyses of gallinacin genes

- For the pairwise sequence alignment of *Gal 2, Gal 3, Gal 4* and *Gal 5* genes, 17 SNPs with identity ratio of 97%, lot of SNPs and gaps with low identity ratio of 50%, ten SNPs and one gap with identity ratio of 98% and 18 SNPs with identity ratio of 97% were identified between F and ½F½RIR, respectively. Between F and ½RIR½F, 11 SNP and three gaps with identity ratio of 98%, 15 SNPs with identity ratio of 98%, twenty SNPs and one gap with identity ratio of 96% and a lot of

SNPs and gaps with low identity ratio of 50% were identified in the sequence of *Gal 2, Gal 3, Gal 4* and *Gal 5* genes.

- For the pairwise sequence of *Gal 2, Gal 3, Gal 4* and *Gal 5* genes, 12 SNPs with identity ratio of 98%, 38 SNPs and one gap with identity 94%, a lot of SNPs and gaps with low identity ratio of 50% and 37 SNP and two gaps with identity ratio of 90% were identified between RIR and ½F½RIR and 21 SNPs and one gap with identity ratio of 96% for *Gal 2* and a lot of SNPs and gaps with low identity ratio of 50% between RIR and ½RIR½F.

Molecular characterization of four gallinacin candidate genes

- PCR-RFLP for *Gal 2* gene revealed homozygous genotype in the studied genetic groups (Fayoumi, Rhode Island Red, ½RIR½F and ½F½RIR), so it was excluded from the association study. For *Gal 3* gene, the genotypic frequency of CC was 1.0 in F breed, while in RIR breed TC genotype 0.50 was higher than TT (0.18) and CC (0.32) genotypes and CC genotype 0.54 was higher than TT (0.13) and TC (0.33) genotypes in ½RIR½F and CC genotype 0.84 was higher than TT (0.0) and TC (0.16) genotypes in ½F½RIR cross.
- For *Gal 4* gene, the frequency of GG genotype was 1.0 in F breed and the frequency of GG (0.46) and AG (0.46) genotypes were higher than AA genotype 0.08 in RIR breed and the frequency of GG 0.50 genotype was higher than AA (0.17) and AG (0.33) genotypes in ½RIR½F and genotype frequency of GG 0.75 was higher than AA (0.0) and AG (0.25) genotypes in ½F½RIR cross.

- For *Gal 5* gene, the frequency of AA genotype was 1.0 in F breed and genotype frequency of CC 0.85 was higher than AA (0.10) and AC (0.05) genotypes in RIR breed and genotype frequency of CA 0.79 was higher than CC (0.21) and AA (0.0) genotypes in ½RIR½F and genotype frequency of CA 0.74 was higher than CC (0.17) and AA (0.09) genotypes in ½F½RIR cross.
- The frequency of C allele was higher than T allele in all populations where the highest frequency for C allele of *Gal 3* gene was recorded by F breed 1.0 and the lowest frequency in RIR breed 0.57, while for T allele the highest recorded by R breed 0.43 and the lowest in F breed 0.0. The frequency of G allele was higher than A allele in all populations where the highest frequency for G allele of *Gal 4* gene was recorded by F breed 1.0 and the lowest frequency in R breed 0.69, while the highest frequency for A allele was recorded by RIR breed 0.31 and the lowest in F breed 0.0. The frequency of C allele of *Gal 5* gene was higher than A allele in RIR breed, ½RIR½F and ½F½RIR where the highest frequency for C allele was recorded by R breed 0.87 and the lowest in F breed 0.0, while the highest frequency for A allele recorded by F breed 1.0 and the lowest frequency in RIR breed 0.13.
- The effective number of alleles (Ne) in F, RIR, ½F½RIR and ½RIR½F were 1.00, 1.960, 1.753 and 1.170 for *Gal 3* gene, 1.00, 1.753, 1.734 and 1.280 for *Gal 4* gene and 1.00, 1.296, 1.917 and 1.985 for *Gal 5* gene, respectively.
- The deviations from Hardy-Weinberg equilibrium were not significant for *Gal 3* and *Gal 4* genes and significant for *Gal 5* gene in all populations studied.

- The observed heterozygosity in F, RIR, ½F½RIR and ½RIR½F were 0.0, 0.476, 0.375 and 0.158 for *Gal 3* gene, 0.0, 0.458, 0.615 and 0.250 for *Gal 4* gene and 0.00, 0.053, 0.792 and 0.739 for *Gal 5* gene, respectively, while the corresponding expected heterozygosity were 0.0, 0.490, 0.430 and 0.145 for *Gal 3* gene, 0.0, 0.430, 0.348 and 0.219 for *Gal 4* gene and 0.00, 0.229, 0.478 and 0.496 for *Gal 5* gene.
- All the values of polymorphic information content (*PIC*) in F, RIR, ½F½RIR and ½RIR½F genetic groups were mostly moderate; being 0.0, 0.370, 0.341 and 0.136 for *Gal 3* gene, 0.0, 0.336, 0.332 and 0.210 for *Gal 4* gene and 0.0, 0.210, 0.365 and 0.375 for *Gal 5* gene, respectively. The average of inbreeding coeffeciants (F_{IS}, F_{ST} and F_{IT}) across all loci and genetic groups were -0.052, 0.117 and 0.071, respectively.
- In Fayoumi breed, there were only homozygous genotypes of CC, GG and AA for *Gal 3*, *Gal 4* and *Gal 5* genes, respectively.

Molecular associations among genotypes of immune gallinacin genes and body weights and feed conversions

- For *Gal 3* gene, the genotype TT in RIR breed was heavier in body weights at 2, 6, 8 and 10 weeks of age than TC and CC genotypes, while the genotype TC had lower and significant feed conversions at 2^{nd}, 6^{th}, 8^{th} and 10^{th} week of age than CC genotype. In ½RIR½F crossbred, the genotype TT had heavier significant body weights than TC and CC genotypes, while the genotype TT had lower and significant feed conversions than CC genotype. The CC genotype in ½F½RIR crossbred had heavier significant body weights than TC

genotype, while the CC genotype had lower and significant feed conversions than TC genotype.

- For *Gal 4* gene, the genotype AG in RIR breed had significant heavier body weights than GG genotype at 6^{th} and 8^{th} week of age, while the GG genotype had lower significant feed conversions than AA and AG genotypes at 6^{th} weeks of age. In ½RIR½F crossbred, the genotype AG had significantly heavier body weights than AA and GG genotypes and the genotype AG had lower and significant feed conversions than AA and GG genotypes. The genotype GG in ½F½RIR crossbred had heavier significant body weights than AG genotype and the genotype GG had lower and significant feed conversion than AG genotype.
- For *Gal 5* gene, the genotype AA in RIR breed had heavier significant body weights than CC and CA genotypes at 4, 6, 8 and 10 weeks of age, while genotypes CC and CA had lower significant feed conversions than AA genotype at the 4^{th} week of age. In ½RIR½F crossbred, the CA genotype was significantly heavier than that of CC genotype at 4 week of age and the genotype CA was significantly lower in feed conversions than that of CC genotype at the 4^{th} week of age. The genotype AA in ½F½RIR crossbred had significant heavier body weights than CC genotype and the genotype AA had lower significant feed conversions than that of CC genotype.

Molecular associations among genotypes of immune gallinacin genes and immunological traits

- For *Gal 3* gene, F breed have only the genotype CC with a lower *Salmonella typhimurium* count of 1.7 $\log_{10}$ cfu/*ml*, ceacal *pH* of 7.1 and higher *Enterococcus faecium* count of 2.1 $\log_{10}$ cfu/ml and antibody titers of 1.4, 1.3 and 1.3 OD 450 nm in IgA, IgG and IgM, respectively. In RIR breed, the genotype CC had a lower *Salmonella typhimurium* count of 2.0 than 3.1 $\log_{10}$ cfu/*ml* for TT genotype, while CC genotype had higher significant *Enterococcus faecium* count of 2.8 than 1.6 and 1.7 $\log_{10}$ cfu/*ml* for TT and TC genotypes. For ceacal *pH*, there were non-significant differences between the genotypes. On the other hand, the genotype CC had a higher significant IgA and IgM antibody titers of 1.3 and 1.3 OD 450 nm than those for TC and TT genotypes, while the genotype TT had significant IgG antibody titers of 1.3 than those for TC and CC genotypes. In ½RIR½F crossbred, the TC genotype had a lower significant *Salmonella typhimurium* count of 1.6 than 2.9 $\log_{10}$ cfu/ml for TT genotype, and TT and TC genotypes had higher significant *Enterococcus faecium* count of 2.3 and 2.0 than 1.0 $\log_{10}$ cfu/*ml* for CC genotype. For ceacal *pH*, there were non-significant differences between the genotypes, while the genotype TT had higher significant IgA, IgG and IgM antibody titers by 1.3, 1.4 and 1.3 OD 450 nm than those for CC genotype, respectively. In ½F½RIR crossbred, the TC genotype had higher count of 3.0 for *Enterococcus faecium* count than that of 1.9 $\log_{10}$ cfu/*ml* for CC genotype. The TC genotype had significant higher ceacal *pH* value of 7.2 than that of 4.6 for CC genotype and the genotype TC had higher significant antibody titers of IgA and IgM by 1.0 and 1.0 than those of 0.7 and 0.7 OD 450 nm for TC genotype.

- For *Gal 4* gene, F breed had only the genotype GG with a lower *Salmonella typhimurium* count of 1.7 $\log_{10}$ cfu/*ml*, ceacal *pH* of 7.3 and higher *Enterococcus faecium* count of 2.1 $\log_{10}$ cfu/*ml* and antibody titers of 1.4, 1.5 and 1.4 OD 450 nm in IgA, IgG and IgM, respectively. In RIR breed, the genotypes GG and AA had lower significant *Salmonella typhimurium* count of 1.8 and 1.9 $\log_{10}$ cfu/*ml* than that of 3.0 $\log_{10}$ cfu/*ml* for AG genotype and higher significant *Enterococcus faecium* count of 1.8 and 1.9 than that of 1.0 $\log_{10}$ cfu/*ml* for AG genotype, while the genotype GG had higher significant IgA, IgG and IgM antibody titers of 1.4, 1.4 and 1.5 OD 450 nm than those for AG and AA genotypes. In ½RIR½F crossbred, the genotype AA had a higher count of 2.2 for *Enterococcus faecium* count than that of 1.7 $\log_{10}$ cfu/*ml* for GG genotype, while the genotype AG had higher significant IgA, IgG and IgM antibody titers of 1.4, 1.4 and 1.3 OD 450 nm than those for GG and AA genotypes. In ½F½RIR crossbred, the genotype AG had a higher significant *Enterococcus faecium* count of 2.3 $\log_{10}$ cfu/*ml* than that of 1.6 for GG genotype and there were non-significant differences among the genotypes in ceacal *pH* and antibody titers.
- For *Gal 5* gene, F breed have only the genotype AA of 1.0 for *Salmonella typhimurium* count, ceacal *pH* of 7.3 and *Enterococcus faecium* count of 2.1 $\log_{10}$ cfu/*ml* and higher antibody titers of 1.7, 1.5 and 1.6 OD 450 nm for IgA, IgG and IgM, respectively. In RIR breed, the genotype CC had a lower significant *Salmonella typhimurium* count of 2.3 than that of 3.0 $\log_{10}$ cfu/*ml* for AA genotype, and higher *Enterococcus faecium* count of 1.9 than those of 1.0 and 1.0 $\log_{10}$ cfu/*ml* for AA and CA genotypes. The genotypes AA and CC had

higher significant IgA (1.5 and 1.4) and IgG (1.6 and 1.4) antibody titers of than those of 0.8 and 0.9 OD 450 nm for CA genotype, while the genotype CA had a higher significant IgM antibody titer of 1.7 OD 450 nm than those for CC and AA genotypes. In ½RIR½F crossbred, the differences among genotypes were non-significant for *Salmonella typhimurium* and *Enterococcus faecium* counts, ceacal *pH* and antibody titers. In ½F½RIR crossbred, the genotype AA had lower significant *Salmonella typhimurium* count of 1.0 than those of 2.1 $\log_{10}$ cfu/*ml* for CC genotypes, while the genotype CA had a higher significant *Enterococcus faecium* count of 2.2 than these of 1.0 and 1.0 $\log_{10}$ cfu/*ml* for AA and CC genotypes. The differences between the genotypes were non-significant for ceacal *pH*, while the genotypes AA and CC had higher significant IgA and IgG antibody titers of (1.4 and 1.3) and (1.3 and 1.3) OD 450 nm than those for CA genotype.