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Allelic Polymorphism of GH, GHR and IGF-1 Genes and Their Association with Growth and Carcass Traits in Mazandaran Native Fowl

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Abstract

This investigation aimed to study polymorphism of growth hormone (GH), growth hormone receptor (GHR), and insulin like growth factor 1 (IGF-1) genes and their associations with growth and carcass traits in Mazandaran native chicken. 200 male chicks were reared and slaughtered at 12 weeks of age. Traits including live weight at 4, 8 and 12 weeks of age, body weight and weights of heart, liver, gizzard, spleen, and abdominal fat, pH of meat, water holding capacity of the meat and intramuscular fat were recorded. Before dissecting birds, blood samples were collected and DNA was extracted. The frequency of alleles (+ and -) were determined to be 0.627 and 0.373 for GH locus, 0.715 and 0.285 for GHR locus, and 0.407 and 0.593 for IGF-1 gene locus, respectively. Hardy-Weinberg equilibrium study using Chi-square test showed that the studied population was not in Hardy-Weinberg equilibrium. Analysis of phenotypic and genotypic data showed a significant association between genotypes of GH gene and live weights at 8 and 12 weeks of age and carcass weight. Significant associations were found between the GHR gene with live weight at 12 weeks of age and carcass weight and also between IGF-1 gene with abdominal fat and intramuscular fat ($P < 0.05$). Generally, it can be concluded that GH, GHR and IGF-1 genes can be used as candidate genes for improving growth and carcass traits in Mazandaran native fowls.

Introduction

Native fowls are one of the most important genetic resources of a country because of their resistance to specific environmental conditions and diseases. In many developing countries, genetic pools of native birds are the breeding foundation in the poultry sector (Hoffmann *et al*., 2004). However, there is little information about the productive and reproductive capacities and features of native birds (Ghazikhani shad *et al*., 2007). Native birds are basic genetic resources in Iran for breeding programs in their local habitats. Thus, understanding genetic resources can improve breeding programs and production efficiency (Dehghanzadeh *et al*., 2004). Important economic traits are controlled by many genes with high and low impacts. The major gene model suggested that a small number of genes could

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have major contributions on genetic variation. Recent advances in molecular genetics allowed for the identification and sequencing of these genes. Genetic progress in recent decades has significantly improved performance of poultry (Havenstein *et al*., 2003).

Poultry growth hormone is a polypeptide hormone produced and secreted from the anterior pituitary and is able to cast changes in physiological functions such as growth, body condition, egg production, aging, reproduction and immune responses. Therefore, genes involved in the production of this hormone can be candidate genes in marker-assisted selection to improve poultry performance (Nie *et al*., 2005). In order for growth hormone to induce effects such as growth and lipid metabolism, corresponding receptors on the surface of target cells are needed. Gene expression of growth hormone receptor and its connection to growth hormone is essential for growth and fat metabolism (Garrett *et al*., 2008). Insulin-like growth factors (IGF) regulate the function and performance of growth hormone in poultry. IGF hormones are important regulators in stimulating growth, protein synthesis, and proliferation and differentiation of various cell types (Lei *et al*., 2005). Because of the biological functions of this gene, IGF-1 is a strong candidate gene for meat quality traits and growth forecast in animal genetic improvement programs (Kadlec *et al*., 2011).

Traditional selection methods based on phenotype have significantly increased meat production and growth rate of poultry in recent decades. However, selection of a favorable phenotype does not always translate to favorable genotype selection. The influence of environmental variance on phenotypic variance would lead to differences between phenotypes and genotypes that reduce selection accuracy (Bai *et al*., 2006). Further, selection based on phenotypes of meat quality traits is not possible before slaughter. Therefore, molecular markers can assist selection and improve efficiency and production performance. In the future, a combination of conventional and new molecular methods will be preferred in selective breeding programs in poultry (Emara and Kim, 2003). Due to its high repeatability and accuracy, RFLP markers are able to detect polymorphism in every locus of the genome (Naghavi *et al*., 2013).

The aim of this research was to identify polymorphisms in loci of growth hormone (GH), growth hormone receptor (GHR), and insulin like growth factor 1 (IGF-1) genes to determine their genotypic and allelic frequencies, and study the association between polymorphism of genotyping patterns of these genes and growth and carcass traits in Mazandaran native chicken using PCR-RFLP technique.

Materials and Methods

200 male chicks of Mazandaran native fowls were selected by cloacal sexing technique. All chickens were reared in similar conditions and slaughtered at 12 weeks of age. Live weight of birds at the age of 4, 8 and 12 weeks were measured, and after slaughter, carcass weight and the weights of heart, liver, gizzard, spleen and abdominal fat were measured with a digital scale with an accuracy of 0.01 g. Pectoral muscle samples were kept in a freezer at -20°C for 10 days, and after thawing, carcass quality traits including pH and water holding capacities of meat and intramuscular fat were measured at room temperature. The tissue pH meter device was used to determine pH of the meat. To determine water holding capacity, the weight of the meat before and after centrifugation (4 min, 252 g) was measured, then put in the oven (for 24 hrs at 70°C) and re-weighed. Water holding capacity was calculated using the following formula:

$$WHC(\%) = \frac{(WAC - WAO)}{WBC} \times 100$$

WHC: Water holding capacity, WAC: Weight after centrifugation, WAO: Weight after oven and WBC: Weight before centrifugation.

Intramuscular fat was measured using Soxhlet method. Before slaughter, 2 mL of blood from a vein under the wing of birds were collected in tubes containing EDTA anticoagulant. Blood samples were kept in a freezer at -20°C until DNA extraction. The extraction of DNA from blood samples was performed using the Cinnagen kit. Spectrophotometer and 1% agarose gel electrophoresis were used to determine the quantity and quality of extracted DNA samples. Primers were used to reproduce a fragment of 1050 bp from intron 4 region of GH gene and a 718 bp of intron 2 of GHR gene (Enayati and Rahimi Mianji, 2011). To reproduce 813 bp fragments of the promoter region of IGF-1 gene, primers used by Piryonesi *et al*. (2012) were utilized. The sequence of primers pair was: F: 5′-CTAAAGGACCTGGAAGAAGGG-3′ and R: 5′-

AACTTGTCGTAGGTGGGTCTG-3′ for GH gene, F: 5′-GGCTCTCCATGGGTATTAGGA-3′ and R: 5′-GCTGGTGAACCAATCTCGGTT-3′ for GHR gene, and F: 5′-CATTGCGCAGGCTCTATCTG-3′ and R: 5′-TCAAGAGAAGCCCTTCAAGC-3′ for IGF-1 gene. The optimal conditions for PCR (12 µL final volume) included 6 µL Master, 1.5 µL of each primer (10 pmol/µL), 1.5 µL DNA (the concentration of 50 to 100 ng to µL) and 1.5 µL twice distilled water.

The amplification of GH gene fragment was performed using a primitive denaturing phase of 94 °C for 4 min followed by 35 cycles containing 30 s at 92°C, 62°C for 120 s, 72°C for 90 s and a final amplification at 72°C for 7 min before storing the sample at 4°C. For GHR gene amplification, the PCR conditions were 94°C for 5 min followed by 35 cycles of 92°C for 30 s, 59°C for 80 seconds, followed by 90 s at 72°C and a final amplification at 72°C for 10 min before storing the sample at 4°C. The amplification of IGF-1 gene fragment was performed using 94°C for 5 min followed by 35 cycles containing 92°C for 60 s, 56°C for 60 s, 72°C for 60 s and a finally 72°C for 10 min before storing the sample at 4°C. Enzymatic digestion with a volume of 12 µL for genes of GH, GHR and IGF-1 was performed on the PCR products with restriction enzymes of Sac-I, Hind-III and Hinf-I at 37°C for 16 hrs, respectively. To view the fragments digested and to determine genotype, 2.5%agarose gel electrophoresis and gel staining with Safe Stain were used.

PopGene 32 software was used to calculate the frequency of alleles, genotypes and Chi-square test. Data obtained using the following statistical model was analyzed by statistical software of SAS 9.12:

$y_{ijk}=M+G_i+d_j+e_{ijk}$

Where, y_{ijk}: observations of growth and carcass traits, M: Overall mean, G_i: effects of genotype, d_j: effects of recording day and e_{ijk}: residual effects.

Results

The sizes of amplified fragments after PCR reactions for GH, GHR and IGF-1 genes were 1050, 718 and 813 bp, respectively. Digestive enzymes also created fragments of 1050, 600 and 450 bp for GH gene, 404, 314, 247 and 157 bp for GHR gene, and 622, 378, 244 and 191 bp for IGF-1 gene (Figures 1, 2 and 3).

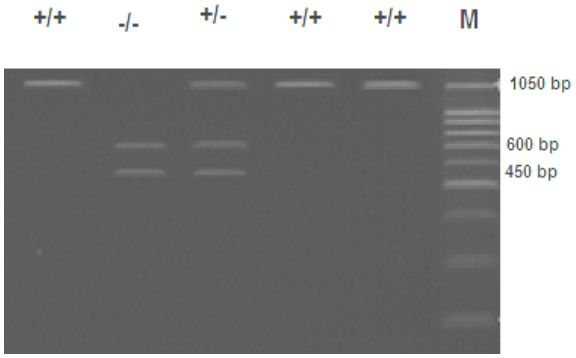

Figure 1. PCR-RFLP pattern of for GH gene with Sac-I enzyme

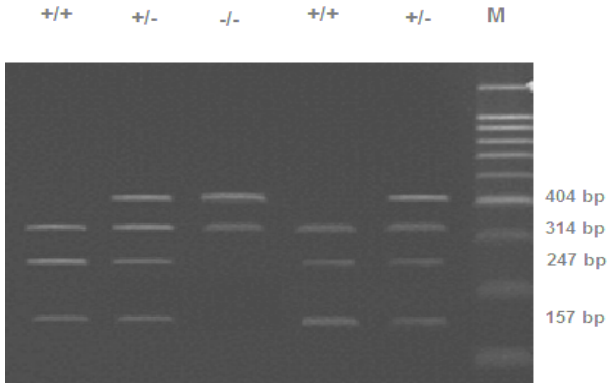

Figure 2. PCR-RFLP pattern of for GHR gene with Hind-III enzyme

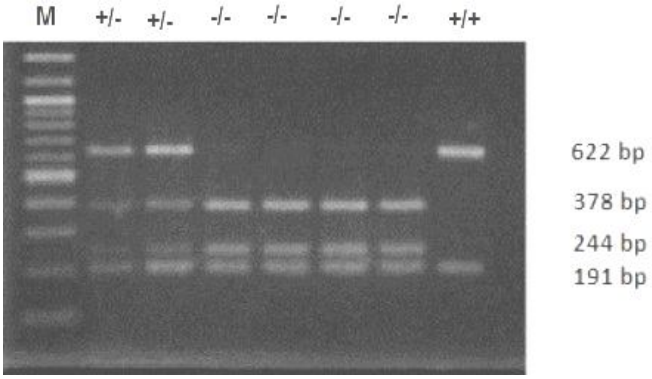

Figure 3. PCR-RFLP pattern of for IGF-1 gene with Hinf-I enzyme

Three genotypes were identified for the desired locus in the GH gene (++, +- and --) with the frequencies of 0.47, 0.325 and 0.205, respectively. Allele frequencies of the + and – were 0.627 and 0.373, respectively. Three genotypes were also recognized for GHR gene locus (++, +- and --) with frequencies of 0.595, 0.24 and 0.165, and allele frequencies of + and - were 0.715 and 0.285, respectively. Lastly, three genotypes were identified for IGF-1 gene (++, +- and --) with frequencies of 0.23, 0.355 and 0.415, and allele frequencies of + and – were 0.407 and 0.593, respectively. Hardy-Weinberg equilibrium using chi-square test for all three genes showed that this population was not in equilibrium for these loci.

There was a significant association between genotypes of GH gene and live weights at 8 and 12 weeks of age and carcass weight. Similar associations were found with GHR gene but only with live weight at 12 weeks of age. IGF-1 gene was associated with abdominal fat and intramuscular fat ($P < 0.05$). The mean comparisons showed that males with ++ genotypes for GH gene have significantly ($P < 0.05$) higher live weight at 8 and 12 weeks and higher carcass weight compared to the other genotypes (+- and --)(Table 1). Roosters with ++ genotypes for GHR also had significantly ($P < 0.05$) higher live weight at 12 weeks of age and higher carcass weight compared to +- and -- genotypes (Table 2). Roosters with ++ and +- genotypes for IGF-1 gene had significantly ($P < 0.05$) higher abdominal and intramuscular fat compared to genotype -- (Table 3). There were no other significant associations between the studied loci genotypes and desired traits.

Table 1. Least squares means comparison of GH genotypes with their standard error for growth and carcass traits of Mazandaran native fowl

Trait	genotype		
	+/+	+/-	-/-
Body weight at 4 weeks (g)	300.44 ± 18.72	279.56 ± 21.05	271.48 ± 20.19
Body weight at 8 weeks (g)	896.12 ± 52.64 [a]	757.84 ± 56.27 [b]	736.10 ± 51.02 [b]
Body weight at 12 weeks (g)	1557.53 ± 91.39 [a]	1306.30 ± 87.88 [b]	1272.14 ± 85.27 [b]
Carcass weight (g)	1136.61 ± 69.26 [a]	940.32 ± 61.33 [b]	915.84 ± 62.12 [b]
Heart weight (g)	10.54 ± 0.98	10.19 ± 0.9	10.24 ± 0.93
Liver weight (g)	36.47 ± 2.56	35.31 ± 2.72	34.78 ± 2.36
Gizzard weight (g)	32.84 ± 3.17	31.26 ± 3.15	31.49 ± 2.91
Spleen weight (g)	2.56 ± 0.23	2.49 ± 0.27	2.38 ± 0.24
Abdominal fat weight (g)	9.61 ± 1.14	9.11 ± 1.02	8.78 ± 1.05
Meat pH	6.23 ± 0.25	6.10 ± 0.32	6.18 ± 0.22
Water holding capacity (%)	46.17 ± 1.38	46.93 ± 1.15	45.96 ± 1.47
Intramuscular fat (%)	3.75 ± 0.39	3.61 ± 0.32	3.52 ± 0.26

[a,b] Least squares means within a row without common superscript differ significantly ($P < 0.05$).

Table 2. Least squares means comparison of GHR genotypes with their standard error for growth and carcass traits of Mazandaran native fowl

Trait	genotype		
	+/+	+/-	-/-
Body weight at 4 weeks (g)	295.81 ± 20.43	282.37 ± 19.61	276.04 ± 19.15
Body weight at 8 weeks (g)	848.57 ± 50.32	784.26 ± 52.18	763.10 ± 49.66
Body weight at 12 weeks (g)	1523.42 ± 88.76	1311.93 ± 85.61 [b]	1284.42 ± 86.30 [b]
Carcass weight (g)	1112.79 ± 64.15 [a]	943.80 ± 64.37 [b]	924.57 ± 62.91 [b]
Heart weight (g)	10.37 ± 0.95	10.24 ± 0.89	10.21 ± 0.94
Liver weight (g)	36.19 ± 2.14	35.29 ± 2.31	35.05 ± 2.11
Gizzard weight (g)	32.63 ± 3.24	32.17 ± 3.02	31.36 ± 3.14
Spleen weight (gr)	2.58 ± 0.25	2.33 ± 0.23	2.41 ± 0.29
Abdominal fat weight (g)	9.47 ± 1.09	9.16 ± 1.17	8.9 ± 1.28
Meat pH	6.29 ± 0.31	6.08 ± 0.27	6.14 ± 0.19
Water holding capacity (%)	45.92 ± 1.54	46.98 ± 1.20	46.02 ± 1.36
Intramuscular fat (%)	3.60 ± 0.33	3.68 ± 0.35	3.55 ± 0.27

[a,b] Least squares means within a row without common superscript differ significantly ($P < 0.05$).

Table 3. Least squares means comparison of IGF-1 genotypes with their standard error for growth and carcass traits of Mazandaran native fowl

Trait	genotype		
	+/+	+/-	-/-
Body weight at 4 weeks (g)	281.85 ± 16.43	275.13 ± 19.62	284.07 ± 19.18
Body weight at 8 weeks (g)	812.86 ± 49.60	765.53 ± 44.31	800.13 ± 53.65
Body weight at 12 weeks (g)	1469.83 ± 72.45	1394.37 ± 81.28	1481.51 ± 79.48
Carcass weight (g)	1057.68 ± 60.04	1003.19 ± 63.47	1081.13 ± 58.52
Heart weight (g)	10.11 ± 0.85	10.39 ± 0.97	10.44 ± 0.89
Liver weight (g)	34.18 ± 2.93	35.66 ± 2.28	35.20 ± 2.79
Gizzard weight (g)	32.35 ± 2.86	31.95 ± 2.53	32.14 ± 3.42
Spleen weight (g)	2.48 ± 0.26	2.41 ± 0.23	2.79 ± 0.31
Abdominal fat weight (g)	10.16 ± 1.02 [a]	9.98 ± 0.91 [a]	7.24 ± 1.08[b]
Meat pH	6.17 ± 0.31	6.21 ± 0.19	6.09 ± 0.26
Water holding capacity (%)	46.31 ± 1.14	46.15 ± 1.22	46.27 ± 1.28
Intramuscular fat (%)	3.88 ± 0.32 [a]	3.86 ± 0.29 [a]	3.01 ± 0.30[b]

[a,b] Least squares means within a row without common superscript differ significantly ($P < 0.05$).

Discussion

In the present study, we elucidated the effects of polymorphism of growth hormone gene on egg production traits in native chickens. We found that the most common genotype was ++ and the least common was – in both GH and GHR genes. In contrast, for IGF-1 gene, the highest and the lowest genotypic frequencies were genotypes of – and ++, respectively. Moreover, the highest allele with the greatest frequency was -, a finding in agreeance of previous work on native chickens (Piryonesi *et al*., 2012). Hardy-Weinberg disequilibrium in the study population was likely due to the small population size or other factors of disturbance, such as selection. Others have also reported a significant association between GH gene polymorphism with body weight of broilers at the age of six weeks (Asghari Ghelghachi *et al*., 2013).

Another study linked GH gene polymorphism with body weight at all ages and daily weight gain at one to four weeks (Nie *et al*., 2005). Similarly, GHR gene polymorphism has also been linked with body weight in broilers and layers (Ouyang *et al*., 2008) as well as body weight and abdominal fat weight in quail (Wang, 2010). IGF-1 gene polymorphism has previously been shown to be associated with intramuscular fat trait in broilers, similar to our results (Zhou *et al*., 2005). Researchers also reported a significant association between polymorphisms in the IGF-1 gene promoter region and growth and carcass composition of broiler (Zhou *et al*., 2005).

Our results show that polymorphisms in the GH, GHR and IGF-1 gene loci are significantly associated with some growth and carcass traits in Mazandaran native chickens. Therefore, the marker-assisted selection can be considered a desirable option for use in breeding programs. Including this data in the optimal selection index can increase genetic progress and improve the response to and accuracy of selection for these traits.

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