POLYMORPHISMS OF GROWTH HORMONE GENE IN A NATIVE CHICKEN POPULATION: ASSOCIATION WITH EGG PRODUCTION

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Abstract

A total of 142 chicken blood samples were collected and a specific primer set was used to amplify a fragment of growth hormone locus using PCR. PCR products were digested with SacI and MspI restriction endonucleases. The amplified fragment digested with SacI enzyme revealed two “+” (wild type) and “−” (normal type) alleles with the frequency of 0.898 and 0.102, respectively. The amplified fragment digested with MspI enzyme revealed three A, B and C alleles with the frequency of 0.599, 0.102, and 0.299, respectively.

Frequencies of +/+ , +/− and −/− were 0.817, 0.162, and 0.021, respectively, and those of AA, AB, AC, BB, BC, and CC were 0.338, 0.113, 0.409, 0.007, 0.070, and 0.063, respectively, in the studied population. The results of $\chi^2$ and likelihood ratio tests showed that this population was at Hardy-Weinberg equilibrium with respect to the marker locus. Marker-trait association analysis revealed statistically significant differences between “SacI-RFLP” genotypes for egg production and rate of laying eggs. The relationship between the molecular marker and these traits can be useful to improve the chicken breeding programmes.

Key words: chicken, growth hormone, genes, polymorphism.

Growth hormone (GH) and the transforming growth factor-β subfamily are the most important groups of hormones that play crucial role in physiological functions such as growth and reproduction (7). The chicken growth hormone (cGH) gene is one of the most important genes that can affect chicken performance traits because of its important function in growth and metabolism (25). The cGH gene contains four exons and five introns with an overall length of 4.1 kb (10). The study was carried out on polymorphisms in the cGH gene by restriction fragment length polymorphisms or DNA sequencing (28).

Studies on the genomic DNA from chicken for singe nucleotide polymorphisms in the growth hormone gene with the use of denaturing high-performance liquid chromatography and sequencing, revealed a total of 46 SNPs, among which four were in the 5' untranslated region, one in the 3' untranslated region, five in exons, and other 36 in introns (19). Association analysis also showed that AvaI genotypes in the third intron of cGH was connected with weight and percentage of abdominal fat pad weight and percentage (29).

Fars native chicken breeding center was settled in Fars province (Iran) several years ago. Genomic information may be transferred faster than the genetic progress; however, such studies are rare in these indigenous chickens. Because of the important role of GH in reproduction (23) and due to lack of information on the allelic attribute of GH locus in breeder hens of the Fars native chicken breeding centre, the GH gene polymorphism in this population was studied.

Material and Methods

Indigenous chicken population. Blood samples were collected from 142 laying hens, randomly selected from an indigenous chicken flock belonging to the Fars Native Chicken Breeding Centre, Iran. This centre was established in 1986 with the aim of promoting indigenous chicken breeding. To form a basis population, the fertilised eggs were collected from cold areas, tropical, and temperate Fars province. Replacement hens and roosters have been under the selection on the basis of age at puberty (first lay), 12-week body weight, 3-month egg production, and mean egg weight at 28, 30, and 32 weeks of age. Genomic
DNA was extracted from blood samples using a modified salting out method.

**PCR amplifications and genotyping.** A pair of primers was designed for genotyping the polymorphisms (Table 1). The primer pair PM1 was used to amplify the 1164-bp fragment of the chicken GH gene containing 2 RFLP of C-2983T and C-2896T.

The PCR was performed in a final volume of 20 μL containing 100 ng of genomic DNA, 0.2 μM of the primer, 200 μM of dNTPs, 1.75 mM MgCl₂, 2 U of Taq DNA polymerase (Sinagene Co., Tehran) and 1× reaction buffer, on a thermocycler with the following profile: initial denaturation of 5 min at 94°C; 35 cycles of 94°C for 30 s, 62°C (primer pair p1) or 54°C (primer pair p2) for 30 s, and 72°C for 2 min with a final elongation of 10 min at 72°C.

The fragment amplified by primer pair PM1 was digested with SacI and MspI endonucleases, and then electrophoresed on 1.5% agarose gel for genotyping the polymorphisms of C-2983T and C-2896T, respectively.

**Allelic frequency.** The allelic frequencies of two polymorphisms were calculated and the test for Hardy-Weinberg equilibrium at each site was conducted separately using the software programme Popgene 1.2 (20).

**Marker-trait association analysis.** Association of single polymorphisms with egg production was analysed using the GLM procedure of SAS (21). The model included genotype, hatch number, age at puberty, and weight at initiation of laying and duration period as fixed effects. Type III sums of squares were used in each F-test. Values were considered significant at P<0.05 and presented as least squares means ± standard errors.

Laying rate was calculated according to the following formula: laying rate % = egg number/day of production 100.

**Results**

**Genotypic and allelic frequencies.** The electrophoretic result of RFLP analysis of the fragment obtained from primer pair PM1 is shown in Figs 1 and 2. Three genotypes were found at the site of -2983 (+/+; +/−, −/−) and six genotypes at the site of -2896 (AA, AB, AC, BB, BC, CC). Allelic and genotypic frequencies are shown in Tables 2 and 3. The frequencies of + and - alleles (SacI-RFLP) were 0.898 and 0.102, respectively; and those of A, B and C (MspI -RFLP) alleles were 0.599, 0.102, and 0.299, respectively.

The studied population was found to be in Hardy-Weinberg equilibrium. The values of chi-square and likelihood ratio test are shown in Table 4.

### Table 1

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Length</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM1</td>
<td>1164</td>
<td>Sense</td>
<td>CTAAAGGACCTGGAAGAAGGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antisense</td>
<td>AACTTGTCGTAGGGTGGTCTG</td>
</tr>
</tbody>
</table>

**Fig. 1.** The electrophoretic result of SacI PCR-RFLP of chicken GH gene. Lane 1: Molecular weight marker genotype.
Fig. 2. The electrophoretic result of MspI PCR-RFLP of chicken GH gene. Lane 1: Molecular weight marker genotype

Table 2
Allelic and genotypic frequencies of the SacI-RFLP sites

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Allelic frequency</th>
<th>Genotypic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SacI-RFLP</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.898</td>
<td>0.102</td>
</tr>
</tbody>
</table>

Table 3
Allelic and genotypic frequencies of the MspI-RFLP sites

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Allelic frequency</th>
<th>Genotypic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MspI-RFLP</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>0.599</td>
<td>0.102</td>
</tr>
</tbody>
</table>

Table 4
Chi-square values and likelihood ratio test

<table>
<thead>
<tr>
<th>Restricted Enzymes</th>
<th>Test</th>
<th>Degree of freedom</th>
<th>Value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MspI</td>
<td>Chi-square</td>
<td>3</td>
<td>2.87&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Likelihood ratio</td>
<td>3</td>
<td>2.99&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.39</td>
</tr>
<tr>
<td>SacI</td>
<td>Chi-square</td>
<td>1</td>
<td>2.1&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Likelihood ratio</td>
<td>1</td>
<td>1.7&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Ns: not significant

**Marker-trait association analysis.** Amongst genotypes of the “SacI-RFLP”, “+/+” genotype produced more eggs than “+/-” and “-/-” genotypes (P<0.05), whereas no significant difference were found between the “+/-” and “-/-” genotypes (Table 5). Amongst “SacI-RFLP” genotypes, rate of laying eggs of “+/-” and “-/-” genotypes were not significantly different from each other, but rate of laying eggs of “+/-“ genotype significantly differ from “+/+” and “-/-” genotypes (P<0.05). In addition, amongst all “SacI-RFLP” genotypes, mean egg weight (28, 30, and 32 weeks of age) was not significantly different. Amongst “MspI-RFLP” genotypes, laying performance (egg number and rate of lay) and mean egg weight (28, 30, and 32 weeks of age) of all genotypes were not significantly different from each other (Table 5).
In the study, a 1164 bp fragment was amplified in the GH locus. This fragment, after digestion with SacI restriction enzyme, produced four fragments with 144, 450, 570, and 1020 bp, which is in accordance with results reported by Kansaku et al. (9). When the 1164 bp fragment was digested with MspI restriction enzyme, it produced four fragments with 482, 578, 586, and 682 bp (Fig. 1). Three polymorphisms were found in the GH gene of Fars indigenous chickens. The frequencies of + (SacI-RFLP) and A (MspI-RFLP) were 0.898, and 0.599, respectively; therefore, these alleles seem to be the preponderant genes affecting egg production in the studied population. The Hardy-Weinberg equilibrium in population indicated that selections in this population were not based on the genetic selection.

In the fourth intron of the GH gene a SacI polymorphism was reported to be correlated with the number of tissues with tumours in Marek’s disease virus-infected White leghorn chickens (16).

As regards the structure of GH gene identified in other animals, like rats (2), cattle (17), sheep (4), pigs (26), humans (6, 8), goats (11), and mice (5), the cGH gene comprises five exons and four introns. The size of the cGH gene is about 3.5 kb (17, 24). RFLPs have been characterised in the introns of cGH gene of White leghorn and it has been suggested that the alleles identified were linked to egg production phenotype, resistance to Marek’s disease, and avian leukosis (12). Genotyping using PCR-RFLPs method were performed in various populations of Chinese native chickens and it was recommended that an allele present in intron 1 might be linked to laying performance (17). In other animals, growth hormone gene polymorphisms have been reported, for example a polymorphism observed in intron 3 of bovine growth hormone (bGH) gene was found to be linked to milk protein content (14). In fact, regulatory elements have been identified in the intron region of the GH gene in various animals (19). A glucocorticoid regulatory element (GRE), which may be responsible for the transcriptional control of the human GH gene, has been located in the first intron of this gene (22, 16). A pituitary-specific transcription factor, GHF1, which was recommended to be involved in the tissue-specific expression of the GH gene, has also been identified in intron 3 of the rainbow trout GH gene (3). These findings suggest that introns in the GH gene might play a crucial role in the regulation of GH gene expression.

In the study, chickens with +/- produced more eggs than those with other genotypes. Chickens with +/- and -/- also had a greater laying rate than those with +/- and -/. Therefore, it may be assumed that the GH gene affected egg production by regulating reproduction in the chickens. GH genotype was reported to be associated with the age at first egg (AFE) and hen day rate of egg production (HDR) was from 274 to 385 d (HDR2), from 386 to 497 d (HDR3), and from AFE to 497 d (total hen/day, egg production HDRT) (7). There was no significant correlation with body weight at housing (HBWT), egg specific gravity (SPG), and egg weight (EWWT).

Selection for abdominal fat appears to affect allele frequencies, and some alleles were associated with juvenile body weight, egg weight, and egg specific gravity (7, 12). A 50 bp deletion in intron 4 of the cGH gene was found in Chinese native Taihe Silkies chickens (18). The cGH gene in Yellow Wai Chow native breed was shown to have one silent substitution, 31 insertions, and other substitutions in introns (23). Genotypes have been characterised in the introns of cGH gene of White leghorn and it has been suggested that the alleles identified were linked to egg production phenotype and avian leukosis (12).

Lei et al. (14) reported that an SNP with G to A substitution of GH gene was associated with abdominal fat pad weight, abdominal fat pad ratio, and crude fat content of the breast muscle. Recently, the effects of bovine growth hormone gene polymorphisms at codons 127 and 172 were assessed on carcass traits and fatty acid compositions in Japanese Black cattle using allele specific-multiplex PCR (1). It was reported that GH

### Table 5

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Egg number</th>
<th>Laying rate (%)</th>
<th>Mean egg weight (g) (28, 30, and 32 weeks of age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SacI-RFLP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/-</td>
<td>50.48 ±0.8a</td>
<td>78.02 ±1.39a</td>
<td>47.04 ±0.29ab</td>
</tr>
<tr>
<td>+/-</td>
<td>38.93 ±1.78a</td>
<td>67.88 ±3.03a</td>
<td>48.14 ±0.66ab</td>
</tr>
<tr>
<td>+/-</td>
<td>35.36 ±5.11a</td>
<td>86.78 ±8.58a</td>
<td>46.34 ±1.79ab</td>
</tr>
<tr>
<td>MspI-RFLP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>48.86 ±1.42m</td>
<td>76.02 ±3.31m</td>
<td>47.15 ±0.45m</td>
</tr>
<tr>
<td>BB</td>
<td>46.95 ±2.43m</td>
<td>71.26 ±5.81m</td>
<td>48.08 ±0.79m</td>
</tr>
<tr>
<td>AC</td>
<td>46.27 ±1.27m</td>
<td>71.37 ±2.1m</td>
<td>47.1 ±0.4m</td>
</tr>
<tr>
<td>BC</td>
<td>48.66 ±9.55m</td>
<td>46.16 ±22.24m</td>
<td>43.13 ±3.01m</td>
</tr>
<tr>
<td>CC</td>
<td>47.34 ±3.07m</td>
<td>77.38 ±7.15m</td>
<td>45.51 ±0.97m</td>
</tr>
</tbody>
</table>

ab means with similar superscript do not differ significantly, Ns: not significant
gene polymorphisms influenced carcass traits and fatty acid composition in these cattle.

In conclusion, an association between egg number and lay rate and SacI-RFLP genotypes were found. These results indicate that GH gene could be a genetic locus or linked to a major gene significantly affecting egg number and rate of lay traits in chickens, and the relationship between these traits may be useful for molecular marker-assisted selection (MAS) to improve the chickens breeding programs.

References


