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Association of growth hormone gene polymorphism with growth and fatness traits in Arian broilers

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Abstract

The aim of the study was to investigate the associations of GH gene polymorphism on chicken growth and body composition traits in Arian broilers. Blood samples randomly collected before slaughter from four different commercial Arian broiler lines (A, B, C and D; 400 samples) at 6 weeks of age. Genomic DNA was extracted and a fragment of 776 bp in size amplified using PCR-RFLP method. To determine the restriction site in intron 1 of GH gene of the animals, the *MspI* endonuclease and the resultant digested products were run on 2% agarose gel studied. Birds were slaughtered at 6 weeks of age and body composition was determined. The treatment of the fragment at GH loci with *MspI* restriction enzyme was revealed A1, A2 and A3 alleles. According to the results, recorded allele was: A_1 allele (414, 217, 125 bp); A_2 allele (125, 147, 237, 267 bp) and for allele A_3 (237, 539 bp), respectively. The comparison of least square means of different genotypes indicated that polymorphism in the GH gene was significantly associated with body weight, ickdrumst weight and percentage of drumstick weight at 6 weeks of age ($P<0.05$). According to the results, GH genes could be candidate gene that can affect some body composition traits in Arian broiler chickens.

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Introduction

Meat quality is a complex structural and functional process that depends on species, genetic background, metabolic status of the antemortem animal, the protein complement of the muscle, and environmental factors. Meat quality relies on several important characteristics, including appearance, color, taste, fat content, texture, and tenderness. Fatness and muscle fiber traits are the major components of meat quality (Lei *et al.*, 2007).

Growth hormone (GH) axis and the transforming growth factor-b subfamily are the most important groups of genes that are involved in a wide variety of physiological functions such as growth, and reproduction. The chicken growth hormone (cGH) gene is considered as one of the most important candidate genes that can influence chicken performance traits because of its crucial function in growth and metabolism (Vasilatos-Younken *et al.*,2000). The cGH gene contains 4 exons and 5 introns with an overall length of 4.1 kb and 5.2 kb in the chicken and duck respectively (Kansaku *et al.,* 2008). Genomic DNA from four divergent chicken breeds was screened for single nucleotide polymorphisms (SNPs) in the cGH gene using denaturing high-performance liquid chromatography and sequencing (Nie *et al.,* 2005). They have found a total of 46 SNPs of which 4 were in the 5' untranslated region, 1 in the 3' untranslated region, 5 in exons and with the remaining 36 in introns. They have found that, among other correlations, G + 1705A was significantly associated with body weight at all ages measured (Nie *et al.*, 2005). Association analysis also shows that AvaI genotypes in the third intron of cGH are related to abdominal fat pad weight and abdominal fat percentage (Zhang *et al.,* 2007).

Previously, Wu *et al.,* (2008) reported there is significant correlation on GH and meat quality in Anka and Rugao broilers. Moreover, it is reported there is positive associated on GH intron 1 and body composition and fatness in duck (Zhang *et al.,* 2010). Based on the previous researches and our hypothesis about possibility of interaction between GH gene and growth parameters, the aim of the current study was to investigate the associations of GH gene polymorphism on Arian chicken growth and body composition traits.

Material and methods

Animal and Blood Samples

Four different commercial broiler lines (A, B, C and D) Arian broiler lines were used in this study (Babol Kenar region, Mazandaran provnce, Iran). Each line was included 4800 chickens which 100 birds from each were randomly selected to perform the study (100 birds from each line; totally 400 birds). At 6 weeks of age before slaughtered blood samples (2ml in EDTA containing tubes) randomly collected from wing vain using disposable syringes in all birds (400 samples) of four different commercial broiler lines (A, B, C and D) and stored at -20 C° until used at hematology laboratory of Iranian Animal Science Research Institute (Karaj, Iran).

Establishment of a PCR-RFLP assay

The PCR primers for the chicken GH gene were used (forward: 5'-ATC CCC AGG CAA ACA TCC TC-3' (PM3-F); reverse: 5'-CCT CGA CAT CCA GCT CAC AT-3' (PM3-R) (Kuhnlein *et al.*, 1997). DNA amplification of each individual bird was performed according to the following conditions: the PCR was performed in a total volume of 25 µL, containing 2 µL of genomic DNA, 10 pmol of each oligonucleotide primer, 2 µL 25 mM MgCl2, 2 µL of 1 mM deoxynucleotide triphosphate mixture, and 1 U of Taq DNA polymerase; cycle parameters were 95 °C for 4 min then 35 cycles of 94 °C for 30 sec, 60 °C for 2 min, and 72 °C for 1.5 min, with a final extension step for 2 min at 72 °C; the PCR products with length 776 bp were digested at 37 °C overnight with 10 U of Hinf I. Restriction digests were electrophoresed for 4 h at 80 V on a 3.5% agarose gel with ethidium bromide, and individual PCR-RFLP fragment sizes in each sample were determined, based on a standard DNA molecular weight marker, by viewing the banding pattern under UV light on the transiluminator.

Carcass characteristics

At 6 weeks of age birds were slaughtered and body weight, carcass, breast, drumstick, wing, lean, abdomen fat weights and percent determined.

Statistical analysis

Data were processed in excel and PCR-RFLP deprived results were analyzed using the PopGene Version 1.31 for genotype polymorphism (Yeh *et al.,* 1999). Phenotypic data include body, carcass, breast, drumstick, wing, lean, abdomen fat weights and percent were analyzed using GLM procedure via MINITAB 14 statistical software. The following model was used:

$y_{ijkl} = \mu + G_i + Sex_j + Line_k +$ Sire (Line) + dam (Line Sire) + e_{ijkl}

Where *Yijkl* is trait analysed in Gene I, II and III of birds m; m is the overall mean of population; genotype (G); fixed effect of Sire; fixed effect of dam and *eijkl* is the random residual error. For treatment showing a main effect, means have compared by least square means of different (LSD).

Results and discussion

The Genomic DNA fragment of 776 bp in size amplified using PCR-RFLP method on 1.5% agarose gel is shown in figure 1.

The PCR-RFLP pattern of digestion of PCR products using MspI enzyme on 2% agarose gel is presented in figure 2. The treatment of the fragment at GH loci with *MspI* restriction enzyme was revealed A_1, A_2 and A_3 alleles. According to the results, recorded allele was: A_1 allele (414, 217, 125 bp); A_2 allele (125, 147, 237, 267 bp) and for allele A_3 (237, 539 bp), respectively.

Table 1. Frequencies of alleles and genotypes on GH in Arian broilers.

Loci	Genotype frequencies												Allele frequencies		
	A_1A_1	N	A_1A_2	N	A_1A_3	N	A_2A_2	N	A_2A_3	N	A_3A_3	N	A_1	A_2	A_3
Line A	0.0808	8	0.202	20	0.2222	22	0.2222	22	0	0	0.2727	27	0.2929	0.3232	0.3838
Line B	0.1157	11	0.3157	30	0.0315	3	0.03157	30	0.1894	18	0.0315	3	0.4316	0.2842	0.2842
Line C	0.0612	6	0.1836	18	0.0714	7	0.2755	27	0.1428	14	0.2653	26	0.2908	0.2347	0.4745
Line D	0.24	24	0	0.09	0.07	4	0.38	28	0.08	8	0.17	17	0.4750	0.1250	0.4000

According to the 3 allele, 6 new genotypes were detected which was similar to previous reports on GH intron 1 gene in Chinese native bird (Ip *et al.,* 2001). Furthermore, Pipalia (2003) reported, polymorphism GH intron 1 gene is presented only in A_1 and A_3 allele which 3 genotypes detected for there locations and the frequency of A_1 is more abundant in different domestic poultry strains. The same result was reported by Thakur *et al.* (2006). Based on the study of Ip *et al.,* (2001) on 4 hybrids of native Chinese birds and broiler or layer strains, similar findings were found in Chinese birds and broilers but the significant results were found between Chinese birds and leghorn layers which 95% for A_1 and zero for A_2, respectively. Moreover, 3 alleles (A_1, A_2 and A_3) and 6 genotypes were reported in Chinese chickens which were 0.54 and 0.18 for A_3 and A_2, respectively. They reported A_2 can be a new location for mutation and *MspI* enzyme activity.

Table 2. Genotypic data of GH hormone for growth and body composition at 6 weeks of age in Arian broilers

Loci	A_1A_1	A_1A_2	A_1A_3	A_2A_2	A_2A_3	A_3A_3	P value
Body weight	2544± 54 ^{a}	2402 ± 55 ^{b}	2451±44 ^{ab}	2550± 38 ^{a}	2521± 30 ^{ab}	2485±45 ^{ab}	0.024
Carcass weight	1737±40	1670±41	1679±33	1764±28	1742±22	1735±33	0.251
Brest weight	576±16	550±16	536±12.7	590±1.3	573±9.6	585±13.7	0.32
drumstick weight	509±13 ^{a}	481±13 ^{b}	482±10.7 ^{b}	497±9.3 ^{ab}	506±7.6 ^{a}	484±10.7 ^{b}	0.03
Wing weight	202±4.7	194±4.3	192±3.7	205±3.3	200±2.7	200±3.3	0.12
Lean weight	384±9.7	380±10.7	370±8.7	388±4	385±5.7	382±7	0.22
% Abdomen fat	26±2	26±2.3	24±1.6	28±1	25±1.6	26±1.6	0.49
% Carcass weight	68±0.53	69±0.54	68±0.43	69±0.37	69±0.3	69±0.43	0.84
% Brest weight	22.6±0.32	22.6±0.33	22±0.27	23±0.23	22.6±0.18	23±0.27	0.147
% Drumstick weight	19.98±0.26 ^{ab}	20±0.26 ^{a}	20± 0.21 ^{a}	19.5±0.18 ^{b}	20±0.14 ^{a}	19.45±0.21 ^{b}	0.048
% Wing weight	7.1±0.1	8.1±0.1	7.8±0.08	8±0.07	7.9±0.05	8±0.08	0.138
% Lean weight	15±0.22	15.9±0.22	15.1±0.18	15.2±0.16	15.3±0.12	15.3±0.12	0.34
% Abdomen fat	1.02±0.07	1.05±0.09	0.99±0.06	1.11±0.05	1.02±0.04	1.04±0.06	0.175

There are significant differences between groups with different codes in a column (superscript letters a, b; $p \leq 0.05$).

Frequencies of alleles and genotypes on GH

Frequencies of alleles and genotypes on GH in Arian broilers are shown in table 1. As shown in the table 1 the most frequency was seen in allele A_3 in lines A and C, whereas it was in allele A_1 for lines B and D. The observed differences can relate to the strain and line differences. In this regard, the same results were reported in Chinese native poultry (Ip *et al.,* 2001). It seems the same results are indicates similarity between Chinese native poultry and Arian broilers but this results are vice versa for Leghorn strain and Arian Broilers.

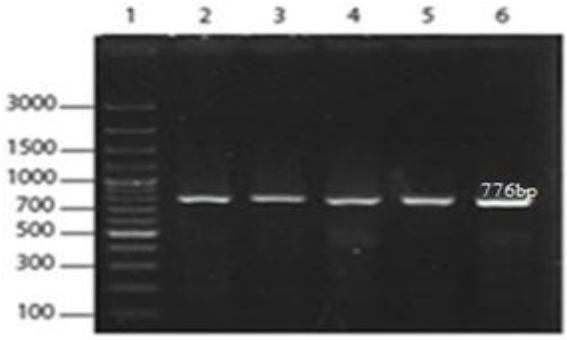

Fig. 1. The Genomic DNA fragment of 776 bp in size amplified using PCR-RFLP method on 1.5% agarose gel.

Based on the previous research on Mazandaran domestic poultry, Enayati *et al.* (2009) polymorphism in GH was higher for A_2 allele (0.81) than A_1 (0.19).

Genotypic data of GH hormone for growth and body composition include body, carcass, breast, drumstick, wing, lean, abdomen fat weights and percent at 6 weeks of age in Arian broilers is presented in table 1.

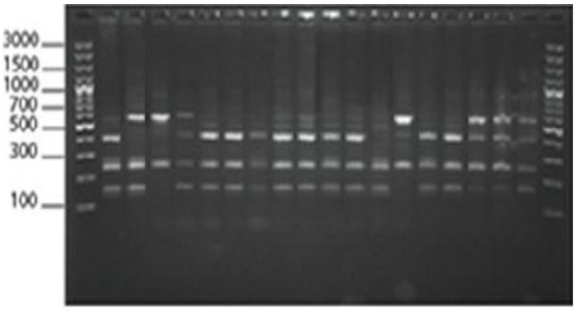

Fig. 2. The PCR-RFLP pattern of digestion of PCR products using MspI enzyme on 2% agarose gel.
A_1A_2 A_2A_3 A_3A_3 A_1A_3 A_1A_1 A_1A_1 A_1A_2 A_1A_1 A_1A_1 A_1A_2 A_1A_1 A_2A_2 A_3A_3 A_1A_2 A_1A_1 A_1A_3 A_1A_3.

According to the results, there is significant difference between GH and drumstick weight and percent in Arian broilers at 6 weeks of age ($P<0.0.5$). In our results, birds with A_2A_2 had better Lean weight and percent than those with A_1A_3 allele. It has been reported that, there is a significant difference between GH and body composition e.g. body weight, breast and drumstick percent in Leghorn and Fayoumi strains at 2, 4, 6 and 8 weeks of age. Additionally, birds with A_2A_2 genotype had better body weight compared to the other groups (Li *et al.,* 2008). Our result was similar to the previous results. Furthermore, in a study on native Anka and Rugao strains, a significant associated was observed between GH and body composition and abdomen fat at 6 weeks of age (Musa and Chen, 2007). The results of this study results were difference with it. In addition, Wu *et al.,* (2008) reported there is a significant correlation between GH and meat quality in Anka and Rugao hens. Moreover, it is reported there is positive associated on GH intron 1 and body composition and fatness in duck (Zhang *et al.,* 2010). Conversely, no correlation was found on GH polymorphism and productive traits in Mazandaranian native poultry (Khadem *et al.,* 2010). It seems the logical reasons for observed differences can because of different strategies of used line breed system or incorporation of marker and QTL. According to the results of this study, it seems there is a significant associated between GH and carcasses composition in Arian broilers. Also, these results can be a good index for proper selection and genetically improvement of domestic poultry. Finally, authors recommends further researches need to clarified direct interaction of GH gene polymorphism with growth and fatness traits in broilers.

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