Research Journal of Biological Sciences 4 (8): 933-936, 2009
ISSN: 1815-8846

Investigation of Leptin's Receptor Gene Polymorphism, by Using PCR-RFLP Technique in Native Poultry Population of Khouzestan Province

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Abstract: Leptin, the product of the obese (ob) gene, is a 16-kDa hormone that has been shown to play an important role in the regulation of food intake, energy expenditure and hypothalamus endocrine function in response to nutritional changes. Chicken leptin receptor is expressed in the hypothalamus, but also in other tissues such as pancreas, where leptin inhibits insulin secretion and thus, may have a key role in regulating nutrient utilization in this species. However, further studies have shown that leptin receptors are also expressed in many other tissues and have suggested that leptin is involved in more diverse biological functions than previously thought. The ability of insulin and leptin to regulate chicken leptin receptor gene expression suggests a direct role of leptin in the control of hepatic metabolism. In order to study of Leptin's Receptor gene (LEPR) polymorphism, by using PCR-RFLP technique in native poultry population of Khouzestan province, a research was planed and done in molecular lab of Ramin agricultural and natural resources. In this research, a fragment of extracted DNA from native poultry blood's samples was amplified with PCR technique. This 2900 bp fragment consists of number 9-11 exon's of LEPR. This fragment was digested with *HaeIII* restricted enzyme. The pictures that were tacked from agarose gel showed 2 allele (A and B) for this fragment. The allele frequencies were 31.19 for A and 61.81 for B. The genotype frequencies were 18.81, 24.75 and 54.44 for AA, AB and BB genotypes, respectively. The studied population was in Hardy-Weinberg disequilibrium.

Key words: Leptin receptor gene, local poultry, polymorphism, hypothalamus, PCR-RFLP

INTRODUCTION

In farm animals, the control and the prediction of fatness is of a high economic interest. The exaggerated adipose tissue development in farm animals negatively affects whole body metabolism, production efficiency, reproduction and meat quality (Taouis *et al*., 2001). Leptin, the product of the ob gene, has been reported to suppress appetite by regulating satiety-centre activities in the brain via its receptor (LEPR) and to affect body weight (Friedman and Halaas, 1998 reported by Niv-Spector *et al*., 2005). Leptin is secreted by mammalian adipocytes and functions as a hormonal sensing mechanism for fat deposition Emilsson *et al*. (1997) and Livnah *et al*. (1999) reported by Niv-Spector *et al*. (2005). Adipocytes produce and secrete more leptin as fat storage increases, signaling the brain to reduce food intake and increase energy expenditure. The homeostatic nature of this mechanism has been the subject of many studies over the last few years (Christopher *et al*., 1999). In poultry, the animal's rapid growth rate has led to excessive body fat associated with impairment of total body metabolism and to disorders in reproductive functions and muscular development resulting in low performance with high mortality (Taouis *et al*., 2001).

As in mammals, chicken leptin expression is regulated by hormonal and nutritional status. This regulation is tissue-specific and with a high sensitivity in the liver compared to adipose tissue. The blood leptin levels are regulated by the nutritional state with high levels in the fed state compared to the fasted state (Taouis *et al*., 2001). In chickens, leptin is expressed mainly in the liver, where its receptor gene expression has also been reported and in adipose tissue.

In view of the key role played by the liver in lipogenesis in avian species, the hepatic expression of leptin may have physiological significance (Cassy *et al*., 2003). The reported sequence of leptin from human, cow, pig, sheep, mouse, rat, dog and most recently, chicken, shows a high degree of sequence conservation. This sequence similarity suggests a common function or mechanism of the peptide hormone across species. The role leptin plays in metabolism and food intake regulation in domestic animals, selected for increased growth and

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energy conversion, is largely unknown (Christopher *et al*., 1999). Variations at DNA level contribute to the genetic characterization of livestock populations and this may help to identify possible hybridization events as well as past evolutionary trends (Vivek *et al*., 2005).

In livestock, such variations In DNA may also be associated with, or linked to, economic traits, which are governed by many genes each having a small effect (Gelderman, 1997). However, the major gene model suggests that only a few genes may account for relatively large proportion of the genetic variation (Lande, 1981), such major genes being the genes usually involved in the biology of a trait and are the candidate genes for marker identification. There is also, the possibility that major genes may be linked with some Quantitative Trait Loci (QTL) contributing to a major part of the variation in traits. Leptin is a protein involved intricately in the growth and metabolism of animals and which plays an important role in the regulation of feed intake, energy metabolism, growth and reproduction of cattle (Ramsay and Cranwell, 1999) and thus, the leptin gene is a potential candidate gene for QTL studies (Vivek *et al*., 2005). Important associations between SNP within the leptin gene with lean yield, fatness (fat yield and subcutaneous fat) and tenderness were detected (Schenkel *et al*., 2005).

MATERIALS AND METHODS

This study was done in molecular lab of Ramin agricultural and natural resources university of Ahvaz in fall and winter of 2008-2009. Seventy one hens (male and female) were selected randomly from native chicken farm of Khuzestan province (this farm was by the control of Agricultural Jahad Organization of the province) and 30 from local village of the province. Sampling was done with gathering blood samples from the wing vein using EDTA as an anti-coagulating agent. After extracting DNA, for assigning DNA quality, using horizontal electrophoresis, the DNA was run on the agarose gel (3.5%). Then, special sequence of receptor leptin genes were amplified by special primers (Forward: 5´-AGAG CGTAGCGTCCAAGAAG-3´ and reverse: 5´-TTCACCAC ATCTGCTGGAAC-3´) by PCR techniques. Consequently, 2900 bp of the DNA fragment was amplified. PCR products were digested by *Hae III* restricted enzyme. Eventually, the length of digested products were measured using horizontal electrophoreses and agarose gel (2.5%).

Statistical analysis: The data were analyzed by Popgene statistical-genetic software. In order to study Hardy-Weinberg equilibrium in the studying fragment in this population chi-square (χ^2) test were done.

RESULTS AND DISCUSSION

In order to study of polymorphisms of the amplified fragment, we need the restricted enzyme that has cutting cite (*s*) on the fragment. When, we have changes on the DNA fragment, we will show a different fragment in length or if we have a mutation on the cutting cite (*s*) we would see the different number of fragments. As a result, we can realize different genotypes using of different fragment in length or numbers.

For this reason, the *Hae III* restricted enzyme that has cutting cites on this fragment was used. Pictures of the results showed three genotype (as a result of two allele) (Fig. 1).

Conventionally, we named them A and B alleles. The digested products were run on the agarose gel (2.5%) for one an hour. Then using ethidumbromid for staining the bonds were observed with UV rays radiate. *Hae III* restricted enzyme has one cutting cite on the A allele and during digestion it produce two bond with 820 and 2080 bp in length (Fig. 1 and 2).

Gene and genotype frequencies were calculated from data of the digested products with *Hae III* restricted enzyme. From 30 rural blood samples, we observed 24 samples with BB genotype and 6 samples with AB genotype (we didn't show any AA genotype). As show in Table 1, BB frequencies were 80 and for AB were 20%.

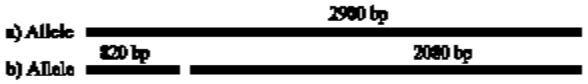

Fig. 1: Results of *HaeIII* restricted enzyme on the PCR products of leptin's receptor gene in native poultry population of Khouzestan province

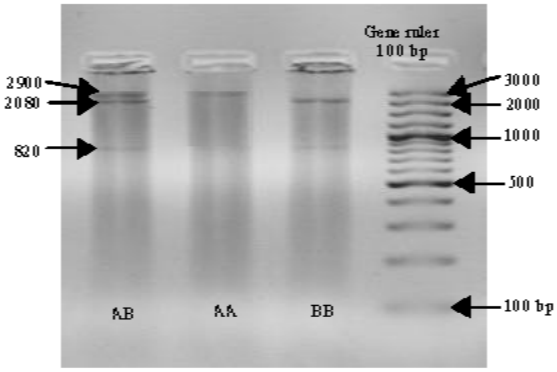

Fig. 2: Electroforesing digested products with *HaeIII* restricted enzyme on agarose gel showed different bonds. The marker that used were 100 base pares

Table 1: Genotypes and alleles frequencies of rural chicken's blood samples (Khouzestan, Iran)

	Genotypes			Alleles	
Variables	AA	AB	BB	A	B
Numbers	0	6	24	6	54
Frequencies (%)	0	20	80	10	90

Table 2: Chi-square (χ^2) test in the 30 blood samples of rural area (Khouzestan, Iran)

	Observations	Expectations		
Genotypes	(O)	(E)	$(O-E)^2/E$	X^2
BB	24	24.25	0.0027	0.304^{NS}
AB	6	5.49	0.0471	-
AA	0	0.25	0.2542	-

Table 3: Genotypes and alleles frequencies of Agricultural Jahad Organization flock (Khouzestan, Iran)

	Genotypes			Alleles	
Variables	AA	AB	BB	A	B
Numbers	19.00	19.00	33.00	57.00	85.00
Frequencies (%)	26.76	26.76	46.48	40.14	59.86

Table 4: Chi-square (χ^2) test in the 71 blood samples of Agricultural Jahad Organization flock (Khouzestan, Iran)

	Observations	Expectations		
Genotypes	(O)	(E)	$(O-E)^2/E$	X^2
BB	33	11.32	5.21	14.41^{**}
AB	19	34.36	6.88	-
AA	19	25.32	3.33	-

Table 5: Chi-square (χ^2) test in the population. As a result we showed that the studying flock is in disequilibrium (18.59>3.84 and 6.63)

	Observations	Expectations		
Genotypes	(O)	(E)	$(O-E)^2/E$	X^2
BB	57	47.72	1.810	18.59^{**}
AB	25	43.57	7.091	-
AA	19	9.72	8.870	-

As a result, frequencies of A and B alleles were calculated 10 and 90%, respectively. Chi-square (χ^2) test on rural consequences showed that these samples are in Hardy-Weinberg equilibrium (Table 2).

Seventy one samples that were obtained from Agricultural Jahad Organization of the Khouzestan province flock were observed 19, 19 and 33 samples for AA, AB and BB genotype, respectively.

Genotype frequencies were 26.76, 26.76 and 46.48% for AA, AB and BB, respectively. As a result, we have 40.14% for A and 59.86% for B frequencies (Table 3). Chi-square (χ^2) test on this results showed that this flock are on Hardy-Weinberg disequilibrium (Table 4).

Altogether in 101 samples genotype frequencies were calculated 18.81, 24.75 and 56.44 for AA, AB and BB genotypes, respectively. A allele frequency was calculate 31.19% and for B it calculated 68.81% (Fig. 3 and 4).

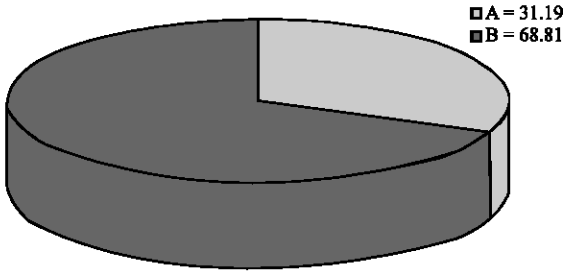

Fig. 3: A and B allele frequencies in studying flock

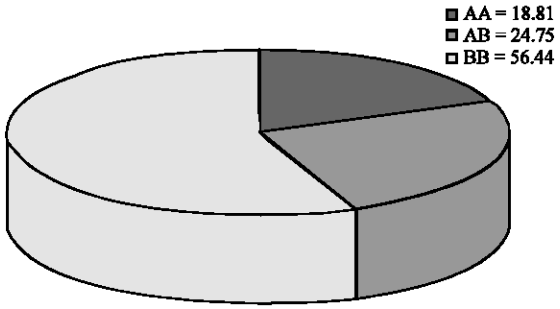

Fig. 4: AA, AB and BB genotype frequencies in studying flock

The results of χ^2-test showed that the population were in disequilibrium of Hardy-Weinberg equilibrium ($p<0.01$) (Table 5).

CONCLUSION

From rural results, we can deduce that randomly copulations that occur on villages without any selection and migration on ChLEPR or its effects. It seems that B allele has more positive effects on the metabolism and economic traits than A allele.

Because the breeding works that have done on the farm of the Agricultural Jahad Organization for increasing yield, rate of produce and economically products, increased B allele. This research is the first one that used these primers, the results of it, isn't comparable with other essays. In fact, this is the first investigation of chicken leptin's receptor gene polymorphism with these special primers, by using PCR-RFLP technique in native poultry.

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