American Journal of Animal and Veterinary Sciences 6 (2): 84-87, 2011
ISSN 1557-4555

Genetic Polymorphism Exon 9-11 at the Leptin Gene Receptor in Breeder Hens of Mazandaran Native Fowls

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Abstract: Problem statement: Leptin a 16 kD protein synthesized by white adipose tissue and involved in regulation of feed intake, energy balance, fertility and immune function. In order to evaluate the leptin gene receptor polymorphism, we have used a Restriction Fragment Length Polymorphism (RFLP) method. **Approach:** Blood samples were collected from randomly chosen 100 native fowls breeding station of Mazandaran. The DNA extraction was based on salting out method and used amplified polymerase chain reaction technique. Exon and intron 9-11 of the fowl leptin gene receptor was amplified to produce a 382 bp fragment. The PCR products were electrophoresed on 1% agarose gel and stained by etidium bromide. **Results:** Then, they were digested of amplicons with Tsp509I and revealed two alleles A and B. Data were analysed using PopGene 32 package. In this population, AA, AB, BB genotype have been identified with the 69.14, 30.16, 0.7% frequencies. A and B alleles frequencies were 0.84, 0.16, respectively. x^2 test didn't signify Hardy-Weinberg equilibrium in this population ($p<0.05$). **Conclusion:** The main objectives of the current strategy in commercial broiler breeding programs aim to increased growth rate, increased breast muscle yield, decreased abdominal fat pad content, increased feed efficiency and increased overall fitness. The obtained results in the present study indicated that the A allele frequency for leptin gene receptor loci was the dominant allele.

Key words: Leptin gene receptor, genetic polymorphism, Restriction Fragment Length Polymorphism (RFLP), mazandaran native fowls, etidium bromide, pivotal role, leptin bind, exogenous administration, selective neuropeptides

INTRODUCTION

Leptin a 16 kDa hormone that has been shown to play an important role in the regulation of feed intake, energy expenditure and hypothalamus endocrine function in response to nutritional changes (Friedman and Halaas, 1998; Elmquist *et al*., 1999). The leptin gene is located on chromosome 6 in the mouse and chromosome 7 in humans and encodes a protein that shows a high degree of homology between species. Mutations in this ob gene revealed the pivotal role of leptin in energy balance (Zhang *et al*., 1994). Recent studies have demonstrated that leptin is produced by other tissues, such as brain, pituitary gland, skeletal muscles, and stomach. In mammals leptin is expressed primarily in adipose tissue (Zhang *et al*., 1994) and at a lower level in the placenta and stomach (Masuzaki *et al*., 1997; Bado *et al*., 1998). Organization of this gene is conserved among mouse, human and bovine, presenting three exons and two introns (Taniguchi *et al*., 2002). Leptin receptors have been located on neurons producing NeuroPeptide Y (NPY) and when activated by leptin binding, it is hypothesized to function in part by down regulating the production of hypohypothalamic NPY (orexigenic effector) to inhibit ingestive behavior (Schwartz *et al*., 1997). Several studies have showed that exogenous administration of leptin decreased feed intake in chicks, which is similar as described in mammals, but the anorexigenic effect within chicken hypothalamus was mediated via selective neuropeptides, such as NPY and orexin (Denbow *et al*., 1997; Dridi *et al*., 2005). The objective of the present study was to genetic polymorphisms exon 9-11 at the leptin gene receptor in breeder hens of Mazandaran native fowls using PCR-RFLP methodology. Association between different allelic and genotypic forms of gene and economical important traits can be found if the polymorphisms are seen. Finding association can improve accuracy and genetic gain in fowls.

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MATERIALS AND METHODS

In this study blood samples were collected from, 100 randomly chosen chicken, in Native fowls breeding station of Mazandaran located in the North of Iran. Approximately, 5 mL blood sample was gathered in EDTA tube and was transferred to -20°C freezer. Genomic DNA was isolated by using DNA Extraction Kit and was based on salting out method. Exon and intron region from a portion of the 9-11 repetitive domain of the leptin gene receptor amplified to a product of 382 bp using primers based on the sequence of the fowl. Spectrophotometer was used for investigating quality and quantity of DNA.

Primers design: One set of primers were designed using Primer3 primer design software (Rozen and Skaletzky, 2000-www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). These primers were designed based on the chicken sequence leptin gene receptor (GenBank-NC006095.2) Gallus gallus chromosome 8, reference assembly (based on Gallus_gallus-2.1). The primers were analyzed on NetPrimer (http:// www. premierbiosoft.com/netprimer netprlaunch/netprlaunch.html), in order to avoid secondary structures, such as hairpins and loops and primer dimmer. Specific primer pairs were prepared from sina gene (Iran) company in lyophilize form and were solved in double striled water and stored at -20°C. Blood purified DNA showed better quantity and quality.

Method of detection: The sequences of the forward and reverse primers for the amplification of the leptin gene receptor were: F 5´-ATAGCTTTGAATGTTGGTGTTCTG-3´ and R 5´-AGCTGTGATACTGACTGCCTGT -3´. The polymerase chain reaction for the leptin gene receptor was performed using a buffer PCR1X, 200 µM dNTPs, 3 µM MgCL2, 8 pmol each primer, 0.15 U taq DNA polymerase, 200 ng genomic DNA and H2O up to a total volume of 25 µL. 35 cycle of preliminary denaturation at 93° (4 min), denaturation at 94°C (1 min), annealing at 58°C (1 min), extention at 72°C (1 min) and final extension at 72°C (4 min). The PCR products were separated by 1% (w/v) agarose gel electrophoresis. The amplified fragment of leptin gene was digested with Tsp509I 11 µL of PCR production with 2 µl buffer, 5U (0.5) of Tsp509I and 11.5 µL H2o up to a total volume of 25 µL, following the manufacturers instruction for 12-16 h at 37°C. The digestion products were electrophoresed on 1% agarose gel in 1X TBE and visualized by eithdium bromide staining for 1 h at 85 V.

Statistical analysis: Pop Gene 32 package (Yeh *et al*., 1999) was used to calculate genotypic Estimates and allelic frequencies and to detect state of population about Hardy-Weinberg equilibrium.

RESULTS

The specific primer pairs were designed from sequence of leptin receptor gene in genbank NCBI site. The amplified leptin gene receptor resulted in a DNA fragment with 382 bp including the sequences of Exon and intron regions from a portion with PCR technique Fig. 1. The Tsp509I restriction enzyme has restriction site of ((AATT)) in the amplified segment and cut this site after second ((A)) base. If the enzyme cut the segment, two alleles (A and B) were observed, resulting in three genotypes. The Tsp509I digests the allele A amplimer, but not allele B. The animals with both alleles were assigned as AB genotype, whereas those possessing only A or B alleles as AA or BB genotypes, respectively. Genotype AA showed two band pattern (bands of approximately 276 and 106 bp). Genotype BB-one-band pattern (approximately 382), while AB animals displayed a pattern with all three- band (382, 276, 106) Fig. 2. x^2 test didn't signify Hardy-Weinberg equilibrium in this population($p<0.05$). The observed and expected hetrozygosity were 0.19 and 0.27, respectively.

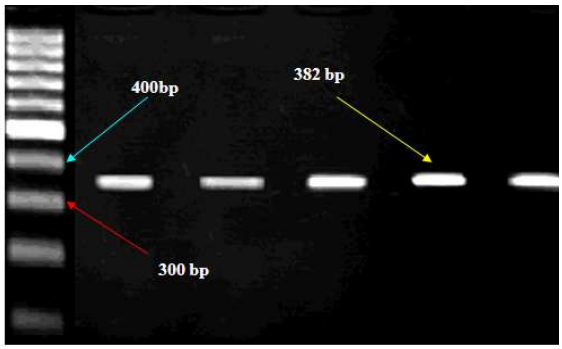

Fig. 1: various samples obtained from PCR

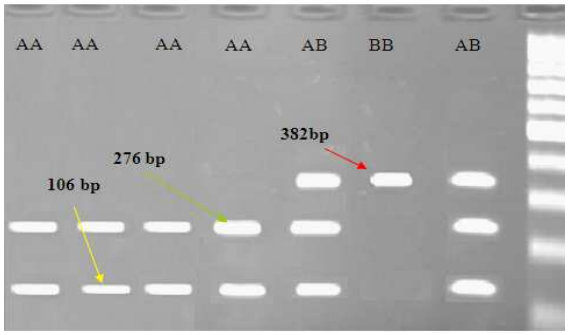

Fig. 2: Results of analysis PCR-RFLP for leptin gene receptor by restriction enzyme Tsp509I on 1% agarose gel on ladder 100bp (fermentas)

DISCUSSION

The digestion of all samples showed breeder hens Mazandaran native fowls were polymorph for leptin receptor gene. This result showed that there were polymorphism in leptin gene receptor segment, as previously observed by Mokhtarzadeh *et al*. (2009). A and B allele frequencies were 0.84 and 0.16, respectively. The genotype frequencies within 100 breeder hens examined were 69.14 for AA, 0.7 for BB and 30.16 for AB. To confirm of accuracy of digestion, this process was performed twice. The low diversity and the difference between effective and true allele number and is due to more frequency of allele A compared to allele B, that reduced frequency in this locus. This number is more, if there are more loci with same combination of alleles. Investigation of mRNA of leptin receptor gene eighteen exons (Almeidate *et al*., 2003). Cloning study revealed expression of leptin gene in chicken's liver (Taouis *et al*., 1998). Also, observed the polymorphisms of leptin receptor gene were surveyed in Khoozestan native fowl population using RFLP-PCR. It showed that change in restriction site of HaeIII generated different restricted segments. Three genotypes and two alleles were seen in Khoozestan fowls (Mokhtarzadeh *et al*., 2009). The allelic frequencies of leptin gene receptor in Khoozestan fowls were 31.19 and 61.81 for alleles A and B and genotypic frequencies were 18.81, 24.75 and 54.44 for genotypes AA, AB and BB, respectively. Results showed deviation from Hardy-Weinberg equilibrium in population of Khoozestan fowls. Association study revealed allele A had positive effect than allele B on economical traits. The observed and expected heterozygosities were 0.243 and 0.568. Obtained results from Mazandaran native fowls are antonymous with Khoozestan fowls. The frequency of mutant allele is higher than wild type allele in Mazandaran native fowls. It could due to physiological role of allele A in Iranian fowls. Inbreeding and family selection can be one of the major factors for enhancing of AA genotype in Mazandaran native fowls. Reared chickens in Mazandaran native fowls breeding station are prepared from state center and in addition it is a closed population and therefore is open for disequilibrium factors. Inbreeding coefficient is high in the closed population that, in turn, caused to decreases of diversity in population. Increasing effective population size, controlling mating and preparing independent populations with large number of primitive individuals are necessary for preventing decrease of diversity in Mazandaran native fowls.

CONCLUSION

The main objectives of the current strategy in commercial broiler breeding programs aim to increased growth rate, increased breast muscle yield, decreased abdominal fat pad content, increased feed efficiency and increased overall fitness. The obtained results in the present study indicated that the A allele frequency in leptin gene receptor loci was the dominant allele. It can be concluded that the incidence of higher A allele frequency for leptin gene receptor loci may be as a long term selection strategy sued in this population. Further association analysis will be required to clarify the effects of these marker genotypes on production traits in this breeder flock.

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