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Research Article

Identification of single nucleotide polymorphisms of Hsp70 gene in a commercial broiler strain

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Abstract

The aim of this study was to identify single nucleotide polymorphisms of heat shock protein (hsp70) at exon region in Ross 308 commercial broiler strains during 6 to 7 week of age. Hsp70 coding region was amplified by PCR technique using specific primers with 359bp. Quality was performed by loading in an agarose gel and single nucleotide polymorphism was identified by direct sequencing technique. The results of the sequences were determined by using the Multi-Align and Chromas software's and possible single nucleotide polymorphisms were detected among the different samples. A comparison of the sequence of samples specified in the Multi-Align showed that sequences of samples are different in four specific loci. Sequence comparison of different samples by Multi-Align revealed that mutations happened at G36, G44, G132 and G335 loci. Sequence was compared with obtained data from NCBI database using NCBI Blast-Multi-Align site. The results showed missing T and A at locus G36 and locus G44 respectively. Also the transition of G to A at locus G132 and C to G at locus G335 was observed. The frequency of A and G at G132 is 0.3438 and 0.6265 respectively, also the frequency of G and C at G335 was 0.3312 and 0.6687 respectively. The results show that there is polymorphism in hsp70 in Ross308 which can be used as a tool in future breeding program.

Keywords: hsp70 gene; broilers Ross308; single nucleotide polymorphism

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Introduction

A common event observed in chicken during thermo-resistance is the high expression of heat shock protein (HSP) gene (Mahmoud et al., 2003; Hagiwara et al., 2007). The intensity of the response to heat stress is determined by the rate, duration and magnitude of the change in temperature (Cheng et al., 1982). The environmental conditions, genotype, and state of acclimation of the organism determine the threshold temperature for the induction of HSP gene (Vayda and Yuan, 1994).

Heat stress is one of the most challenging environmental conditions affecting commercial poultry. Compared to other species of domestic animals, broiler chickens are more sensitive to high ambient temperatures. They have no sweat glands, a rapid metabolism, and high body temperature. Furthermore, fast-growing lean broilers generate more heat than their free-living counterparts living in the wild (Geraert et al., 1993). These physiological characteristics, in combination with confined housing, make it difficult for broilers to regulate their heat balance (Van der Hel et al., 1992; Geraert et al., 1996; Mashaly et al., 2004).

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Pale, soft, exudative-like changes in meat quality have been observed in broilers exposed to acute or short-term heat stress immediately pre-slaughter (Northcutt et al., 1994; Sandercock et al., 2001). Genetic association between growth rate of broiler and response to high environmental temperature (Cahaner and Leenstra, 1992; Settar et al., 1999; Deeb and Cahaner, 2001), nutritional treatments (Bonnet et al., 1997), and management techniques (Wiernusz and Teeter, 1996; Zhou et al., 1998; Basillio et al., 2001; Yalçın et al., 2003) were studied to examine ways to cope with the heat stress.

Heat stress in birds is one of the major concerns in the poultry industry, since it causes high mortality and/or low productivity, especially during the hot season (Eberhart and Washburn, 1993). When the ambient temperature is high, the chicken show less ability to heat (Yahav, 2009). It is well demonstrated that economic losses occur due to high environmental temperature in broiler production (Cahaner and Leenstra, 1992; Yalçın et al., 1997; Berrong and Washburn, 1998). The most common problem are reduced consumption, lack of proper feed efficiency, carcass quality, weight loss and reduced immune defense during heat stress (Bottje and Harrison, 1985). Heat shock protein (hsp70) protects proteins and prevents their extra accumulation (Sugimoto et al., 2003). Studies on the identification of hsp70 gene polymorphism has shown that birds that were more resistant to heat stress, have only one allele hsp70 form, while other species showed two different alleles for the gene (Mazzi et al., 2003). Mahmoud (2000) analyzed the hsp70 gene in birds submitted to heat stress and found polymorphic sites located upstream from the coding region. The birds that were more resistant to heat showed only one PstI hsp70 allele of 6.48 kb, whereas the other breeds showed two different alleles for that gene.

The purpose of this study was to evaluate the presence of polymorphisms in exon regions of the hsp70 gene. The existence of polymorphisms opens up the possibility that one of them might be associated with the phenotype that shows more tolerance to heat.

Materials and Methods

In this study, 4 ml blood was collected in EDTA test tube from a total of 80 pieces of broiler chickens (Ross 308) at the age of 6 to 7 weeks. Genetic material from each sample was extracted and stored at -20°C by modified salting out method. PCR amplification was performed using primers designed by Mazzi et al. (2003) (Table 1). The reactions was set up and placed in the thermal cycler.

Table 1: Primer sequences used in PCR

Primer	Sequence
F	5' - AACCGCACCACACCCAGCTATG - 3'
R:	5' - CTGGGAGTCGTTGAAGTAAGCG - 3'

PCR was performed in a 25µl reaction volume containing: 100ng genomic DNA, 0.2µM of each primer, 1x buffer (including 2mM MgCl2), 100µM dNTPs (dATP, dTTP, dCTP and dGTP) and 1 unit of Taq DNA polymerase. The cycling protocol was 2 min at 95ºC, 35 cycles of denaturing at 95ºC for 1 min, annealing at 64.5ºC for 1 min, extending at 72ºC for 1 min, with a final extension at 72ºC for 10 min.

The result of amplified gene region was used for identification of single nucleotide polymorphism (SNP) by direct sequencing method. The results of define sequences for all samples were used for any SNP by Chromas and then in Multi-Align software's. Estimates genotype, alleles frequencies, heterozygosity (He) and polymorphism information content (PIC) were directly calculated, and Hardy-Weinberg equilibrium for population was analyzed using x^2 and G^2 test, which was performed by PopGene software (version 32).

Results

The polymorphism and genetic diversity are the number of observed alleles, number of effective alleles and heterozygosis which was calculated by the method of Nei (1987). Table 2 shows the number of observed alleles, number of effective alleles, Shannon index information for G132 and G335 loci, as well it indicated rate of expected and observed heterozygous and homozygous at above loci.

To study the single nucleotide polymorphisms (SNP) in the exon region of the gene hsp70, all PCR products were evaluated in the reaction (Sequencing). The results of sequencing chromes software revealed in all the samples fragment length of 359 bp were amplified. In order to identify single nucleotide polymorphisms, sequence for each of the samples was evaluated in the software and a text file containing sequence chromes were prepared. Then this file was

Table 2: Information of polymorphism at G132 and G335 loci

Locus	Sample Size	Na	Ne	I	Obs_Hom	Obs_Het	Exp_Hom	Exp_Het*	Nei	Ave_Het
Hsp70 G132	160	2	1.8221	0.6435	0.7625	0.2375	0.5460	0.4540	0.4512	0.4212
Hsp70 G335	160	2	1.7955	0.6351	0.3375	0.6625	0.5542	0.4458	0.4430	0.4430

Na: number of observed alleles, Ne: number of effective alleles, I: Information Shannon index, Obs_Hom: homozygous observed, Obs_Het: heterozygotes observed, Exp_Hom: homozygous expected, Exp_Het *: heterozygous expected, Nei: Nei-Index, Ave_Het: Average heterozygous.

Fig. 1: Sequences of genotype was discovered in Multi Align Software

Table 3: The genotype and allele frequencies at G132 locus

Frequencies	Alleles	Frequencies	Genotype
0.3438	A	0.5375	GG
0.6562	G	0.2375	GA
		0.225	AA

Table 4: The genotype and allele frequencies at G335 locus

Frequencies	Alleles	Frequencies	Genotype
0.3312	G	0	GG
0.6687	C	0.6625	GC
		0.3375	CC

used online by Multi-Align software according to Figure 1.

This software can put together the same sequence of samples. Thus, differences arising from single bases at amplified fragment length genome will detected in samples. Single nucleotide polymorphisms have been identified in the application of Multi Align, all sequences samples were examined by Chromas software to determine the genotypes and the curves mapped for the bases, and genotype was identified. The results were according to the Table 3 and Table 4. In this study, the frequency of genes and genotypes for 80 Ross 308 broilers was done.

Hardy-Weinberg equilibrium, Chi-square and G–square test were examined for all broilers at G132 and G335 (Table 5 & 6). The results of Table 5 shows population in the G-square and Chi-square test cannot be stand under Hardy-Weinberg equilibrium at G132 ($P<0.01$). Also according to this table, the number of sample that observed in GG homozygous is greater than GA and AA. This observation suggests that the selection was conducted strongly.

The results of Table 6 shows that population in the G-square and Chi-square test were not under Hardy-Weinberg equilibrium at G335 ($P<0.01$). Also according to this Table, the number of people who observed in GG homozygous is greater than GA and AA. Also according to this table, the number of observed heterozygosity in the locus was higher than the homozygous.

Discussion

In the present study, four SNPs in the HSP70 exone region were found in broiler chickens. According to the result, Mazzi et al. (2003) amplified a 359bp fragment length polymorphism in the hsp70 gene which is consistent with our findings. A comparison of the sequence of samples specified in the Software Multi Align showed differences in four specific loci. Comparison of different samples demonstrated mutations in the locus G36, G44, G132, G335. Sequences were compared with the NCBI data base using the Blast-Multi Align in NCBI site. Comparison determined sequences with the sequence obtained from NCBI database showed the missing T at locus G36 and A in the G44, as well as the replacement of G by A at position G132 and C by G at locus G335.

Zhang et al. (2002) discovered the polymorphism in the coding region of hsp70 gene in chicken with the ability to tolerate heat through PCR-SSCP and sequencing. Polymorphisms in the hsp70 gene with PstI were also observed in other species such as pig (Ruohonen-Lehto et al., 1993). In addition, Zhang et al. (2002) detected through PCR-SSCP and sequencing, polymorphism regulatory and coding regions of the hsp70 gene in chickens with different heat tolerance capability. Huang et al. (2002) detected single nucleotide polymorphisms (SNPs) in the 5' flanking region of the hsp70-2 gene in boars.

Table 5: Results of the test and χ^2 & G^2 and probability levels in Hardy-Weinberg equilibrium at G132

Genotype	Observed people	expected People	$(0-E)^2/E$	2*O*Ln(O/E)	(χ^2_T)	Probability (χ^2_T)	(χ^2_G)	Probability (χ^2_G)
GG	43	34.3396	2.1841	19.3414				
GA	19	36.3208	8.2600	-24.2261	18.474635	0.000017	18.339121	0.000018
AA	18	9.3396	8.0305	23.6198				

Table 6: Results of the test and χ^2 & G^2 and probability levels in Hardy-Weinberg equilibrium at G335

Genotype	Observed people	expected People	$(0-E)^2/E$	2*O*Ln(O/E)	(χ^2_T)	Probability (χ^2_T)	(χ^2_G)	Probability (χ^2_G)
GG	0	8.6667	8.6667	0.0000				
GC	53	35.6667	8.4237	41.9840	19.196262	0.000012	26.951486	0.0000
CC	27	35.6667	2.1059	-15.0325				

Conclusion

It appears that selective breeding has resulted in alterations in the physiology and concomitantly the ability to withstand high ambient temperature compared with resistant. Domestication and selective breeding are leading to more susceptibility to stress rather than resistant. Heat-shock proteins (Hsp), known as stress proteins and play important roles in the folding, translocation, and refolding/degradation of proteins can affect the heat resistant. In this study, we identified four Hsp SNP in Ross broiler strain for first time, which displayed conserved Hsp characteristics in their predicted heat stress behaviour. Two of them do not contain replacement and no genotyping (G36, G44) showing only missing model and the others exhibited replacement model and were named as G132 and G335. These results suggest that despite intense selection systems in broilers, polymorphisms of Hsp70 may participate in the defence against heat stress. It is clear that genetic differences between different strains can affect body weight in any ages and size, and these differences can affect resistance to heat stress. Therefore, gene expression studies can be valuable in this case.

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