UDC 636.52/.58:575.113/.118

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GENETIC STRUCTURE OF TWO LINES OF HENS OF THE COMBINED DIRECTION OF PRODUCTIVITY ON LOCUSES MSTN AND TLR4

The purpose. To study genetic structure of populations of hens of line G2 (Plimutrok Belyi) and line 38 (Rodisland Krasnyi) on locuses *MSTN* and *TLR4*. **Methods.** Polymerase chain reaction — polymorphism of length of destruction fragments. **Results.** Frequencies of alleles A and G on locus *MSTN* have made 0,33 and 0,67 in line G2; 0 and 1 in line 38; of alleles A and B on locus *TLR4* — 0,3 and 0,7 in line G2; 0,66 and 0,34 in line 38. **Conclusions.** Gene *TLR4* is polymorphic in both lines, and *MSTN* — only in line G2. On gene *MSTN* the deflexion from genetic equilibrium in line G2 is fixed. The probed populations are essentially differ on genetic structure on both locuses.

Key words: genetic structure, hens, restriction analysis, gene of myostatinum *(MSTN)*, gene of tall-like receptor 4 *(TLR4)*.

Lately, increasing attention is given to studying of functional polymorphism of candidate genes and researching of genetic-populational structure of lines and breeds of poultry on DNA-markers with the aim of marker-assisted selection [1, 3, 5, 9]. Such candidate genes include myostatin gene (*MSTN*), which is negative regulator of the growth of skeletal muscles, and toll-like receptor gene 4 (*TLR4*), that takes part in the immune response activation [4, 6-8, 10]. Mutations that have been selected for studying – transition G2109A (HpaII-polymorphism) in *MSTN* first exon, which associated with body weight, breast muscle weight and abdominal fat weight, and transversion G3954C (Sau96I -polymorphism) in *TLR4* second exon, which associated with bacterial load on spleen in some chicken breeds [4, 7, 10]. For Ukrainian population of dual-purpose chicken (line G2 of White Plymouth Rock – meat-and-egg and line 38 of Rhode Island Red – egg-and-

meat productivity types of breeds) data about the genetic structure on mentioned mutations are not available, and this determines the relevance and the newness of this investigation.

The purpose. To study the genetic structure of population of chicken of line G2 of White Plymouth Rock breed and line 38 of Rhode Island Red breed on *MSTN* and *TLR4* loci.

Materials and methods. Investigations were conducted in laboratory of disease prevention and molecular diagnostics of State Poultry Research Station of NAAS. It was chosen on 50 chicken from line G2 and line 38 by the method of random selection. The biological material from chicken was taken by the method “a blood drop on paper”. DNA was extracted by “ДНК-сорб В” kit (“АмплиСенс”, RF).

Individuals were genotyping by PCR-RFLP method. PCR was set by DreamTaq PCR Master Mix kit (“Thermo Scientific”, USA) by such protocol: 1 cycle – 94°C/5 min; 35 cycles – 94°C/30 s, 62°C/30 s, 72°C/30 s; 1 cycle – 72°C/5 min. *MSTN* fragment (298 bp) was amplified using primer pair 5′-aaccaatcgtcggttttgac-3′, 5′-cgttctctgtgggctgacta-3′; *TLR4* (257 bp) – primer pair 5′-cctggacttggacctcag-3′, 5′-ggactgaaagctgcacatc-3′ (final concentration – 0,2 μM). Then, it was performed restriction using HpaII enzyme for *MSTN* and Sau96I enzyme for *TLR4* by manufacture’s protocols (“Thermo Scientific”, USA). Restriction fragments were separated in 1,5% agarose gel, lengths of fragments were determined by molecular mass marker M-50. The visualization of DNA- fragments was performed using ethidium bromide under UV-light.

To analyze genetic structure we calculated genotype and allele frequencies and estimated accordance of genotype frequencies distribution to Hardy-Weinberg equilibrium using χ^2 by common methods [2].

Results. Genotypes of individuals were determined by specific restriction patterns of each amplified fragment. *MSTN* gene of HpaII-polymorphism has two alleles – A (restriction sites for HpaII is absent within amplified fragment) and G (single restriction site). On electropherogram AA genotype is corresponded to

single fragment (298 bp), GG – two fragments (259 and 39 bp), AG – three fragments (298, 259 and 39 bp). Amplified fragment of *TLR4* gene have one polymorphic and two monomorphic restriction sites for Sau96I, that determines the existing of alleles A (without polymorphic restriction site) and B (with polymorphic restriction site). On electropherogram AA genotype was determined by fragments of length of 128, 118, 10 bp, BB genotype – 119, 89, 39, 10 bp, AB –
128, 119, 89, 39, 10 bp.

On *MSTN* locus genotypes frequencies in line G2 of White Plymouth Rock breed were: AA – 0,20; AG – 0,27; GG – 0,53, and frequencies of A and G alleles were 0,33 and 0,67 respectively. In line 38 of Rhode Island Red breed were revealed only individuals of GG genotype, that is evidence of absence of polymorphism on this locus. *TLR4* locus was found as polymorphic in both lines. In line G2 genotypes frequencies were: AA – 0,06; AB – 0,48; BB – 0,46. In line
38 genotypes values were: AA – 0,44; AB – 0,44; BB – 0,12 respectively. Frequency of allele A in line G2 was 0,30; in line 38 – 0,66; allele B - 0,70 та 0,34 respectively; that is population of White Plymouth Rock chicken was characterized by prevalence of allele B frequency, and population of Rhode Island Red chicken, on the contrary, of allele A.

It was shown through χ^2 method that it takes place the deviation from Hardy- Weinberg equilibrium on *MSTN* gene in population of line G2 of White Plymouth Rock breed. On *TLR4* gene both population is in the condition of genetic equilibrium. Data about factual and theoretical ratio of number of individuals with different genotypes is represented is table.

Results of investigation suggest significant differences in genetic structure between studied populations on both investigated loci, despite on fact that both lines belong to combined (or dual-purpose) type of productivity. Received data on genetic structure suggest the advisability of further work with both studied genes in line G2 and with *TLR4* gene in line 38 in a cut of marker-assisted selection.

Genetic structure of investigated lines of chicken of *MSTN* and *TLR4* loci (Vivarium of SPRS of NAAS, 2014)

Locus	Genotype	Line G2				Line 38			
		O	E	$(O-E)^2/E$	χ^2	O	E	$(O-E)^2/E$	χ^2
MSTN	AA	10	5,5	3,727	8,320	0	-	-	-
	AG	13	22,0	3,682		0	-	-	
	GG	27	22,5	0,911		50	-	-	
TLR4	AA	3	4,5	0,500	1,021	22	21,8	0,002	0,016
	AB	24	21,0	0,429		22	22,4	0,007	
	BB	23	24,5	0,092		6	5,8	0,007	

Note. O – factual and E – theoretically expected number of individual with marked genotypes.

Conclusions.

It was found that *TLR4* gene is polymorphic in both lines, whereas *MSTN* – only in line G2. On *TLR4* gene investigated population is in the condition of genetic equilibrium, whereas on *MSTN* gene in line G2 is observed the deviation from Hardy-Weinberg equilibrium. Studied populations have the significant differences on genetic structure on both loci.

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