OVINE DRB1 POLYMORPHISM AND ITS ASSOCIATIONS WITH BODY WEIGHT, MILK CONTENTS AND IMMUNOLOGICAL PARAMETERS

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Abstract

The DRB1 gene is known as a part of the Major Histocompatibility Complex (MHC) with important effects on disease resistance and immunological response in mammals. Single strand conformational polymorphism (SSCP) analysis and direct DNA sequencing methods were employed to study the second exon of DRB1 gene polymorphism in Mehraban and Lori-Bakhtiari sheep, using 155 Mehraban and 167 Lori-Bakhtiari (totally 322) ewes. Eleven different SSCP patterns with 26 SNPs (five new mutations) were found in both breeds. Associations of different genotypes with some traits including body weight, milk contents, milk somatic cell count and some immunological parameters were also studied. The observed genotypes had significant associations ($P < 0.05$) with immunological parameters including serum total protein, $\alpha 2$ -globulin, β -globulin, γ -globulin and total globulins and body weight at 3 months of age (weaning). The effects of the genotypes on serum albumin and $\alpha 1$ -globulin, body weights at 9 and 12 months of age and milk lactose tended to be significant ($P < 0.10$). The results of the present study indicated that the DRB1 exon 2 is highly polymorphic and could be considered as an important fragment in marker assisted selection, especially for improvement of immunity in sheep.

Key words: *DRB1* gene, polymorphism, production, immunity

The candidate gene study is widely used for detection of genes affecting important traits in farm animals and more polymorphic loci are more valuable in candidate gene studies, because a more polymorphic gene is more likely to have a significant association with the studied traits.

The Major Histocompatibility Complex (MHC) contains the most polymorphic loci known in vertebrates (Sommer, 2005). The MHC class II genes encode glycoproteins which bind and present pathogenic peptide fragments to circulating CD4+ T cells (Fremont et al., 1996). Six genes of MHC class II, DRB1, DRA1, DQA1, DQB1, DQA2, and DQB2 have been transcribed (Deverson et al., 1991; Fabb et al., 1993).

The DRB1 exon 2 encodes the β 1 domain which constitutes a part of the peptide binding region of the DR molecules. The DRB1 exon 2 is highly polymorphic in mammals (Ripoli et al., 2004; Dukkupati et al., 2006) and highly variable residues in this region are likely to be related to some functionalities such as disease resistance or susceptibility. Therefore, the DRB1 exon 2 is a principal target in genotyping studies designed to investigate the association of MHC diversity with important economic traits, especially resistance or susceptibility to diseases (Dukkupati et al., 2006).

Diversity in the exon 2 of DRB1 has been identified using a range of methods such as polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis (Gruszczyńska et al., 2005), cloning and sequencing (Ballingall et al., 2008), single-stranded conformational polymorphism (SSCP) method (Arrieta-Aguirre et al., 2006) and direct sequence analysis of PCR products (Arrieta-Aguirre et al., 2006; Ballingall et al., 2008).

The objectives of the present study were evaluation of DRB1 exon 2 polymorphism and study of its associations with some production traits and immunity parameters in Mehraban and Lori-Bakhtiari sheep.

Material and methods

Animals and sampling

A total of 155 Mehraban and 167 Lori-Bakhtiari ewes (totally 322 ewes) were randomly selected from different regions of Hamedan and Chaharmahal-o-Bakhtiari provinces in west Iran, respectively. The studied populations were large enough with no selection program or immigration. Both breeds were maintained under semi-extensive farm conditions and allowed to graze on medium vegetation ranges. Averages of birth and weaning (90 d) weights are 3.88 kg and 21.58 kg in Mehraban sheep and 4.9 kg and 27.7 kg in Lori-Bakhtiari sheep, respectively. Daily milk yield in both breeds is less than 1 kg/d (Salehian Dehkordi and Zamani, 2015). Averages of lactation length in Mehraban and Lori-Bakhtiari sheep are 177 and 168 d, respectively and generally, milk yield peaks around 2nd week of lactation and decreases afterwards (Izadifard and Zamiri, 1997).

Blood samples (5 ml per ewe), used for DNA extraction, were collected from jugular vein by EDTA-containing venoject tubes. Body weights were measured at birth, 3 (weaning), 6, 9 and 12 months of age. Two milk samples per ewe were collected in 4th and 5th weeks of the first lactation. Blood serums were prepared using the blood samples collected from jugular vein in 24th month of age. Serum electrophoresis was performed by an automated capillary electrophoresis (Sebia, France, software version 4.51). Serum total protein was measured by a BT3000 plus (Biotechnica Instruments SpA, Rome, Italy).

DNA and PCR conditions

Genomic DNA was extracted from whole blood using CinnaGen DNA extraction kit (CinnaGen Co, Iran) and stored at -20°C . A pair of primers was used to amplify a

285-bp fragment in exon 2 of *DRB1* gene according to Arrieta-Aguirre et al. (2006), as follows:

Forward: 5'-TATCCCGTCTCTGCAGCACATTTC-3'

Reverse: 5'-TCGCCGCTGCACACTGAAACTCTC-3'

Polymerase chain reaction (PCR) was performed in a mixture containing 10x PCR buffer, 200 mM dNTPs, 1 U of *Taq* DNA polymerase, 0.4 μ M forward and reverse primers, 50–100 ng DNA, 1.5 μ M $MgCl_2$ and sterilized distilled water to make a final volume of 50 μ L. PCR reactions were run on a thermal cycler (Master Cycler, Eppendorf, Germany) under following conditions: initial denaturation for 5 min at 95°C followed by 32 cycles consisting of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 30 s and a final extension at 72°C for 5 min. The PCR products were run on 1% agarose gel in a voltage of 100 V for 25 minutes to ensure that the reaction worked.

Single-strand conformational polymorphism (SSCP) analysis

Five μ L of the PCR products were mixed with 5 μ L of denaturing loading dye [95% (w/v) deionized formamide, 0.05% (w/v) xylene cyanol, 0.05% (w/v) bromophenol blue and 0.02 M EDTA]. The PCR products were denatured at 95°C for 10 minutes and immediately chilled on ice for 10 minutes. The total volume was applied on 12% polyacrylamide gel (19:1 acrylamide to bisacrylamide). Vertical electrophoresis was carried out in a TBE buffer (100 mM Tris, 9 mM boric acid, and 1 mM EDTA) at an ambient temperature of 10°C and voltage of 220 V for 7 hours. The gels were silver stained according to Bassam et al. (1991).

DNA Sequencing

Two samples from each SSCP pattern were randomly selected for DNA sequencing. The PCR products were sequenced using the same primers of PCR (Bioneer Co., Korea). Sequence alignments, translations and comparisons were carried out using ExPASy translation tools website (available at <http://us.expasy.org/translate-tool/>) and MegAlign module of DNASTAR software (DNASTAR Inc., Madison, WI, USA) by Clustal W method. The sequences of PCR amplicons were compared with 100 NCBI sequences.

Protein structure

The amino acid sequences and secondary structures were predicted for different SSCP patterns and a referring sequence (GenBank accession number FR848373.1). EditSeq of the DNASTAR software (DNASTAR Inc., Madison, WI, USA) and ExPASy translate tools (<http://us.expasy.org/translate-tool/>) were used to enter mutations and translation of nucleotide to amino acid sequences, respectively. Secondary protein structures were predicted using the Protean section of DNASTAR software (DNASTAR Inc., Madison, WI, USA).

Statistical analysis

Frequencies of different patterns in two populations were compared using a two-way chi-square test. The Proc FREQ of SAS software (SAS Institute, 2004) was

employed for calculation of genotypic frequencies and chi-square test. Associations of different SSCP patterns with following production traits: body weights at birth, weaning and 6, 9 and 12 months of age; averages of milk fat, protein, lactose, solid-not-fat (SNF) and Log of milk somatic cell count (Log SCC) per ewe, and some immunity parameters, including serum levels of total protein and globulins, albumin, α 1-globulin, α 2-globulin, β -globulin, γ -globulin and albumin to globulins ratio (A/G) were investigated using the following model:

$$Y_{ijkl} = \mu + B_i + D_k + B_i \times D_k + F_j(B_i) + e_{ijkl}$$

where:

Y_{ijkl} is the phenotypic value,

μ is overall mean,

B_i is the effect of i^{th} breed (1 and 2),

D_k is the effect of k^{th} genotype (1 to 11),

$B_i \times D_k$ is interaction of i^{th} breed and k^{th} genotype,

$F_j(B_i)$ is the effect of j^{th} flock (1 to 3) within the i^{th} breed,

e_{ijkl} is the residual effects.

Least square means were compared by Bonferroni adjustment, assuming error level of 0.05, when the effects of the observed genotypes were significant ($P < 0.05$) or tended to be significant ($P < 0.1$) in analysis of variance. Proc GLM of SAS software (SAS Institute, 2004) was employed for general linear model analysis and comparison of means.

Results

SSCP patterns and frequencies

The SSCP analysis of the studied fragment showed 11 different SSCP patterns (Figure 1). However, the first pattern was only found in Mehraban sheep. Frequencies and percentages of the observed SSCP patterns are presented in Table 1. The most frequent SSCP patterns in Mehraban sheep were the patterns 4 and 10, with frequencies of 24.4% and 22.6%, respectively. The patterns 4 and 6 with frequencies of 28.74% and 16.76%, respectively, were the most frequent patterns in Lori-Bakhtiari sheep. The pattern 4 was the most frequent SSCP pattern in both breeds. The two-way chi square test showed a significant difference ($P < 0.05$) in SSCP pattern frequencies in two breeds (Table 1).

DNA sequence and translation

The DNA sequencing showed a specific sequence for each SSCP pattern. Therefore, 11 SSCP patterns had 11 different sequences and generally, 26 SNPs were observed in the studied population (Table 2). In comparison to NCBI reference sequences, new mutations were found in the patterns 1, 8, 9 and 11. A comparison between the observed sequences and a NCBI sequence is presented in Table 2. To-

tally, five new mutations were observed at 45, 46, 157, 159 and 164 bp of the studied fragment.

Figure 1. Eleven SSCP patterns of the studied fragment of *DRB1* gene exon 2

Table 1. The frequencies of different SSCP patterns in the studied populations

SSCP Pattern	Mehraban		Lori-Bakhtiari	
	observed number	frequency (%)	observed number	frequency (%)
1	6	3.9	0	0
2	15	9.7	11	6.58
3	10	6.5	4	2.4
4	38	24.4	48	28.74
5	13	8.4	24	14.37
6	13	8.4	28	16.76
7	5	3.3	5	3
8	7	4.6	4	2.4
9	7	4.4	11	6.58
10	35	22.6	24	14.37
11	6	3.9	8	4.8
Total	155	100	167	100
$\chi^2 = 22.3$		P-value = 0.012		

The nucleotide sequence changes deduced some amino acid (AA) changes in the codified AA sequences. The most frequent changes in AA sequences were observed in the pattern 11 (Table 2). The pattern 9 had the most frequent heterozygous positions and the pattern 5 was completely homozygous (Table 2).

Table 2. Comparison of nucleotide sequences and deduced AA substitution between the observed patterns and a referred GenBank sequence (accession number FR848373.1)

Nucleotide substitutions													Predicted AA changes												
Position	GB	1	2	3	4	5	6	7	8	9	10	11	Position	GB	1	2	3	4	5	6	7	8	9	10	11
45	G	-	-	-	-	-	-	-	-	-	-	C*	15	E	-	-	-	-	-	-	-	-	-	-	D
46	T	C*	-	-	-	-	-	-	-	C*	-	-	16	C	R	-	-	-	-	-	-	-	R	-	-
50	G	-	-	-	A	-	-	-	-	-	-	-	17	R	-	-	-	H	-	-	-	-	-	-	
81	C	-	-	-	G	-	G	-	-	-	-	-	27	F	-	-	-	L	-	L	-	-	-	-	
87	A	-	-	-	C	-	-	-	-	C	-	C	29	E	-	-	-	D	-	-	-	D	-	D	
92	A	-	-	-	W	-	-	-	-	-	-	-	31	Y	-	-	-	-	-	-	-	-	-	-	
97	T	-	-	-	-	-	C	-	-	-	-	C	33	Y	-	-	-	-	-	H	-	-	-	H	
143	A	W	-	W	-	-	T	W	W	W	-	T	48	Y	Y/F	-	Y/F	-	-	F	Y/F	Y/F	-	F	
147	A	R	-	G	G	G	G	G	G	R	G	G	49	R	-	-	-	-	-	-	-	-	-	-	
154	G	-	-	-	A	-	-	-	-	-	-	-	52	A	-	-	-	T	-	-	-	-	-	-	
157	G	-	-	-	-	-	-	-	-	-	-	C*	53	E	-	-	-	-	-	-	-	-	-	H	
159	G	-	-	-	-	-	-	-	-	-	-	C*	53	E	-	-	-	-	-	-	-	-	-	H	
164	G	-	-	-	-	-	-	-	T*	-	-	-	55	G	-	-	-	-	-	-	-	V	-	-	
173	A	-	-	-	-	-	-	-	R	-	-	-	58	D	-	-	-	-	-	-	D/G	-	-	-	
178	A	-	R	-	G	-	R	-	-	-	-	R	60	K	-	K/E	-	E	-	K/E	-	-	-	K/E	
201	G	-	C	-	-	-	C	-	-	-	-	C	67	E	-	D	-	-	-	D	-	-	-	D	
202	A	-	T	-	-	-	T	-	-	-	-	T	68	I	-	F	-	-	-	F	-	-	-	F	
211	C	-	-	-	-	-	A	-	-	-	-	A	71	R	-	-	-	-	-	S	-	-	-	S	
213	G	-	-	-	-	-	C	-	-	-	-	C	71	R	-	-	-	-	-	S	-	-	-	S	
215	G	-	-	-	-	A	-	A	-	-	-	-	72	R	-	-	-	-	K	-	K	-	-	-	
217	C	-	-	-	-	-	A	-	-	-	-	A	73	R	-	-	-	-	-	-	-	-	-	-	
220	A	-	G	-	-	-	-	-	-	-	-	-	74	T	-	A	-	-	-	-	-	-	-	-	
223	G	R	-	A	-	-	-	-	-	R	-	-	75	E	K/E	-	K	-	-	-	-	-	K/E	-	
224	A	-	-	-	-	-	C	-	-	-	-	C	75	E	-	-	-	-	A	-	-	-	-	A	
259	A	-	-	G	-	G	K	G	G	R	G	R	87	I	-	-	-	G	-	F/V	G	G	I/V	G I/V	
260	T	-	-	K	-	G	-	G	G	K	K	-	87	I	-	-	G/V	-	G	-	G	G	S/I	G/V -	

GB: Referred GenBank sequence; Heterozygous positions are presented by W, R and K (W: A/T; R: A/G and K: G/T); New mutations are marked by * symbol.

Figure 2. Secondary protein structures predicted for some of the observed SSCP patterns (6, 7, 10 and 11) and a referred sequence (GenBank accession number FR848373.1)

The patterns 1 and 9 had a transition as T→C at position 46, deducing an AA substitution as Cys→Arg (C→R) at position 16 of the codified AA sequence. The pattern 11 had several mutations such as G→C mutation at position 45, deducing an AA change as Glu→Asp (E→D) at position 15 and two G→C mutations at positions 157 and 159, indicating a deduced AA change as Glu→His (E→H) at position 53 of the codified AA sequence. Another mutation found in the pattern 8 was G→T at the position 164, with a deduced AA substitution as Gly→Val (G→V) at position 55 of the codified AA sequence.

Protein structures

Secondary protein structures predicted for some observed sequences and a NCBI sequence (GenBank accession number FR848373.1) are presented in Figure 2. Slightly different secondary protein structures were predicted for the observed genotypes and the NCBI sequence. The pattern 11 had the most obvious changes and the pattern 10 had the least noticeable changes of predicted secondary protein structure in comparison to the referred GenBank sequence (Figure 2). The most obvious changes of the secondary protein structures predicted for different SSCP patterns were found in alpha amphipathic regions (Figure 2).

Associations with the studied traits

Descriptive statistics of the studied traits and results of the association study are presented in Tables 3, 4 and 5. The genotypes of the studied fragment had a significant association ($P<0.05$) with weaning weight, where the animals with patterns 6 or 9 had significantly ($P<0.05$) higher weaning weights than those with patterns 1 or 2 (Table 4). The effects of the observed genotypes on 9 month body weight tended to be significant ($P<0.1$). In comparison of least square means, the lambs with the pattern 1 had the lowest body weight at 9 months of age (Table 4). The animals with the pattern 1 showed the lowest weaning and 9 month body weights. The observed genotypes did not have any significant effect on birth weight and 6 and 12 month body weights, milk contents and Log of milk somatic cell count (Table 4).

Table 3. Descriptive statistics of the studied traits

Trait	Number	Average	SD	Min	Max
1	2	3	4	5	6
Body weights					
Birth (kg)	322	4.43	0.97	2.10	7.50
Weaning (kg)	322	26.90	5.44	13.84	41.00
6 month (kg)	322	35.07	4.46	25.50	53.00
9 month (kg)	322	43.87	4.73	32.86	58.00
Yearling (kg)	322	49.40	4.58	38.80	64.00
Milk contents					
fat (%)	322	7.89	1.74	3.46	12.3
protein (%)	322	5.83	1.30	3.31	10.18
lactose (%)	322	3.83	0.60	1.62	6.21
solids not-fat (%)	322	12.39	1.04	9.05	15.41
SCC (1000/ml)	322	1077.79	3653.18	7.00	42230.50

Table 3 – contd.

1	2	3	4	5	6
Serum parameters					
total proteins (g/dl)	322	7.07	0.51	6.00	8.70
albumin (g/dl)	322	3.38	0.33	2.02	4.37
globulins (g/dl)	322	3.54	0.57	2.41	5.51
albumin/globulins	322	0.99	0.22	0.50	2.90
α 1 globulin (g/dl)	322	0.22	0.05	0.17	0.90
α 2 globulin (g/dl)	322	0.84	0.21	0.13	1.79
β globulin (g/dl)	322	0.30	0.09	0.19	0.70
γ globulin (g/dl)	322	2.22	0.64	1.05	4.41

SCC: Somatic cell count; SD: Standard deviation.

Table 4. Association of the SSCP patterns with production traits

Pattern	BW (kg)	WW (kg)	BW6 (kg)	BW9 (kg)	BW12 (kg)	Milk fat (%)	Milk protein (%)	Milk lactose (%)	Milk SNF (%)	Log SCC (log 1000/ml)
1	3.57	20.26 c	30.46	39.16 b	46.43	8.37	5.12	4.12 a	11.75	2.18
2	4.39	24.75 b	33.89	42.73 ab	48.40	8.41	5.63	4.16 a	11.97	2.26
3	4.53	26.05 ab	35.32	44.89 a	50.36	7.77	5.28	3.85 a	12.16	2.18
4	4.48	26.70 ab	34.82	43.10 ab	48.36	7.83	5.98	3.65 a	12.34	2.41
5	4.51	27.54 ab	35.28	44.05 a	49.22	7.87	5.88	3.93 a	12.66	2.31
6	4.81	29.49 a	36.44	45.59 a	51.03	7.46	6.20	3.78 a	12.65	2.54
7	4.43	28.03 ab	37.58	46.44 a	52.29	7.37	5.81	4.02 a	12.79	2.01
8	4.08	26.50 ab	35.08	44.23 a	50.23	8.23	5.45	3.75 a	12.28	2.24
9	4.23	29.38 a	36.51	44.61 a	50.80	7.61	6.08	3.86 a	12.66	2.21
10	4.26	25.68 b	34.19	43.47 ab	49.28	8.18	5.60	3.81 a	12.20	2.20
11	4.28	27.51 ab	36.03	44.63 a	49.48	7.70	5.95	3.85 a	12.72	2.02
SEM	0.190	0.772	0.872	0.973	1.088	0.425	0.273	0.147	0.223	0.153
P-values										
Pattern	0.509	0.031	0.189	0.052	0.115	0.611	0.951	0.062	0.334	0.299
Breed	0.008	<0.001	<0.001	0.003	0.049	0.446	0.020	0.047	0.002	0.003
P×B	0.492	0.330	0.218	0.1313	0.131	0.270	0.125	0.181	0.057	0.453

BW, WW, BW6, BW9 and BW12: birth weight, weaning weight and body weights at 6, 9 and 12 months of age; SEM: Standard error of means; P×B: pattern × breed interaction; Least square means with same letter are not significantly different at 0.05 level.

The effects of different patterns on the studied immunity parameters are presented in Table 5. The patterns had significant associations ($P < 0.05$) with serum total protein and globulins, α 2-globulin, β -globulin and γ -globulin. The effect of the observed patterns on serum albumin and α 1-globulin, tended to be significant ($P < 0.1$). In comparison of means, the ewes with the pattern 5 had the highest serum levels of total proteins and globulins, α 1-globulin, β -globulin and γ -globulin and lowest level of α 2-globulin (Table 5).

Table 5. Association of the SSCP patterns with serum parameters

Pattern	A/G	Total Proteins (g/dl)	Albumin (g/dl)	Globulins (g/dl)	α 1 globulin (g/dl)	α 2 globulin (g/dl)	β globulin (g/dl)	γ globulin (g/dl)
1	0.95	7.01 b	3.45 a	3.34 b	0.21 b	0.84 a	0.29 b	2.02 b
2	0.96	7.04 b	3.32 a	3.51 b	0.21 b	0.77 ab	0.30 b	2.21 b
3	1.05	7.14 ab	3.45 a	3.57 ab	0.23 ab	0.89 a	0.30 b	2.15 b
4	1.02	6.97 b	3.43 a	3.40 b	0.21 b	0.89 a	0.30 b	2.04 b
5	0.90	7.62 a	3.33 a	4.09 a	0.25 a	0.66 b	0.41 a	2.90 a
6	0.98	7.02 b	3.28 a	3.57 ab	0.21 b	0.88 a	0.30 b	2.21 b
7	0.97	7.04 b	3.38 a	3.54 ab	0.21 b	0.85 a	0.27 b	2.22 b
8	0.99	6.90 b	3.26 a	3.52 b	0.21 b	0.84 a	0.28 b	2.10 b
9	0.96	6.96 b	3.31 a	3.51 b	0.20 b	0.82 ab	0.27 b	2.24 b
10	1.02	6.96 b	3.45 a	3.41 b	0.21 b	0.85 a	0.28 b	2.10 b
11	0.98	7.08 b	3.43 a	3.54 ab	0.21 b	0.83 a	0.27 b	2.22 b
SEM	0.048	0.110	0.065	0.122	0.011	0.036	0.019	0.111
P-values								
Pattern	0.110	<0.001	0.056	0.003	0.066	<0.001	<0.001	<0.001
Breed	0.013	0.243	<0.001	0.044	0.016	0.001	0.056	0.005
P×B	0.163	<0.001	0.018	<0.001	0.009	<0.001	<0.001	<0.001

A/G: albumin to globulins ratio; SEM: Standard error of means; P×B: pattern × breed interaction; Least square means with same letter are not significantly different at 0.05 level.

Discussion

Several studies have confirmed that the DRB1 exon 2 is highly polymorphic in human, rat, mouse, cattle and other species (Ripoli et al., 2004; Dukkupati et al., 2006). In human, an average of 18.6 alleles has been detected in 22 populations (Salamon et al., 1999). In another study 19 alleles were identified in Latxa sheep, whose eight alleles had not been published previously (Arrieta-Aguirre et al., 2006). In Iranian breeds of sheep, such as Shal, Zandi, Arabi, Sangsari and Makuie, the exon 2 of DRB1 was also highly polymorphic (Nikbakht et al., 2012; Lotfi et al., 2012; Jamshidi et al., 2010; Ashrafi et al., 2014 a). Ballingall et al. (2010) studied the allelic diversity of Ovar-DRB1 exon 2 in a cohort of 214 sheep, consisting of 15 breeds and crossbreds and identified genomic equivalents of 15 alleles identified by Herrmann-Hoesing et al. (2008) and all 10 full-length coding sequences described by Ballingall et al. (2008). In Makuie sheep 18 genotypes were determined in DRB1 exon 2 (Ashrafi et al., 2014 a). Likewise, 12 novel alleles and 30 previously reported alleles were identified in DRB1 exon 2 of three Chinese sheep breeds (Polat et al., 2015). In the present study, detection of 26 SNPs is in accordance with previous reports on high polymorphism of the DRB1 exon 2 (Ripoli et al., 2004; Dukkupati et al., 2006; Ashrafi et al., 2014 a, b).

In comparison to the GenBank sequences, five new mutations were detected in the patterns 1, 8, 9 and 11. In addition to these five new mutations, another mutation

found in the pattern 4, as G→A at position 154 deducing an AA substitution as Ala →Thr (A→T) at position 52 of the codified protein sequence, seems to be a new mutation in sheep. This mutation has been reported in *Bos taurus* (GenBank accession numbers AB523811.1, AB558416.1 and AB523840.1).

Any change of AA sequence could result in change of secondary structure of protein (Figure 2) and consequently the third and fourth structures of protein. Therefore, significant associations of the observed genotypes could be attributed to some changes in structural properties of the corresponding codified proteins.

Though the observed genotypes had significant association only with weaning weight, the observed genotypes had similar effects on body weights at different ages. For example, the genotype 1 had the lowest BWs at 3, 6, 9 and 12 months of age. This diversity of significance is likely due to temporary environments and some random variations in weight gain trajectories between animals. However, despite significant association of the observed genotypes with weaning weight, the production traits were little or not affected by the DRB1 polymorphisms, while immunological parameters were considerably affected.

In the present study, associations of the observed genotypes with body weights at 3 and 9 months of age and immunological parameters are in accordance with some previous reports. Association of DRB1 exon 2 polymorphism with body weight traits has been reported in Makuie sheep. In that study, 18 genotypes were detected with positive effect of AB genotype on average weight gain at 9 and 12 months of age (Ashrafi et al., 2014 b). Likewise, particular MHC alleles associated with body weight in Soay sheep (Paterson et al., 1998), clean fleece weight, greasy fleece weight, fibre diameter and body weights at birth and 15 months of age in Merino ewes (Bot et al., 2004). In another study, a total of 16 DRB1 microsatellite alleles in German Merino sheep associated with reproductive performance and this association was attributed to individual variability of humoral immune response, cell recognition or tissue differentiation between carriers of various MHC genotypes (Mir and Gelderman, 2008).

The MHC polymorphism might affect the resistance or susceptibility to diseases. It has been found that the BoLA class II DRB3 allele may play a role in higher milk somatic cell count, as a measure of udder health (Dietz et al., 1997). Likewise, Kelm et al. (1997) confirmed the potential use of DRB3.2 alleles (DRB3-2*8 and DRB3-2*16) as markers of mastitis susceptibility. Sayers et al. (2005) identified 8 and 7 Ovar-DRB1 alleles in Texel and Suffolk breeds, respectively, two alleles common to both breeds, and suggested important role of DRB1 gene in resistance to nematode infection in Suffolk breed. The role of ovine-DRB1 in nematode resistance was also observed in Ghezel sheep (Hajializadeh Valilou et al., 2015). In a recent study there was a strong association between DRB1 exon 2 polymorphism and hydatidosis resistance in Chinese Merino (Shen et al., 2014).

The class II genes of MHC, including DRB1 are members of immunoglobulin super-family genes, specialized for presentation of antigenic peptides mainly derived from extracellular proteins and parasites (Dukkipati et al., 2006). A change of MHC class II genes function against pathogens may change the levels of globulins and total protein levels. Low levels of serum total protein may be related to a low survival

rate, because serum total proteins and immunoglobulin fractions are necessary for immunity and growth (Piccione et al., 2011).

In the present study, serum levels of total proteins, globulins and albumin (Table 3) were in a normal range and close to previously reported values (Chaichisem-sari et al., 2011). Likewise, the A/G ratios (Table 3) were also in the normal range and close to the previous reports (Hobi et al., 2012). Significant associations of the observed genotypes with serum parameters and probably animal resistance in the present study could be attributed to their effects on functionality of MHC class II protein products (Sayers et al., 2005).

The MHC contains many genes with a wide variety of functions associated with immune response and production traits (Bot et al., 2004). Significant association of the observed genotypes with weaning weight and to less extent with 9 month body weight in the present study can be, to some extent, attributed to the effects of MHC on animal resistance or susceptibility to diseases, because a healthy animal generally has a higher production.

The present study is probably one of the first reports on association of DRB1 exon 2 polymorphism with milk contents and serum parameters in sheep. It seems that more studies are still needed to clarify the mechanisms of DRB1 effects on production traits and immunity parameters. The strength and consistency of the association of MHC alleles and production traits need also to be evaluated in other breeds and populations.

Conclusions

The results of the present study indicated that the DRB1 exon 2 is highly polymorphic with noticeable effects on some traits, especially immunological parameters. Significant associations of the observed genotypes of DRB1 exon 2, especially with serum parameters indicate that the corresponding mutations have important effects on immunity and to a lesser extent, production traits. More studies are still needed for use of DRB1 exon 2 in marker assisted selection in sheep.

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