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Study on Haemoglobin Polymorphism in Two Breeds of Iranian Sheep

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Abstract: In the present research, hemoglobin polymorphism was studied in two Iranian important sheep breeds; Moghani and Makouei type. Eighty eight samples from age groups and each breed were selected. Determine of the hemoglobin phenotype carried out with acetate cellulose electrophoresis method. Two hemoglobin phenotypes includes AA and AB were observed in the studied breeds. In Moghani breed AA and AB phenotypes frequency were in 71.6 and 28.4%, respectively and in Makouei breed were as below: AA in 69.3 and AB in 30.7% of the sheep. In sheep with AB phenotype, the amount of B hemoglobin was more than A but difference between A and B haemoglobins levels were not significant between the two breeds. The total amount of hemoglobin in Makouei was more than Moghani breed and there was a significant difference between two breeds concerning total hemoglobin. According to the results of the present research, C and F hemoglobins present to one month of age and by the increase of age, the amounts reduce gradually and reach to around zero after 2 month of age.

Key words: Haemoglobin polymorphism, electrophoresis, HbF and HbC, sheep

INTRODUCTION

Haemoglobin, a protein molecule with tetramer structure, α and β chains. Its plays an important role in the transfer of blood gasses and is found in different phenotypes in sheep. As in other ruminant, sheep has the large amount of polymorphism, which occurs between breeds and within the individual as it develops from embryo to adult. The polymorphism is greatest in the β chain. Differences in the amino acid sequence of the globin moiety are responsible for phylogenetic and intraspecies differences in adult haemoglobin; differences between embryonic, fetal and adults forms within a species; and heterogeneity within individual (Jain, 1986). Sheep has the different haemoglobin types as A, B, C, E, F. Embryonal type (HbE) to maintain a dam to in utero O_2 gradiant and adult type (HbA) for the ex utero environment. Transition from HbE to HbA begins in utero and may not be complete untile months after birth. Fetal haemoglobin (HbF), which replace HbE in utero. As gestation pogresses HbF is gradually replaces by HbA. Sheep and goats have a unique haemoglobin type, HbC. The HbC replaces HbF at birth; within a few months, HbA replaces it. Only sheep with HbA phenotypes A (α_2, β_2^A) or AB (α_2, $\beta_2^{A, B}$) express HbC, which make up 15-30% of their total haemoglobin. Sheep with HbA phenotype B (α_2, β_2^B) do not express HbC. By 30 days of a lamb's life, nearly all of the HbC is displaced by HbA. Erythropoietin is responsible for HbA-to-C switching. The mean maximum haemoglobin type from a series in which A is greater than AB and AB is greater than B. On the basis of electrophoretic mobility, the haemoglobin moving more rapidly toward the anode at pH 8.6 was called HbA and very slow-moving haemoglobin was called HbC. HbF moving more rapidly than HbB and HbB, moving more rapidly than HbC toward the anode (Jain, 1986).

MATERIALS AND METHODS

The study was carried out on two Iranian important sheep breeds (Moghani and Makuei). A total of 176 blood samples were collected according to their age (in 11 age

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groups) and sex. All animals were clinically healthy and free from internal and external parasites. Samples were taken from the Jugular vein using disposable syringes containing heparin (10,000 Iu L^{-1} blood; Novo, Novo Nordisk). Electrophoresis was performed on cellulose acetate strips (350V, 45 min) and after staining with Ponceaus-S, destainig, clearing and drying, the percentage levels of haemoglobin types were determined by densitometery (Helena Co., France). Total haemoglobin concentration was measured by standard laboratory mothod (Jain, 1986). The results were statistically analyzed by using SPSS software, Duncan test was used to determine if there was any significant difference ($p<0.05$) between the different age groups for total haemoglobin and types of haemoglobin. The mean and standard deviation for total haemoglobin, types of haemoglobin were determined for breed, sex and age groups. Correlation between measured parameters was detected by Pearson's method.

RESULTS AND DISCUSSION

After electrophoresis on cellulose acetate, two haemoglobin phenotypes were detected in Moghani and Makuei breeds: AA and AB phenotypes. AA was commonest in the two breeds (Table 1 and 2). The incidence of AB phenotype in Moghani and Makuei breeds was 28.4% (25/88) and 30.7% (27/88), respectively. In sheep with AB phenotype, haemoglobin B was dominant. The amount of B haemoglobin was more than A haemoglobin but no significant difference was observed between the two breeds. The amount of total haemoglobin in Makuei breed was more than Moghani breed and there was a significant difference between two breeds concerning total haemoglobin (Table 3 and 4). According to the results of present research, C and F haemoglobins present until one month of age and by the increase of age, the amounts reduce gradually and reach to around zero after 2 month of age. In the Table 5, the correlation coefficients between measured parameters are shown for each sheep breeds. In the Moghani and Makuei breeds,s Pearson's method showed significant correlation for age with A, F, C haemoglobin amounts, HbA absolute level with HbB amount and HbF absolute level with HbC amount ($p<0.05$).

Haemoglobin A was commonest and BB Phenotype was not seen in Moghani and Makuei sheep. This finding is in contrast with a previous report of Mostaghni (1978) for other Iranian sheep breeds, which predominantly have type B haemoglobin but comformity with report of Mohri *et al.* (2005) for Baloochi and Kordi sheep breeds. Missohou *et al.* (1998) in Senegalese study also reported the dominance of type B haemoglobin in sheep. In a study of the haemoglobin types of the five Iranian sheep breeds, Mostaghni (1978)

Table 1: Total haemoglobin levels (g dL^{-1}) in the Moghani and Makuei sheep breeds according to sex

Breed	Sex	n	Total- Hb (g dL^{-1})	Total (g dL^{-1})
Moghani	Male	38	11.21±1	11.15±0.92
	Female	50	11.11±0.85	
Makuei	Male	38	11.57±0.95	11.62±0.89
	Female	50	11.63±0.94	

There was a significant difference between male sexes in the two breeds ($p<0.05$), There was a significant difference between female sexes in the two breeds ($p<0.05$)

Table 2: Percentage of observed haemoglobin phenotypes, total haemoglobin concentration and absolute levels of A and B haemoglobin according to breed and sex

Breed	Sex	n	Hb-B (g L^{-1})	Hb-A (g L^{-1})	Hb-AA (%)	Hb-AB (%)	Hb-BB (%)
Moghani	Male	38	23.0±4.1	71.0±3.9	94.74	5.26	-
	Female	50	25.6±6.2	68.2±5.1	54.00	46.00	-
Makuei	Male	38	28.0±5.2	67.1±9.8	86.84	13.16	-
	Female	50	27.2±4.4	67.9±6.7	56.00	44.00	-

Table 3: Percentage and absolute levels of A and B haemoglobins in sheeps with AB phenotype according to breed

Breed	Hb-B(%)	Hb-B (g L^{-1})	Hb-A (%)	Hb-A (g L^{-1})
Moghani	55.02±18.87	62.12±16.25	45.08±7.72	39.46±8.48
Makuei	55.05±18.84	62.39±23.27	45.05±14.59	37.91±12.99

Table 4: Change of total haemoglobin levels (g dL^{-1}), C and F haemoglobins percentage in Moghani and Makuei sheep breeds with different ages

		Moghani breed			Makuei breed		
Age	n	Total- Hb(g dL^{-1})	Hb-C (%)	Hb-F(%)	Total-Hb(g/dl)	Hb-C (%)	Hb-F(%)
1-7 day	8	$11.61\pm 0.51^{a,b,c}$	9.37 ± 1.08^{a}	58.9 ± 6.24^{a}	11.15±0.53	9.87 ± 1.37^{a}	55.76 ± 9.73^{a}
7-14 day	8	12.03 ± 1.03^{a}	$1.62^{b}\pm6.4$	55.26 ± 5.31^{a}	11.43± 0.53	6.87 ± 2^{b}	52.47 ± 5.68^{a}
14-31 day	8	$10.33\pm0.84^{d,f}$	$\pm1.21^{c}$ 1.72	12.63 ± 4.73^{b}	11.82±0.73	1.96 ± 1.39^{c}	13.48 ± 5.05^{b}
1-2 month	8	$10.37\pm0.55^{d,f}$	$0.15^{d}\pm0.23$	2.18 ± 0.31^{c}	11.58±0.61	0.30 ± 0.09^{d}	2.18 ± 0.64^{c}
2-3 month	8	9.88 ± 0.82^{f}	0.002 ± 0.001^{d}	0.003 ± 0.001^{c}	10.93±0.91	0.003 ± 0.002^{d}	0.004 ± 0.002^{c}
3-6 month	8	$10.87\pm0.44^{b,c,d}$	0.00	0.00	12.22±0.79	0.00	0.00
6-12 month	8	$11.43\pm0.76^{a,b,c}$	0.00	0.00	11.24±0.77	0.00	0.00
12-18 month	8	$10.97\pm0.82^{b,c,d}$	0.00	0.00	11.28±0.73	0.00	0.00
18-24 month	8	$10.92\pm0.77^{c,d}$	0.00	0.00	11.71±0.88	0.00	0.00
24-36 month	8	$11.56\pm0.63^{a,b,c}$	0.00	0.00	12.15±0.84	0.00	0.00
36-48 month	8	$11.71\pm0.38^{a,b}$	0.00	0.00	11.85±0.79	0.00	0.00

There was a significant difference between age groups with different code (a, b, c, d, f)

Table 5: Correlation coefficient between measured parameters with statistical analysis for the total samples

	Age	Total-Hb	Hb-A	Hb-B	Hb-F	Hb-C
Age						
Pearson Correlation	1	0.138	0.626**	0.057	-0.734**	-0.721**
Sig.(2-tailed)	-	0.318	0.000	0.595	0.000	0.000
Total-Hb						
Pearson Correlation		1	-0.095	0.031	0.370	0.312
Sig.(2-tailed)		-	0.613	0.932	0.001	0.002
Hb-A						
Pearson Correlation			1	-0.412**	-0.745	-0.733
Sig.(2-tailed)			-	0.000	0.211	0.227
Hb-B						
Pearson Correlation				1	-0.181	-0.184
Sig.(2-tailed)				-	0.076	0.072
Hb-F						
Pearson Correlation					1	0.982**
Sig.(2-tailed)					-	0.000
Hb-C						
Pearson Correlation						1
Sig.(2-tailed)						-

** Significant correlation in 0.05 level.n = 176

reported BB and AB phenotypes in the Karakul, Bakhtiari and Ghezel breeds and BB, AB and AA phenotypes in the Mehraban and Naeini breeds. In the present study, the AB phenotype was seen in both sexes but AA phenotype was commonest in male sex of two breeds and significant difference was observed between the two sexes ($p<0.05$).

In the present study, the levels of total haemoglobin are consistent with previous reports for various breeds of Iranian sheep (Mostaghni, 1978; Nikbakht *et al*., 2000; Mojabi *et al*., 2002) and other breeds of sheep (Jain 1993; Meyer and Harvey, 1998).

CONCLUSION

Two haemoglobin phenotypes were detected in Moghani and Makuei breeds: AA and AB phenotypes. AA was commonest in the two breeds and in sheep with AB phenotype, haemoglobin B was dominant. In this sudy, C and F haemoglobins present until one month of age and by the increase of age, the amounts reduce gradually and reach to around zero after 2 month of age.

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