

## Estimation of Inbreeding Coefficients Using Pedigree and Microsatellite Markers and Its Effects on Economic Traits of Shirvan Kordi Sheep

### Research Article

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### ABSTRACT

In this study, pedigree data of 7170 lambs produced from 177 rams and 2182 ewes collected during 1989 to 2009 in the Shirvan Kordi sheep breeding station, were used for calculating inbreeding coefficients. The impact of inbreeding on the studied traits was investigated by including inbreeding coefficients for each animal as a covariate in the animal model. Average inbreeding coefficient of the flock in base year, 1989, was estimated as 0.668%. The low average inbreeding in the flock could be ascribed to the structured breeding regime of the station for preventing mating among relatives. Totally, 23.3% of the animals were inbred with average inbreeding coefficient of 2.87%. Out of these, 1668 animals were inbred including 823 males and 845 females with the mean inbreeding coefficients of 2.81% and 2.93%, respectively. Coefficients of inbreeding were also estimated in this population using 6 microsatellite markers (BM8125, BMS2361, BM6526, BM6526, BMS1004 and BM6444) and 100 blood samples. Within population, inbreeding F-statistic ( $F_{IS}$ ) ranged from -0.1859 (BM6526) to 0.3329 (BM6438). The  $F_{IS}$  estimates were positive for BM6438 and BM6444 loci (0.3329 and 0.2287), respectively and estimates for the other loci were negative. Average inbreeding coefficients based on molecular markers was estimated as 0.2617%. Birth weight, weaning weight, body weight at 6 months, body weight at 9 months, body weight at 12 months, annual wool production and number of lambs per ewe lambing were changed by -0.0131, 0.0795, 0.0013, -0.0653, -0.0921, 0.0083 and -0.023 kg, respectively for every one percent increase in inbreeding.

**KEY WORDS** genetic markers, growth traits, inbreeding, sheep.

### INTRODUCTION

Intensive selection within a single population of finite size reduces the genetic variability and increases the rate of inbreeding (Norberg and Sorensen, 2007; Barczak *et al.* 2009). One definition for inbreeding is the mating of individuals whose relatedness is greater than the average degree of relationship that exists in the population (Lush, 1945), and capable of changing the genotypic frequencies of a population without modifying the gene frequencies. Inten-

sive use of a few breeding animals, where the selection intensity is high, could result in greater rates of inbreeding in the population. The unavoidable mating of related animals in closed populations leads to accumulation of inbreeding and a reduced genetic diversity. Measurement of the effect of inbreeding on economic traits is important in order to estimate the magnitude of change associated with increasing inbreeding. The inbreeding depression has been well documented in many populations for a variety of traits (Lamberson and Thomas, 1984; Ercanbrack and

Knight, 1991; Analla *et al.* 1998; Dario and Bufano, 2003; Van Wyk *et al.* 2009). Inbreeding has a deleterious effect on additive genetic variance as well as on phenotypic values and also affects fitness traits by accumulation of deleterious recessive alleles in the population (Falconer and Mackay, 1996). Heterozygosity and allelic diversities can be lost from small, closed, selected populations at a rapid rate. The loss of diversity and increase in homozygosity may compromise production and / or fitness of inbred animals (Selvaggi *et al.* 2010). Identity by descent occurs because allele from one common ancestor may flow through multiple offspring (MacKinnon, 2003). The main proposed mechanisms for inbreeding depression are those of overdominance and the partial recessive hypotheses (Charlesworth and Charlesworth, 1987).

In the overdominance hypothesis, inbreeding depression is attributable to higher fitness of heterozygotes for the loci in question. For the partial recessive hypothesis, negative fitness consequences are due to the fixation of recessive or partially recessive deleterious alleles (Frankham *et al.* 2002).

Microsatellites has been widely used as reliable molecular markers to study the genetic relationship of different populations and for indirect measures of inbreeding. They are codominant, highly polymorphic, highly abundant, heritable, locus specific, and easily analyzed and therefore suitable for studies on population phylogenesis constitution (Wang *et al.* 2007). With increased availability, microsatellite markers have been widely used to provide indirect measures of inbreeding. Heterozygosity (the occurrence of two different alleles at a specific locus) scored at neutral microsatellite markers has generally been assumed to reflect genome wide heterozygosity. Fitness traits have been found to correlate with inbreeding estimates achieved by using marker-based metrics such as heterozygosity (Coltman *et al.* 1999) and internal relatedness (Amos *et al.* 2001).

This study was carried out using microsatellite markers because they are powerful tools for tracking alleles through a population and to estimate genetic variability and inbreeding (Zajc *et al.* 1997). The inbreeding depression has been well documented in many populations for a variety of traits (Lamberson and Thomas, 1984; Ercanbrack and Knight, 1991; Analla *et al.* 1998; Dario and Bufano, 2003; Alsheikh, 2005; Swanepoel *et al.* 2007; Norberg and Sorenson, 2007; Barczak *et al.* 2009; Van Wyk *et al.* 2009). Marker data collected provides information on population structure, relatedness and inbreeding (Dobson *et al.* 1998; Surridge *et al.* 1999). The objective of this study was to estimate inbreeding coefficient using pedigree and microsatellite markers and its effects on economic traits in Shirvan Kordi sheep.

## MATERIALS AND METHODS

### Data

Pedigree information from 1989 to 2009 of a flock of Kordi sheep maintained at Shirvan Sheep Breeding Station was used. Pedigree file contained information on individual identification number, sex, type of birth, dam and sire as well as birth date and included 7170 registered animals (3332 males and 3838 females), progeny of 177 sires and 2182 dams.

### Pedigree analysis

Inbreeding coefficients of the animals were computed using the CFC program (Sargolzaei *et al.* 2006). Inbreeding coefficient of an individual (F) is the probability of having two alleles at the same locus, which are identical by descent. Coefficients of inbreeding (F) of all animals in the file were computed using the method of Meuwissen and Luo (1992). The evolution of the average coefficient of inbreeding was observed from 1989 to 2009 and the annual increase of inbreeding was estimated by a linear regression over time. Inbreeding depression was estimated as a partial linear regression of performance (corrected for fixed effects) on individual inbreeding coefficients, using an animal model. It is interpreted as the change in the expression of traits per one percent of inbreeding. Traits considered were birth weight (BWT), weaning weight (WWT), body weight at 6 months (BW6M), body weight at 9 months (BW9M), body weight at 12 months (BW12M), annual wool produced (Wool) and the number of lambs born per ewe lambing (LS). The estimation of parameters was performed by using of derivative free restricted maximum likelihood (DFREML) algorithm numerically implemented in the DFREML package program by Meyer (1997), fitting single-trait animal models. The fitted models included fixed effects of sex of lamb in two classes, type of birth in two classes (single and twin), year of birth in 20 classes (1989-2009) and age of dam at lambing in six classes (2-7 years old) as well as linear partial regressions of the trait on inbreeding coefficient of the individual for all the traits except or LS for which the effect of sex of lamb was not included. The following model was used for the analysis:

$$y = Xb + Z_1a + Z_2c + Z_3m + e$$

$$\text{Cov}(a,m) = 0$$

Where:

y: vector of records on the respective traits.

b, a, c, m and e: vectors of fixed effects, direct additive genetic effects, maternal permanent environmental effects, maternal additive genetic effects, and residual effects, respectively.

$X$ ,  $Z_1$ ,  $Z_2$  and  $Z_3$ : corresponding design matrices associating the fixed effects, direct additive genetic effects, maternal permanent environmental effects, and maternal additive genetic effects to vector of  $y$ .

### Microsatellite method

Blood samples (5 mL) of 100 animals were collected from the jugular vein and transferred into vacutainer tubes containing 0.5 molar EDTA as anticoagulant and frozen at  $-20$  °C. Total DNA was isolated from blood samples using the Diatom DNA Kit, according to the manufacturer instructions. The quantity and quality of the isolated DNA was determined using both spectrophotometry and by 0.8% agarose gel electrophoresis. Characteristics of the microsatellite markers used in this study are listed in Table 1. These loci (BM8125, BM6526, BM3438 and BM6444 (Bishop *et al.* 1994), BMS2361 (Stone *et al.* 1997) and BMS1004 (Stone *et al.* 1995), were selected because of their high polymorphic character and high number of alleles previously detected in sheep.

Approximately, 100 ng DNA (adjusted concentration) was used as template for polymerase chain reaction (PCR). The PCR reaction cycle was carried out in a the rmcycler (Biorad) by denaturation at 95 °C for 4 min, denaturation at 95 °C for 45 sec, primer annealing for 45 sec at the desired temperature (55-60 °C) and an extension for 1 min at 72 °C, repeating the cycle 35 times. The final extension step was at 72 °C for 4 min. The PCR amplification was conducted in a 12  $\mu$ L volume. PCR products were analyzed by vertical electrophoresis in 6% non-denaturing polyacrylamide gel (170 V, 3-4 h) and bands visualized by rapid silver staining (Sanguinetti *et al.* 1994). The amplicon sizes were determined by using a 622 bp DNA ladder (pBR322 DNA-MspI Digest) as a standard marker. The genotypes were scored based on the presence of a single band (homozygotes) or double bands (heterozygotes) in the gel.

### Molecular data analysis

Molecular data were analysed using the POPGENE V1.32 (Yeh *et al.* 1999) and Molkin V 3.0 (Gutierrez *et al.* 2005) software. Computed parameters included allele frequencies, observed number of alleles per locus, effective number of alleles, tests of genotype frequencies for deviation from Hardy-Weinberg equilibrium (HWE), observed and expected heterozygosity, F-statistics and the polymorphic information content (PIC) (Botstein *et al.* 1980).

Detailed pedigree information is required to accurately derive inbreeding coefficients. However, in recent years, the developments of molecular techniques offer the opportunity to estimate accurate population parameters even in absence of the pedigree information. The inbreeding coefficient was calculated as the deviation of the observed het-

erozygosity of an individual relative to the heterozygosity expected under random mating (Lukas and Donald, 2002) which was derived as:

$$F_{IS} = 1 - (Ho/He)$$

Where:

F: coefficient of inbreeding.

Ho: observed frequency of heterozygous individuals.

He: expected frequency of heterozygous individuals in the population.

## RESULTS AND DISCUSSION

### Pedigree analysis

The analysis of pedigree revealed that mean level of inbreeding (F) of all animals across all years (1989-2009) was 0.668%. Moreover, the minimum and maximum coefficients of inbreeding for the animals in the flock was 0 and 31.25%, respectively. The low average coefficient of inbreeding could be ascribed to breeding strategies at the station for preventing mating of relatives. Totally, 23.26% of the animals (1668 out of 7170) were inbred with a mean inbreeding coefficient of 2.87%. In the pedigree, 3332 and 3838 of the animals were males and females with mean inbreeding coefficients of 0.693% and 0.646%, respectively. Out of all, 1668 animals were inbred. These included 823 males and 845 females having average inbreeding coefficient of 2.81% and 2.93%, respectively. These results indicated that few matings of close relatives have occurred. Descriptive statistics for inbreeding coefficients for the entire population and the inbred portion of the population are shown in Table 2. Overall, average coefficient of inbreeding in this study were lower than values reported in some previous studies, i.e. 8.08% for Iran-Black sheep (Mokhtari *et al.* 2014), 2.93% for Moghani sheep in Iran (Ghavi Hossein-Zadeh, 2012a), 2.25% for Sakiz lambs (Ceyhan *et al.* 2011), 2.33% for Santa Inês sheep (Pedrosa *et al.* 2010), 1.13% for Sakiz sheep (Ceyhan *et al.* 2009), 1.2% for the Dohne Merino (Swanepoel *et al.* 2007), 1.70% for Thalli sheep (Hussain *et al.* 2006a), 0.72% for Barki sheep (Alsheikh, 2005) and 1.16% for Hissardale sheep (Akhtar *et al.* 2000). However, values reported in some other studies, i.e. 0.5% for Moghani sheep (Dorostkar *et al.* 2012), 0.3% for different breeds of sheep (Barczak *et al.* 2009) and 0.15% for Guilan sheep (Eteqadi *et al.* 2014), were lower than present investigation.

Table 3 illustrates the distribution of animals in classes according to inbreeding coefficients. Maximum number of animals was in the first class (F=0) and the minimum number of animals was in the sixth class (F>25), for all the studied traits.

**Table 1** Characteristics of microsatellite markers

Loci	Primer sequence	Ch. No.	Gene bank accession number	Annealing temperature (°C)	References
BM8125	GGGGTCACAAAAAGTTGGACAT TGGAGTCCACAACAACAAACAA	17	G18475	60 °C	Bishop <i>et al.</i> (1994)
BMS2361	ACACAACCCAAATGTTACCAA ATTGTGCAGAGACCAAGTGC	16	G18984	60 °C	Stone <i>et al.</i> (1997)
BM6526	CATGCCAAACAATATCCAGC TGAAGGTAGAGAGCAAGCAGC	26	G18454	56 °C	Bishop <i>et al.</i> (1994)
BM6438	TTGAGCACAGACACAGACTGG ACTGAATGCCTCTTTGTGC	1	G18435	60 °C	Bishop <i>et al.</i> (1994)
BMS1004	TAAAAAGTCAGAAAGGGAAGCC CTCGACCTCACATACTCAAAGC	15	G18607	58 °C	Stone <i>et al.</i> (1995)
BM6444	CTCTGGGTACAACACTGAGTCC TAGAGAGTTTCCCTGTCCATCC	2	G18444	55 °C	Bishop <i>et al.</i> (1994)

**Table 2** Descriptive statistics for inbreeding coefficients of the studied Kordi sheep population

	Entire population			Inbred portion of the population		
	All	Male	Female	All	Male	Female
No. of animals	7170	3332	3838	1668	823	845
Mean of F (%)	0.668	0.693	0.646	2.87	2.81	2.93
Minimum (%)	0.00	0.00	0.00	0.006	0.006	0.012
Maximum (%)	31.25	26.56	31.25	31.25	26.56	31.25

**Table 3** Distribution of animals in different classes of inbreeding coefficient for various traits

Classes of F	BWT		WWT		BW6M		BW9M	
	Animal	Mean	Animal	Mean	Animal	Mean	Animal	Mean
F = 0	4547	4.31±0.72	4140	22.67±5.22	3625	31.42±6.27	2797	35.42±6.49
0 < F < 6.25	946	4.35±0.70	1047	25.58±5.20	860	31.86±6.25	606	36.42±6.48
6.25 < F < 12.50	90	4.25±0.71	88	23.89±5.19	144	32.44±6.23	52	36.30±6.45
12.50 < F < 18.75	7	4.33±0.70	9	26.30±5.23	8	33.68±6.18	6	37.72±6.39
18.75 < F < 25.00	13	4.41±0.73	12	25.92±5.19	10	34.45±6.25	9	38.11±6.32
F > 25	5	4.30±0.69	7	23.76±5.22	4	29.25±6.28	3	37.00±6.34

  

Classes of F	BW12M		Wool		LS	
	Animal	Mean	Animal	Mean	Animal	Mean
F = 0	2589	42.57±8.23	2529	1.04±0.47	1347	1.05±0.06
0 < F < 6.25	607	44.50±8.25	1016	0.84±0.45	286	1.06±0.06
6.25 < F < 12.50	51	42.60±8.33	62	0.89±0.44	28	1.04±0.05
12.50 < F < 18.75	6	48.37±8.25	9	0.96±0.42	1	1.00±0.04
18.75 < F < 25.00	9	45.33±8.26	7	1.33±0.41	3	1.67±0.5
F > 25	3	44.83±8.22	5	0.98±0.39	1	1.00±0.06

BWT: birth weight trait; WWT: weaning weight trait; BW6M: body weight at 6 months of age trait; BW9M: body weight at 9 months of age trait; BW12M: body weight at 12 months of age trait; Wool: wool produced annually and LS: number of lambs per ewe lambing.

Mean of all traits decreased and increased irregularly by increasing inbreeding coefficients. This could be due to fewer records in the numerically higher classes of inbreeding.

**Inbreeding depression**

Details of the data used for the estimation of inbreeding depression are given in Table 4, where the number of records is shown after editing.

Regression coefficients and their standard errors on inbreeding coefficients were  $-0.0013 \pm 0.0003$  kg for BWT,  $0.080 \pm 0.015$  kg for WWT,  $0.001 \pm 0.0009$  kg for BW6M,  $-0.065 \pm 0.055$  kg for BW9M,  $-0.092 \pm 0.063$  kg for BW12M,  $0.008 \pm 0.0066$ kg for Wool and  $-0.023 \pm 0.012$  lambs for LS.

**Pre-weaning growth traits**

Changes for every one percent increase in inbreeding coefficient for BWT and WWT were  $-0.0131$  and  $0.0795$  kg, respectively. Regression coefficient estimate for BWT ( $-0.0131$  kg/%F) was in close agreement with the results obtained by Analla *et al.* (1999) ( $-0.013$  kg/%F in Merino sheep), MacKinnon (2003) ( $-0.012$  kg/%F in crossbred sheep), Norberg and Sorensen (2007) ( $-0.011$  kg/%F in Danish populations of Texel, Shropshire and Oxford Down) and Mokhtari *et al.* (2014) ( $-0.007$  kg/%F in Iran-Black sheep). However, other researchers including Ceyhan *et al.* (2011), ( $-0.0245$  kg/%F in Sakiz lambs), Selvaggi *et al.* (2010) ( $-0.019$  kg/%F in Leccese sheep) and Hussain *et al.* (2006a) ( $-0.051$  kg/%F in Thalli sheep), reported somewhat higher values.

**Table 4** Number of observations, mean, standard deviation and mean of inbreeding coefficient of studied traits

	BWT*	WWT	BW6M	BW9M	BW12M	Wool	LS**
Records no.	5608	5303	4576	3473	3265	3634	1666
Mean (kg)	4.315	23.28	31.51	35.62	42.95	0.98	1.06
SD (kg)	0.72	5.22	6.27	6.49	8.23	0.47	0.06
Minimum (kg)	2	8	14.2	17	20	0.2	1
Maximum (kg)	7.2	43	53	63	75	6	2
Mean of F (%)	0.63	0.70	0.66	0.66	0.69	0.82	0.60

\* BWT: birth weight trait; WWT: weaning weight trait; BW6M: body weight at 6 months of age trait; BW9M: body weight at 9 months of age trait; BW12M: body weight at 12 months of age trait; Wool: wool produced annually and LS: number of lambs per ewe lambing.

\*\* Unit of measurement for LS is number of lambs.

SD: standard deviation.

The regression coefficients estimates for BWT were higher than those obtained for Moghani sheep (-0.007 kg) by Dorostkar *et al.* (2012), -0.0055 kg for Guilan sheep by Eteqadi *et al.* (2014), -0.0034 kg for Santa Inês sheep by Pedrosa *et al.* (2010) and -0.0064 kg for Elsenburg Dormer sheep by Van Wyk *et al.* (2009). Inbreeding depression may be detrimental to the sustainability of the production systems, leading to economic losses. Regression coefficient estimated for WW in the present study was  $0.080 \pm 0.015$  kg. Thus, weaning weight increased with increase in the level of inbreeding, but this increase was not significant. The findings of Hussain *et al.* (2006b) who reported a regression coefficient of 0.083 kg and Akhtar *et al.* (2000) who observed a change of 0.0213 kg for a 1% increase of inbreeding in Hissardal sheep were in agreement with the finding of present study.

#### Post-weaning growth traits

Regression coefficients for every one percent increase in inbreeding for BW6M, BW9M and BW12M were 0.0013, -0.0653 and -0.0921 kg, respectively. For BW6M, the linear effect of individual inbreeding was non-significant. Akhtar *et al.* (2000) reported a regression coefficient for BW6M of 0.0142 kg in Hissardale sheep, Mehmannaavaz *et al.* (2002) found a value of 0.0034 kg in a flock of Baluchi sheep, Hussain *et al.* (2006b) observed a change of 0.093 kg for Thalli sheep, Eteqadi *et al.* (2014), found a value of 0.0020 kg in Guilan sheep and Dorostkar *et al.* (2012) observed a significant negative effect of inbreeding coefficient (-0.026 kg) on this trait for Moghani sheep.

Higher heritabilities for weaning weight and BW6M resulted in this change, since traits of higher heritability tend to show more additive genetic variation and less dominance deviation (Selvaggi *et al.* 2010).

Regression coefficients for BW in the 9<sup>th</sup> and 12<sup>th</sup> month were more than all the other studied traits. Estimates of -0.0653 and -0.0921 kg per 1% increase in inbreeding coefficient for BW in 9<sup>th</sup> month and BW in 12<sup>th</sup> month, were more than the values reported in other studies. Hussain *et al.* (2006b) reported inbreeding effects of -0.013 and -0.019 kg for Thalli sheep and Dorostkar *et al.* (2012) reported -0.019 and -0.042 kg for Moghani breed, respectively.

#### Annual wool production and number of lambs per ewe lambing

The effect of inbreeding on wool produced annually and the number of lambs per ewe lambing amounted to 0.0083 kg and -0.023 lambs, respectively. For the annual wool production, the linear effect of individual inbreeding, regression was positive but non-significant. On the average, an increase in 1 percent in individual inbreeding increased the wool produced annually by 0.0083 kg. A review by Lamberson and Thomas (1984) showed little effect of inbreeding on fleece weight, but effects were more prominent in Merinos than in other range sheep. The average reduction in greasy fleece weight was 0.017 kg/%F, with a range of -0.006 to -0.029 kg/%F. Similar results were found by Ercanbrack and Knight (1991) in Rambouillet, Targhee and Columbia breeds with an average effect of -0.0127 kg/%F. They reported that the effects of inbreeding on fleece weight of Rambouillet, Targhee and Columbia ewe were curvilinear and amounted to reductions of -0.017, -0.012 and -0.009 kg, respectively. The estimate of regression coefficients for wool produced annually in this study was in agreement with finding of Akhtar *et al.* (2000) who reported a regression coefficient for fleece weight of 0.0013 kg in Hissardale sheep. Mandal *et al.* (2002) showed smaller effects in an Indian coarse-wool sheep breed, -0.0029 kg/%F for ewe inbreeding and non-significant values due to lamb inbreeding. According to MacKinnon (2003), it seems that individual inbreeding does not have large effect on the quality of the fleece, but may slightly decrease fleece weight (i.e. quantity). Swanepoel *et al.* (2007), found a change of -0.0673 and 0.0906 kg, respectively for the individual and dam inbreeding coefficients, in the Dohne Merino sheep breed in south Africa.

The regression coefficient estimated for LS in the present study was -0.023. Analla *et al.* (1999) reported effects of inbreeding for LS in Merino sheep as -0.04 with a 10% increase in inbreeding. Norberg and Sorensen (2007) showed that the regression on direct inbreeding for the number of lambs per ewe lambing was significant for all three breeds i.e. Texel, Shropshire and Oxford Down. Direct inbreeding depression ranged from -0.019 to -0.032 lambs per ewe lambing with 10% increase in inbreeding

which was lower than present investigation based on one percent increase in inbreeding. Van Wyk *et al.* (2009) reported an inbreeding effect of 0.0022 lambs in Elsenburg Dormer sheep.

#### Estimation of inbreeding coefficient using microsatellite markers

All the microsatellite markers were successfully amplified in all animals, and all the loci for this population were in Hardy-Weinberg disequilibrium ( $P < 0.001$ ). In the global test of deviation from Hardy-Weinberg equilibrium (HWE), the deviations from the expected value may be due to a variety of causes: population subdivision owing to genetic drift, artificial selection, inbreeding, the excess of heterozygote individuals than homozygote individuals, migration, high mutation rate in microsatellite and artificial selection in a breed (Lawson *et al.* 1989; Aminafshar *et al.* 2008). Tests of genotype frequencies for deviation from HWE at each locus in this breed, revealed significant departure from HWE.

Deviation from HWE at microsatellites loci have, also been reported in various studies (LuiKart *et al.* 1999; Laval *et al.* 2000; Barker *et al.* 2001; Hassan *et al.* 2003; Elfawal, 2006; Aminafshar *et al.* 2008; El Nahas, 2008; Sharifi Sidani *et al.* 2009).

It is known that a population is considered to be in HWE only when it is able to maintain its relative allele frequencies. Heterozygosity deficiency is one of the parameters underlying departure from HWE.

Heterozygosity deficiency may result from one or more of the following reasons: i) the presence of a null allele which is the allele that fails to multiply during PCR using a given microsatellite primer due to a mutation at the primer site (Callen *et al.* 1993; Pemberton *et al.* 1995); ii) small sample size, where rare genotypes are likely to be included in the samples; iii) the Wahlund effect, i.e. presence of fewer heterozygotes in a population than predicted on account of population subdivision and iv) the decrease in heterozygosity due to increased consanguinity (inbreeding) (Kumar, 2006). As shown in Table 5 from a total of 100 samples, a total of 34 alleles were detected for the 6 microsatellite markers (BM8125, BMS2361, BM6526, BM6438, BMS1004 and BM6444).

The number of alleles per locus ranged from 4 (BM6438) to 7 (BMS1004) with a mean of 5.67 and the number of effective alleles ranged between 2.104 and 6.129. The indices of the observed heterozygosity ( $H_o$ ) per locus varied from 0.35 (BM6438) to 0.90 (BM8125) with an average value of 0.738 for all individuals. F-statistics is primarily concerned with relationship between the genotype frequencies in the total populations and in the sub population for a single locus. This is usually expressed as a ratio of different types of gene diversities, or simply; it is the ratios of gene diversities of heterozygosities rather than the correlation of uniting gametes (Ibrahim *et al.* 2010). The inbreeding coefficient measures the reduction of heterozygosity in an individual because of non-random mating within population and hence  $F_{IS}$  values significantly higher or lower than zero reveal inbreeding or outbreeding, respectively (Radha *et al.* 2011). The Wright's F-statistic ( $F_{IS}$ ) for each locus is shown in Table 5. The  $F_{IS}$  estimates ranged from -0.0277 (BMS1004) to 0.3329 (BM6438). The positive  $F_{IS}$  values ranged from 0.2287 (BM6444) to 0.3329 (BM6438). Out of 6 studied loci, four revealed negative  $F_{IS}$  values, indicating an excess of heterozygotes in the population and the absence of inbreeding at these loci. The highest within-population fixation index was observed for the BM6438 (0.3329) and BM6444 (0.2287) microsatellites, however, others were below zero. The average inbreeding coefficient based on marker data was positive and estimated as 0.2617%, indicating that parents were more related than expected under random mating. In the present study, heterozygotes deficiency was observed at 4 loci and heterozygotes excess at 2 loci. The positive  $F_{IS}$  value ranged from 0.2287 (BM6444) to 0.3329 (BM6438). Four loci revealed negative  $F_{IS}$  value indicating an absence of inbreeding at these loci.

The  $F_{IS}$  value (0.3329) for BM6438 revealed a shortage of heterozygotes (33%) and an excess of homozygotes (67%) in Kordi sheep. Lower rates of inbreeding were observed in three endangered Spanish sheep breeds, namely the Colmenarena, Mallorquina and Rubia de El Molar of 0.103, 0.113 and 0.138 respectively (A'lvarez *et al.* 2009), in Muzzafarnagari sheep of 0.128 (Bhatia and Arora, 2008), Recka sheep of 0.013 (Hoda *et al.* 2010) and a higher value in Vembur sheep, namely 0.295 (Prasad *et al.* 2009).

**Table 5** Population genetic parameters for each microsatellite marker

Locus	No. of alleles		Heterozygosity		$F_{IS}$
	Observed ( $N_a$ )	Effective ( $N_e$ )	Observed ( $H_o$ )	Expected ( $H_e$ )	
BM8125	5.00	4.77	0.900	0.794	-0.1386
BMS2361	6.00	3.79	0.780	0.740	-0.0599
BM6526	6.00	5.03	0.950	0.805	-0.1859
BM6438	4.00	2.10	0.350	0.527	0.3329
BMS1004	7.00	6.13	0.860	0.841	-0.0277
BM6444	6.00	4.25	0.590	0.769	0.2287

$N_a$ : observed number of alleles and  $N_e$ : effective number of alleles.

Inbreeding coefficient measures the reduction of heterozygosity in an individual because of non-random mating within the population and hence  $F_{IS}$  values significantly higher or lower than zero suggest inbreeding or outbreeding, respectively. In closed breeding flocks, success in controlling inbreeding over the long term will depend on the ability to limit genetic relationships between rams entering the breeding flock for mating. In the present study, observed levels of heterozygosity and inbreeding for studied loci, could be explained as the result of a controlled mating system over generations that have been implemented through breeding program aimed to maximize genetic variability and the maintenance of lower inbreeding levels in the flock.

## CONCLUSION

In conclusion, it is evident that inbreeding coefficients should be considered when compiling mating lists to limit the possible negative effects of inbreeding on productive and reproductive traits and to detect animals susceptible to high levels of inbreeding. Slow inbreeding allows natural and artificial selection to operate and to remove the less fit animals. In this flock (which was developed under planned mating to avoid inbreeding) and with moderate increases in inbreeding, natural and artificial selection may be capable of overcoming accumulated inbreeding depression.

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