Genetic Diversity of Sanjabi Sheep Inferred from Microsatellite Markers and Their Association with Fecundity and Body Weight Traits

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Abstract: Currently, microsatellites are the most popular markers in livestock genetic characterization studies. Microsatellite is extensively used for designing the genetic chain map, orienting quantity character loci and evaluating the genetic diversity. The aim of the present study was to detect genetic diversity of Sanjabi sheep breed inferred from microsatellite markers and their association with fecundity and body weight traits. Blood samples were randomly collected from 100 Sanjabi sheep, transported to the laboratory and stored at -4°C for further analysis. DNA was extracted using modified salting-out method and 11 microsatellite loci were amplified by specific primers pairs using Polymerase Chain Reaction (PCR). The PCR products were then separated by electrophoresis on 8% poly acryl-amid gel. Data was analyzed using GENEPOP and SAS software. Statistical analysis showed that all markers had significant effect on fecundity and some of the body weight traits ($p<0.05$).

Key words: Microsatellite, fecundity, body weight, Sanjabi sheep, Kermanshah, PCR

INTRODUCTION

Microsatellite also called Simple Sequence Repeat (SSR) is among the most variable types of DNA sequence in the genome. It has been developed from the late 1980s. It is generally accepted for its abundant quantity, wider distribution, richer polymorphism, dominant heredity and its quicker and convenient inspecting method. Microsatellite is extensively used in designing the genetic chain map, orienting quantity character loci and evaluating the genetic diversity (Takahashi *et al.*, 1998; Saitbekova *et al.*, 1999; Hearne *et al.*, 1992; Farid *et al.*, 2000; Bishop *et al.*, 1994). Microsatellite sequences also known as Short Tandem Repeats (STRs), consist of tandem repeated 1-6 nucleotide motifs dispersed throughout the genome. Their high mutation rate, the abundance, the distribution throughout the genome, the neutrality, the co-dominance nature and easy automation of analytical procedures permit the estimation of within and between-breed genetic diversity (Canon *et al.*, 2001; FAO, 2007). Iran has 27 sheep populations which vary in their genetic potential for production of milk, meat and wool; disease resistance, fecundity etc. (Tavakkolian,

2000). The Sanjabi sheep is one of the local breeds in Iran. This breed has an average adult body weight of 42.3 kg and has 79% single birth and 21% twins. The Sanjabi sheep has better adaptability to extensive management in Western area of Iran, better immunity against diseases, semi high reproduction rate and better meat-wool quality.

Many studies showed that seven microsatellite loci including OarAE101, BM1329, BM143, LSCV043, GC101, OarHH55, OarHH35 and four microsatellite loci including OarFCB128, TGLA377, CSSM47 and OarHH64 had relation with reproductive performance in goat and sheep, respectively (Arora *et al.*, 2010; Guan *et al.*, 2007; Ouyang *et al.*, 2006a, b; Song *et al.*, 2008; Wang *et al.*, 2010). The aim of this study was to detect genetic diversity of Sanjabi sheep inferred from 11 microsatellite markers and their association with fecundity and body weight traits.

MATERIALS AND METHODS

Samples: Blood samples were collected from 100 registered Sanjabi sheep breed rearing in Mehregan

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Breeding Station of Kermanshah Agriculture and Natural Resources Research Center, Iran. Sanjabi sheep is a bulk heavy sheep that upbringing in Kermanshah. For each sheep, the number of lambs, the date of lambing, the season of lambing, the body weight in different times (birth, 45 days, 3, 6, 9, 12 and 15 months) were recorded in this research.

Microsatellite markers: Eleven microsatellite markers including GC101, LSCV043, TGLA377, CSSM47, OarFCB128, OarAE101, BM1329, BM143, OarHH55, OarHH35 and OarHH64 were selected as their level of polymorphism detected in previous studies (Guan *et al.*, 2007; Ouyang *et al.*, 2006a, b; Song *et al.*, 2008; Wang *et al.*, 2010).

DNA extraction and PCR amplification: Fresh blood samples were randomly collected from 100 Sanjabi sheep and stored at -4°C until the analysis was performed. Genomic DNA was isolated from white blood cells using a standard protocol (Miller *et al.*, 1988). Microsatellites were amplified by Polymerase Chain Reaction (PCR). PCR was performed in a final volume of 25 μL containing double-distilled water 18.5 μL, 10×buffer 2 μL, $MgCl_2$ 1-2 μL (25 mmol L^{-1}) dNTPs 1 μL (2.5 mmol L^{-1}), for each primer 0.5 μL (10 mmolL^{-1}), Taq DNA polymerase 0.5 μL (5 u μL^{-1}) and DNA template 0.5 μL (200 ng μL^{-1}). The primer sequences, volume of $MgCl_2$ and annealing temperature are shown in Table 1. The cycling conditions were as follows: an initial denaturation step of 3 min at 94°C followed by 35 cycles of 1 min at 94°C, 45 sec at 60-63°C and 1 min at 72°C. The last polymerization step was extended for 5 min at 72°C. The amplified products were separated by electrophoresis of denaturing poly acryl-amid gel (8%) and were fixed and developed using the silver nitrate method.

The product sizes were determined with the help of 10 bp DNA ladder as a standard marker. The genotypes were scored based on the presence of a single band (homozygote) or double bands (heterozygote) in the gel.

Data analysis: Gene and genotypic frequencies were estimated by direct counting. Deviations from Hardy-Weinberg (HW) equilibrium based on hypothesis of heterozygote deficiency were estimated by GENEPOP software (Raymond and Rousset, 1995). Heterozygosity (H), Polymorphic Information Content (PIC), effective allele Number (Ne) of each locus were computed by SAS software (SAS Institute, 2003). The effect of animal genotypes on fecundity and body weight traits were analyzed using General Linear Model (GLM) procedure of SAS software. The following model was used:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + e_{ijkl}$$

Where:

Y_{ijkl} = Analyzed traits
μ = The overall mean of population
a_i = Fixed effect of animal genotype
b_j = Fixed effect of year
c_k = Fixed effect of season
e_{ijkl} = The random residual error

Table 1: The primer sequences, volume of $MgCl_2$ with annealing temperature used in PCR

Locus	Primer sequences (5'-3')	25 mmol L^{-1} $MgCl_2$ (μL)	Annealing temp. (°C)
GC101	F-ATCCTCACCCTTCAAACAG		
	R-CTGGGGAGTTTTCTCTGAC	2.0	62.5
LSCV043	F-CCAGAATATAGAGTTTTGTCAAG		
	R-GCCTGATTTGATTTTGTCAAG	1.5	61.0
TGLA377	F-GACTGTCATTATCTTCCAGCGGAG		
	R-GATCTCTGGTTGAAATGGCCAGCAG	1.5	63.0
CSSM47	F-TCTCTGTCTCTATCACTATATGGC		
	R-CTGGGCACCTGAAACTATCATCAT	1.5	61.0
OarFCB128	F-CAGCTGAGCAACTAAGACATACATGCG		
	R-ATTAAAGCATCTTCTCTTTATTTCCTCGC	1.0	60.0
OarAE101	F-TTCTTATAGATGCACTCAAGCTAGG		
	R-TAAGAAATATATTTGAAAAACTGTATCTCCC	1.5	62.0
BM1329	F-TTGTTTAGGGCAAGTCCAAAGTC		
	R-AACACCGCAGCTTCATCC	1.0	60.0
BM143	F-ACCTGGGAAGCCTCCATATC		
	R-CTGCAGGCAGATTCTTTATCG	1.5	63.0
OarHH55	F-GTTATTCCATATTCTTTCCTCCATCATAAGC		
	R-CCACACGACAACTAAAACCCAGC	2.0	62.5
OarHH35	F-AATTGCATTCAGTATCTTTAACATCTGGC		
	R-ATGAAAATATAAAGAGAATGAACCACACGG	2.0	62.5
OarHH64	F-CGTTCCCTCACTATGGAAAGTTATATATGC		
	R-CACTCTATTGTAAGAATTTGAATGAGAGC	1.5	61.0

RESULTS AND DISCUSSION

Microsatellite analysis: All loci were successfully amplified in samples collected from Sanjabi sheep. A total of 64 alleles were detected for the 11 studied loci and the number of alleles/locus ranged from 3 (TGLA377) to 9 (GC101, LSCV043) with mean of 5.82 for observed number of alleles and 4.05 for the effective number of alleles. The chromosomal location and size range of alleles were determined for each of 11 markers (Table 2). All of the markers were found to have high polymorphism, with PIC ranging from 0.63 (TGLA377) to 0.82 (OarHH35). Overall, the mean H_o across loci was 0.64 while the mean H_e was 0.77. The H_o was highest for LSCV043 (0.79) and lowest for BM143 (0.34) and the H_e was highest for CSSM47 (0.96) and lowest for OarHH55 (0.70). In this study, microsatellite markers were on Hardy-Weinberg disequilibrium (p>0.01) (Table 2).

Association between locus and fecundity and body weight traits: In this research, 84 genotypes were detected for all microsatellite markers. The genotype number was highest for OarHH35 (13) and lowest for TGLA377, BM143 and OarHH64 (5) (Table 3). Duncan's test was used in comparison of different genotype means of microsatellite markers for fecundity and body weight traits. As shown in Table 3, statistical analysis indicated that all markers had significant effect on fecundity and some of the body weight traits (p<0.05).

The Sanjabi breed showed a significant (p<0.01) deviation from Hardy-Weinberg equilibrium at all loci. This deviation might represent nonrandom mating, selection or the presence of null alleles. Sun *et al.* (2010) selected five microsatellite loci OarAE101, BM1329, BM143, OarHH35 and OarHH55 to study their correlation with fecundity of HU sheep breed. The result of variance analysis showed that there were extreme significant

Table 2: Number of alleles (observed n_a, effective n_e), chromosomal location and size range of alleles, heterozygosity (observed H_o, expected H_e) and Polymorphism Information Content (PIC) for each of 11 markers

Marker	No. of alleles			Minimum allele size	Maximum allele size	Heterozygosity			H-W (χ^2)
	Chr	Observed (n_o)	Effective (n_e)			Observed (H_o)	Expected (H_e)	PIC	
GC101	6	9.00	4.09	190	278	0.75	0.71	0.75	190.18[**]
LSCV043	6	9.00	4.77	73	173	0.79	0.74	0.79	127.31[**]
TGLA377	2	3.00	2.76	85	108	0.64	0.70	0.63	12.83[**]
CSSM47	2	5.00	4.08	140	185	0.75	0.96	0.65	161.90[**]
OarFCB128	6	4.00	3.92	92	127	0.74	0.91	0.77	44.13[**]
OarAE101	6	6.00	4.67	103	157	0.65	0.79	0.78	157.31[**]
BM1329	6	7.00	4.21	135	194	0.69	0.76	0.77	243.57[**]
BM143	6	4.00	3.39	100	135	0.34	0.70	0.70	146.58[**]
OarHH55	6	5.00	3.04	121	167	0.42	0.67	0.67	157.35[**]
OarHH35	4	8.00	5.63	100	174	0.68	0.83	0.82	221.25[**]
OarHH64	4	4.00	3.97	110	141	0.66	0.75	0.74	105.47[**]
Mean	-	5.82	4.05	-	-	0.64	0.77	0.73	142.53[**]

Table 3: Least significant range of locus genotypes for fecundity and body weight traits in Sanjabi sheep

Locus	Genotype	N	Fecundity	BW					
				45 days	W3	W6	W9	YW	W15
GC101	AB	6	1.31±0.084[ab]	11.40	18.30	24.22	29.06	34.27	35.15[b]
	BC	5	1.20±0.097[b]	11.68	18.46	26.16	30.44	34.92	37.46[a]
	BF	6	1.35±0.086[ab]	11.68	18.38	25.87	30.28	33.78	36.65[ab]
	DC	4	1.27±0.095[b]	12.05	18.53	26.32	30.95	35.70	38.50[a]
	FC	3	1.13±0.136[b]	11.90	19.63	24.70	30.27	35.03	38.46[a]
	EF	4	1.41±0.143[a]	11.67	18.97	26.52	31.20	35.07	38.78[a]
	FF	21	1.23±0.069[b]	11.61	18.86	24.54	30.23	35.04	38.17[a]
	FG	13	1.23±0.061[b]	11.20	17.60	23.30	30.70	37.10	39.20[a]
	FH	12	1.25±0.189[b]	11.54	18.25	26.45	30.88	35.21	37.70[a]
	GH	14	1.22±0.069[b]	11.67	18.99	26.33	31.14	36.00	39.36[a]
	HH	8	1.19±0.097[b]	12.52	19.53	25.95	30.07	35.08	38.35[a]
	HI	4	1.21±0.124[b]	12.52	18.72	25.35	30.42	34.70	37.98[a]
LSCV043	AA	4	1.02±0.134[b]	10.55[b]	18.45[ab]	26.20	32.40[b]	36.15[b]	39.27[b]
	BC	6	1.28±0.131[a]	12.80[a]	20.37[a]	27.43	32.22[b]	36.23[b]	38.96[b]
	DD	11	1.22±0.067[a]	12.24	19.52[ab]	27.10	32.21[b]	36.50[b]	37.86[b]
	DE	14	1.18±0.065[a]	1.77[ab]	18.68[ab]	25.81	30.84[b]	34.89[b]	37.86[b]
	DG	7	1.27±0.172[a]	11.17[ab]	18.68[ab]	25.02	30.54[b]	34.39[b]	37.50[b]
	EF	18	1.35±0.050[a]	11.78[ab]	18.79[ab]	26.46	29.31[b]	34.47[b]	37.50[b]
	EG	16	1.28±0.063[a]	11.17[ab]	18.65[ab]	25.02	28.93[b]	37.01[b]	37.33[b]
	EH	13	1.28±0.077[a]	11.76[ab]	17.22[b]	25.03	31.51[b]	33.22[b]	37.12[b]

Table 3: Continue

Locus	Genotype	N	Fecundity	BW 45 days	W3	W6	W9	YW	W15
	GI	10	1.20± 0.084[a]	11.70[ab]	19.20[ab]	27.30	32.85[a]	37.72[a]	40.01[a]
TGLA377	AA	20	1.36±0.0450[a]	11.60	18.32	25.83	31.11[ab]	35.93[ab]	38.64[a]
	AB	16	1.20±0.0700[ab]	11.75	18.97	27.23	31.85[a]	36.21[a]	38.74[a]
	AC	28	1.17±0.0560[b]	11.60	18.96	24.95	29.58[b]	34.11[b]	37.78[a]
	BC	24	1.19±0.0580[b]	11.71	18.68	25.54	30.76[ab]	34.87[b]	37.92[a]
	CC	12	1.17±0.0740[b]	11.52	18.68	26.02	30.41[ab]	35.27[ab]	33.82[b]
CSSM47	AB	16	1.37±0.0540[a]	11.64[ab]	18.99[ab]	27.06	32.27[a]	36.97[a]	39.69[a]
	AD	11	1.19±0.0730[bc]	10.81[b]	17.79[b]	25.10	29.63[b]	34.67[b]	38.09[ab]
	BC	17	1.08±0.0730[c]	11.91[a]	19.26[a]	25.31	30.01[b]	34.77[b]	36.12[b]
	DD	9	1.17±0.0700[bc]	11.53[ab]	18.30[ab]	25.94	31.46[ab]	36.91[a]	38.95[ab]
	DE	25	1.31±0.0510[ab]	11.61[ab]	18.45[ab]	24.95	29.96[b]	33.91[b]	37.10[ab]
	EE	22	1.15±0.0600[bc]	11.95[a]	18.89[ab]	26.12	30.89[ab]	35.11[b]	38.06[ab]
OarFCB128	AA	9	1.14±0.0810[b]	11.81[ab]	18.86	24.64	29.03[b]	34.51[ab]	38.26
	AB	19	1.38±0.0450[a]	11.40[ab]	18.38	26.13	31.41[a]	36.14[a]	38.72
	AD	14	1.15±0.0730[b]	11.00[b]	18.18	26.15	30.11[ab]	34.53[ab]	37.50
	BC	22	1.16±0.0580[b]	11.71[ab]	18.80	25.68	30.84[ab]	35.37[ab]	36.81
	BD	18	1.26±0.0770[ab]	12.17[a]	19.23	25.99	30.09[ab]	35.53[ab]	36.77
	DC	18	1.18±0.0590[b]	11.79[ab]	18.80	25.11	30.81[ab]	33.91[b]	36.42
OarAE101	AB	13	1.28±0.0690[a]	11.12[ab]	18.07[ab]	25.27	30.39	34.74	38.10
	AC	6	1.26±0.0830[a]	10.63[b]	16.95[b]	25.17	30.24	34.48	37.67
	BB	17	1.23±0.0630[a]	11.87[ab]	19.18[a]	25.95	30.39	35.21	38.47
	BC	7	1.33±0.0860[a]	12.23[a]	19.18[a]	26.19	30.56	35.63	38.32
	BD	10	1.19±0.0770[a]	11.67[ab]	19.92[a]	26.56	30.89	34.72	36.91
	CD	22	1.26±0.0620[a]	12.15[a]	19.13[a]	25.15	30.96	35.30	36.98
	DD	4	1.32±0.1010[a]	11.57[ab]	17.82[ab]	26.27	31.10	36.80	39.27
	EE	14	1.35±0.0620[a]	11.47[ab]	18.66[a]	26.19	31.47	36.06	38.89
	EF	7	1.16±.07900[b]	11.06[ab]	17.93[ab]	25.71	30.67	34.07	39.29
BM1329	AA	9	1.47±0.1120[a]	11.73[a]	18.30[ab]	25.64	31.17	33.17	36.14
	BE	12	1.19±0.0610[b]	11.33[a]	18.53[ab]	26.37	30.69	33.69	35.60
	BG	14	1.29±0.0740[a]	10.80[a]	17.38[b]	25.07	29.89	32.89	35.09
	CC	19	1.27±0.0660[a]	11.14[a]	18.25[ab]	26.69	31.84	33.85	35.75
	CD	16	1.25±0.0570[a]	11.67[a]	18.80[ab]	26.34	30.86	33.05	35.04
	CF	13	1.27±0.0580[a]	11.71[a]	18.75[ab]	25.60	29.74	32.95	34.77
	EF	11	1.25±0.0790[a]	11.94[a]	19.26[a]	23.94	30.86	32.95	34.26
	FF	6	1.32±0.1170[a]	11.95[a]	18.96[ab]	25.35	30.05	33.69	35.13
BM143	AA	19	1.22±0.0590[b]	11.83	18.86[ab]	24.26[b]	29.59[b]	34.84[ab]	38.20[ab]
	AB	21	1.31±0.0630[a]	11.57	18.59[ab]	25.94[ab]	30.78[a]	35.68[a]	38.89[a]
	BC	15	1.25±0.0610[a]	11.10	17.87[b]	25.35[ab]	30.11[ab]	33.71[b]	36.87[b]
	CC	27	1.29±0.0490[a]	11.83	19.11[a]	26.83[a]	31.41[a]	35.52[a]	38.40[ab]
	CD	18	1.27±0.0630[a]	11.47	18.26[ab]	25.69[ab]	31.07[ab]	36.27[a]	35.75[b]
OarHH55	AA	16	1.364±0.058[a]	11.81[ab]	18.85	26.15	30.84[ab]	36.14[a]	39.27
	AC	12	1.241±0.071[ab]	11.71[ab]	18.80	26.13	31.40[a]	35.52[ab]	38.72
	BB	13	1.141±0.062[bc]	11.78[ab]	18.80	25.99	30.84[ab]	33.90[b]	37.50
	CC	29	1.142±0.055[bc]	12.70[a]	19.22	24.63	30.11[ab]	34.52[ab]	36.80
	DC	21	1.374±0.049[a]	11.48[ab]	18.18	25.68	29.26[b]	34.50[ab]	36.72
	CE	9	1.025±0.096[c]	11.09[b]	18.37	25.11	30.84[ab]	34.22[ab]	38.25
OarHH35	AB	5	1.16±0.1280[ab]	11.93[ab]	19.23	25.26	29.55	33.66[ab]	37.80
	AD	4	1.04±0.1130[b]	11.70[ab]	18.45	26.00	29.88	34.97[ab]	38.37
	BD	9	1.12±0.0880[b]	11.47[ab]	18.24	25.21	29.69	34.30[ab]	37.60
	BF	8	1.20±0.0840[b]	11.41[ab]	19.00	26.77	31.15	35.03[ab]	38.14
	CC	6	1.01±0.0940[b]	10.56[b]	18.10	25.82	30.77	35.00[ab]	37.62
	DD	7	1.12±0.0100[b]	11.78[ab]	19.10	25.20	29.88	32.86[b]	36.40
	DE	8	1.13±0.0840[ab]	11.63[ab]	18.98	27.00	31.42	35.38[ab]	37.74
	DF	10	1.13±0.0950[b]	12.46[a]	19.65	24.77	31.46	36.21[ab]	39.12
	FF	11	1.41±0.0510[a]	11.00[ab]	17.76	25.73	30.85	35.70[ab]	38.50
	FG	9	1.18±0.0770[ab]	11.78[ab]	18.79	25.59	30.74	34.36[ab]	33.77
	FH	6	1.08±0.1100[b]	11.75[ab]	18.82	25.35	28.97	36.75[a]	39.14
	GH	11	1.28±0.0630[ab]	11.61[ab]	18.40	26.53	31.80	36.46[a]	39.29
	HH	7	1.1±0.08900[b]	12.52[a]	19.40	25.03	29.75	34.63[ab]	38.01
OarHH64	AA	17	1.32±0.0490[a]	11.11[b]	18.50[b]	24.61	30.47	35.08	38.19[ab]
	AB	18	1.15±0.0590[b]	11.65[ab]	18.17[b]	26.02	30.46	35.27	35.81[b]
	BD	26	1.36±0.0500[a]	11.61[ab]	18.65[ab]	25.87	30.65	34.98	37.70[ab]
	CC	17	1.58±0.0680[b]	11.56[ab]	18.84[ab]	25.87	30.79	34.98	38.60[ab]
	CD	22	1.16±0.0620[b]	12.26[a]	19.53[a]	26.63	30.99	35.78	38.88[a]

differences among the different genotypes for the producing ability of fertility in OarHH35 locus ($0.01 < p < 0.05$). Also, they found no significant difference among the different genotypes for the producing ability of fecundity in BM1329 and OarHH55 loci ($p > 0.05$). However in this research, we found that all genotypes of studied loci had significant ($p < 0.05$) effect on fertility. By using 19 microsatellite loci for determining the genetic diversity among Chinese indigenous sheep breeds, Zhong *et al.* (2009) detected that the expected heterozygosity was ranging from 0.623-0.737. In Sanjabi breed, the range of expected heterozygosity was found to be 0.7-0.96. Ligda *et al.* (2009) analyzed 28 microsatellite markers in order to estimate their genetic diversity and differentiation in ten Greek sheep breeds. They found non-biased average expected heterozygosis ranged from 0.68 ± 0.134 (Skopelos breed) to 0.76 ± 0.103 (Karagouniko breed) with an average of 0.74 while the average observed heterozygosis ranged from 0.626 ± 0.132 (Skopelos) to 0.74 ± 0.135 (Kefallenias). But in Sanjabi sheep, the H_e and H_o ranging were 0.67-0.96 and 0.34-0.79, respectively.

Bozzi *et al.* (2009) used a panel of 24 microsatellite markers to analyze the genetic diversity of five Italian sheep breeds. They found that all of the investigated breeds showed a significant heterozygote deficiency caused by the high level of inbreeding. In present research with eleven microsatellite markers, the finding showed that the Sanjabi sheep had high PIC and heterozygote caused by the low level of inbreeding. Ivankovic *et al.* (2005) analyzed genetic variation in the Pag island sheep population using seven microsatellite loci. The mean number of alleles per microsatellite locus (8.71) and mean expected heterozygosis (0.833) revealed the high genetic variability. In Sanjabi breed, the mean H_e (0.77) was almost the same with Pag island sheep breed. But there was difference in the mean number of alleles per microsatellite locus (5.82). Arora and Bhatia (2004) searched genetic structure of Muzzafarnagri sheep based on microsatellite analysis. They reported that the mean observed and expected heterozygosity was 0.652 and 0.697, respectively. Also, they detected the mean PIC value (0.636) caused by the high level of genetic variability. In present study, the mean H_o, H_e and PIC were 0.64, 0.77 and 0.73, respectively.

CONCLUSION

The results of this study using 11 microsatellite loci offer a great potential to improve efficiency of economically important traits in Sanjabi sheep breed.

Individual Sanjabi sheep also could be selected for breeding and preservation based on specific knowledge of their genotypes. The results will help to enhance genetic improvement of this breed as well as the management of production and development of high quality products.

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