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Comparison of Fleckvieh and Montbeliarde Stud Bulls with Turkish Simmental Stud Bulls in Terms of Genetic Structure and Diversity

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Abstract

The original Simmental cattle stood out for their potentials of durability, rapid growth and milk quality. They deliver many kinds of breeding types due to their multi-purpose production. The goals of Simmental cattle breeding has been diverted many times since its domestication. Intensive selection programs applied to increase the frequency of alleles associated with economically important traits in the population may cause the frequencies of some alleles to decrease or even disappear. In this study, it was aimed to evaluate the diversity of Simmental cattle subpopulations, which have been subjected to many crossbreeding and selection programs since their existence, by using their sire lines. For this purpose, genetic structure and diversity were evaluated using microsatellite data of 16 Simmental bulls bred in Türkiye and 115 Fleckvieh and 27 Montbeliarde breeding bulls whose semen imported to Türkiye. As a result, it was observed that the genetic structure of Montbeliarde subpopulation had more homogenity than Fleckvieh and Turkish Simmental subpopulations. Besides, the genetic structure of Turkish Simmental subpopulation was similar to Fleckvieh subpopulation.

Introduction

Originating from the Simmen and Bern regions of Switzerland, Simmental cattle is known as the second most bred cattle breed in the world today. The original Simmental cattle stood out for its potentials of durability, rapid growth and milk quality (American Simmental Association, 2023). They deliver many kinds of breeding types due to their multi-purpose production. Globally, they are mostly grown for milk yield, meat yield as well as dual-purpose (i.e., milk and meat yield) (World Simmental Fleckvieh Federation, 2023).

The largest Simmental cattle population is reported to be in Germany (Periši et al., 2009). The German Simmental was obtained by crossing the Swiss Simmental with local breeds and is called Fleckvieh, serving dual-purpose breeding (Periši et al., 2009; World Simmental Fleckvieh Federation, 2018). Simmental cattle bred in Australia is descended from German Fleckvieh strain, and they were backcrossed with Swiss strains. Fleckvieh population bred in Germany and Australia are evaluated via the same genetic evaluation system run common by these two countries (Averdunk and Krogmeier, 2011). In recent years, Fleckvieh bulls are the most preferred Simmental strain among the breeders in Türkiye.

In France, three subpopulations were created from Simmental with crossbreeding with domestic breeds. One of these subpopulations, named as French Simmental, is preferred for carcass yield, while the other subpopulation named Abordance is preferred for high-quality milk composition for the purpose of exclusive cheese production. The third subpopulation named Montbeliarde, which has an average body size, is preferred for milk yield by breeders (Averdunk & Krogmeier, 2011). Montbeliarde has the highest milk yield among all the Simmental subpopulations although it has less than 25% Holstein genotype (Mihai et al., 2019). Montbeliarde stud bulls are the one the preferred subpopulations of Simmental in Türkiye.

At first, the purposes of importing Simmental to the United States and Australia were improving both milk and meat yield of domestic breeds (Averdunk and

Krogmeier, 2011). After that, American Simmental Association (ASA) focused on producing meat yield in contrast to the Europe breeding programs on Simmental. Whereby the US breeding program, the color and the other visual traits of American Simmental were ignored, while the traits on high meat yield and the adaptation of different environmental conditions were focused on (American Simmental Association, 2023).

The goals of Simmental cattle breeding has been diverted many times since its domestication. These goals are continuing to be reshaped as regards to the changing demands of the countries (Periši et al., 2009). Intensive selection programs are conducted mostly on stud bulls (Wiggans et al., 2017). Nowadays, genomic selection and marker-assisted selection (MAS) approaches are frequently used for breeding programs applied to livestock (Arruda et al., 2016). Especially in recent years, GWAS studies conducted with farm animals have created a large QTL data useable for MAS (Arzik et al., 2023; Kizilaslan et al., 2022; Scholtens et al., 2020; Zhang et al., 2014).

One of the biggest difficulty of livestock farming is to offer cheap products to the target consumer without giving up on quality characteristics (Williams, 2005). Intensive selection programs applied to increase the frequency of alleles associated with economically important traits may cause the frequencies of some alleles to decrease or even disappear in the population. This situation, which results in a decrease in genetic diversity, may increase some genetic vulnerabilities leading to the increase of inbreeding depression in the population and hamper the subsequent selection programs (Williams, 2005). Altering the breeding programs will be required to keep up with the changing breeding goals. Changing breeding programs can only show their effects if there is sufficient genetic diversity.

Crossbreeding will cause an increase in genetic diversity, as new alleles will be contributed to the generations. The increased genetic diversity is related to the increased rates of heterozygosity in the crossbred populations (Ganteil et al., 2021). Crossbreeding is important for increasing the adaptation ability of a susceptible breed to different environments but also causes a loss of ancestral identity of breeds for subsequent generations (Hall, 2004). For this reason, there should be a delicate balance among the selection programs, crossbreeding and the management of genetic diversity.

Genetic diversity analysis has a guiding effect in evaluating the current genetic structure of populations and a potential of shaping the future breeding plans. Microsatellite loci are useful markers because they are multi-allelic (Williams, 2005), allowing them to be used efficiently in studies such as genetic mapping in populations, linkage analysis and pedigree inferences, genetic bottlenecks and genetic diversity (Agung et al., 2019; Garkovenko et al., 2018; Knott et al., 1998; Unlusoy, 2022).

In this study, it was aimed to evaluate the diversity of Simmental cattle subpopulation, which have been subjected to many crossbreeding and selection programs since their existence, by using their sire lines. For this purpose, genetic diversity was evaluated using microsatellite data of Simmental bulls bred in Türkiye as well as Fleckvieh and Montbeliarde breeding bulls imported to Türkiye.

Materials and Methods

This research was conducted using the microsatellite fragment analysis data from the International Center for Livestock Research and Training (ICLRT), of the semen samples from 16 Simmental bulls produced in Türkiye and the semen samples of 27 Montbeliarde and 115 Fleckvieh bulls imported to Türkiye. The data, produced using the ABI 3130 Genetic Analyzer device, was subjected to the preliminary quality assessment in this study and then subjected to bioinformatics analysis.

For quality assessment, GeneMapper® Software Version 4.0 was used to visually inspect the background noise to eliminate them. A total of 158 bulls that passed the quality assessment were evaluated for 10 microsatellite loci (BM2113, BM1824, TGLA126, TGLA122, TGLA53, ETH10, SPS115, INRA23, ETH3, and ETH225). The data was manipulated with the R 3.6.3 (R Development Core Team, 2019) program.

Genepop (Rousset 2008) data was generated and converted to the other data format for the different software. Cervus 3.0.7 (Kalinowski 2007) software was used for the number of alleles, the number of genotypes, the observed heterozygosity (Ho), the expected heterozygosity (He), the polymorphic information content (PIC), null allele frequency and significance of deviation from Hardy-Weinberg equilibrium in whole population for each locus. Arlequin 3.5(Excoffier et al., 2005) software was used to calculate number of alleles, number of genotypes, Ho, He and significance of the deviation from Hardy-Weinberg equilibrium in each subpopulation for each locus.

FSAT V2.9.4 (Goudet 1995) software was used for the calculation of the inbreeding coefficient (F_{IS}) values of each subpopulation to measure the degree of inbreeding. P-values for the difference between F_{IS} within population were obtained by Permutation tests with Bonferroni correction (Rice 1989). For evaluating whether F_{IS} within population was significantly different from zero, p values of F_{IS} were calculated from the proportions of permutations that gave larger than observed of F_{IS}. The fixation index (F_{ST}) were calculated for population differentiation by Arlequin (Excoffier et al. 2005) Software based on the approach by Weir and Cockerham (1984). Genetic structure analysis of the subpopulations was performed with STRUCTURE v2.3.4 software (Pritchard et al 2000). The optimal k-value for the structure analysis was revealed using Evanno method (Evanno et al, 2005) by Structure Harvester (Earl et al., 2012).

Results and Discussion

In the study, the microsatellite genotypes have been obtained from 158 stud bulls based on 10 loci (BM2113, BM1824, TGLA126, TGLA122, TGLA53, ETH10, SPS115, INRA23, ETH3, and ETH225). All the loci were informative for each group of the population. The information on the allele content of the study populations was shown in Table 1. Considering all the 10 microsatellite loci, a total of 76 alleles were observed in the whole population. The average number of alleles per locus is 7.6. In a study on Holstein bulls, the number of total alleles was 70 and the average number of alleles was 7.0 for the same 10 microsatellite loci evaluated (Unlusoy, 2023). When we compared this two studies, all populations in this study had more alleles than Holstein bulls of the previous. In this study, BM1824 was the locus with the least polymorphism observed, with 4 alleles, and was observed at the same rate in the entire populations. INRA23 and TGLA53 are the most polymorphic loci with 11 alleles on average, with the most observed in Fleckvieh and the least in Turkish Simmental. The highest number of alleles was observed in Fleckvieh population as 73, while the least number of alleles was in Turkish Simmental as 57. The PIC value, which evaluates the discrimination power of the loci, was observed to vary between 0.537 and 0.840. Since all values were above 0.5, the discrimination power of all loci was found to be quite high.

In the study conducted by Choroszy et al. (2006) on Simmental cattle, the PIC values of 8 microsatellites ranged from 0.591 to 0.851, while Jevrosima et al. (2009) found values between 0.590 and 0.880. Agung et al. (2016) reported values ranging from 0.627 to 0.877 in their study. In a study on Holstein bulls using the same 10 loci, the PIC value was found to be between 0.339 and 0.836 (Unlusoy, 2023). In this study, the null allele frequencies were less than 0.2 for all loci and it is the indicator of enough PCR success according to Dakin and Avise (2004). All microsatellite loci were very informative in this study.

The heterozygosity evaluation was given in Table 2. It shows that the observed heterozygosity varied between 0.509 and 0.852 while the expected heterozygosity (i.e., gene diversity) varied between 0.563 and 0.839 in Fleckvieh. In Montbeliarde, observed heterozygosity varied between 0.222 and 0.926 while expected heterozygosity varied between 0.359 and 0.846. In Turkish Simmental, observed heterozygosity diverted between 0.500 and 0.813 while expected heterozygosity diverted between 0.613 and 0.833. In the studies on Simmental cattle of Chorosy et al. (2006), Jevrosima et al (2009), Agung et al.(2016), observed heterozygosity (Ho) diverted between 0.659 and 0.769; 0.452 and 0.774; 0.559 and 0.767 respectively. In this study it was determined that TGLA126 locus deviated from Hardy-Weinberg equilibrium for whole population ($p<0.001$). However in a study of Holstein stud bulls BM2113 and SPS115 loci were not in Hardy–Weinberg equilibrium (HWE) ($p<0.01$, $p<0.001$ respectively) while TGLA126 was in HW equilibrium (Unlusoy, 2023). According to HWE evaluation of the loci, Holstein bulls and Simmental bulls had different results.

It was observed that INRA23 locus of Fleckvieh population was not in Hardy-Weinberg equilibrium ($p<0.05$). In Montbeliarde population, a significant deviation from HW balance was observed in the TGLA126 locus ($p<0.001$). All the loci were in Hardy-Weinberg equilibrium in Turkish Simmental.

Table 1. Information on the allele content of loci

Locus	**Fleckvieh**		**Montbeliarde**		**Turkish Simmental**		**Whole Population**			
	k	**N**	**k**	**N**	**k**	**N**	**k**	**N**	**PIC**	**F(Null)**
BM1824	4	115	4	27	4	16	4	158	0.693	-0.024
BM2113	7	112	7	26	7	16	8	154	0.722	0.058
ETH10	5	113	5	27	5	16	6	156	0.542	0.030
ETH225	6	115	6	26	5	16	6	157	0.639	0.033
ETH3	5	115	4	27	4	16	5	158	0.682	0.018
INRA23	11	115	9	27	7	16	11	158	0.756	-0.030
SPS115	8	114	5	27	5	16	8	157	0.537	0.031
TGL122	9	115	7	27	5	16	10	158	0.748	-0.011
TGL126	7	114	4	27	7	16	7	157	0.640	0.121
TGL53	11	114	9	27	8	16	11	157	0.840	0.020

k: number of alleles, N: number of genotypes, PIC: polymorphic information content, F(Null): Null allele frequency.

Table 2. Heterozygosity evaluation.

Locus	Fleckvieh Ho	Fleckvieh He	Fleckvieh HW	Montbeliarde Ho	Montbeliarde He	Montbeliarde HW	Turkish Simmental Ho	Turkish Simmental He	Turkish Simmental HW	Whole population Ho	Whole population He	Whole population HW
BM1824	0.809	0.744	NS	0.778	0.713	NS	0.563	0.667	NS	0.778	0.743	NS
BM2113	0.696	0.746	NS	0.654	0.785	NS	0.563	0.734	NS	0.675	0.761	NS
ETH10	0.566	0.611	NS	0.407	0.359	NS	0.813	0.694	NS	0.564	0.590	NS
ETH225	0.643	0.681	NS	0.654	0.745	NS	0.625	0.613	NS	0.643	0.685	NS
ETH3	0.713	0.744	NS	0.593	0.688	NS	0.813	0.718	NS	0.703	0.733	NS
INRA23	0.852	0.749	*	0.741	0.846	NS	0.813	0.758	NS	0.829	0.787	NS
SPS115	0.509	0.563	NS	0.630	0.551	NS	0.500	0.613	NS	0.529	0.567	NS
TGL122	0.809	0.778	NS	0.741	0.787	NS	0.813	0.712	NS	0.797	0.783	NS
TGL126	0.579	0.663	NS	0.222	0.585	***	0.813	0.762	NS	0.541	0.695	***
TGL53	0.816	0.839	NS	0.926	0.843	NS	0.688	0.833	NS	0.822	0.858	NS

Ho: observed heterozygosity, He: expected heterozygosity, HW:significance of Hardy-Weinberg disequilibrium. *: p<0.05, ***: p<0.001, NS: non-significant.

F_{IS} values for each subpopulation and each locus was given in Table 3. It was ranged from -0138 to 0.128 in Fleckvieh, from -0.101 to 0.625 in Montbeliarde and from -0.147 to 0.236 in Turkish Simmental. The F_{IS} values of TGLA126 in Fleckvieh and Montbeliarde subpopulations were statistically significant (p<0.05 and p<0.01 respectively). The within subpopulation F_{IS} values were 0.018 in Fleckvieh (p>0.05), 0.082 in Montbeliarde (p<0.01) and 0.015 in Turkish Simmental (p>0.05) while the F_{IS} value of whole population was 0.022 (p>0.05). It was unveiled that the inbreeding was increased in Montbeliarde subpopulation. In the study of Holstein stud bulls the F_{IS} value of the whole population was not statistically significant (Unlusoy, 2023). In the current study, global heterozygosity deficit among the subpopulations (F_{IT}) was 0.060 (p<0.01) while the fixation index (F_{ST}) was 0.039 (p<0.001). The value of F_{ST} meant that the variance of among the subpopulations explained 3.9% of the total variance and it was statistically significant.

Table 3: F_{IS} evaluation

Locus	F_{IS} Fleckvieh	F_{IS} Montbeliarde	F_{IS} Turkish Simmental
BM1824	-0.088	-0.093	0.161
BM2113	0.067	0.170	0.239
ETH10	0.074	-0.137	-0.178
ETH225	0.055	0.125	-0.020
ETH3	0.041	0.141	-0.137
INRA23	-0.138	0.126	-0.074
SPS115	0.097	-0.147	0.189
TGL122	-0.040	0.060	-0.147
TGL126	0.128*	0.625***	-0.068
TGL53	0.027	-0.101	0.179
Population	0.018	0.082**	0.015

*: p<0.05, **p<0.01, ***: p<0.001, NS: non-significant

Table 4: F_{ST} values based on Weir and Cockerham (above diagonal) and their p value (below diagonal) in three subpopulations.

	Fleckvieh	Montbeliarde	Turkish Simmental
Fleckvieh	-	0.055	0.005
Montbeliarde	***	-	0.067
Turkish Simmental	NS	***	-

***: p<0.001, NS:non-significant

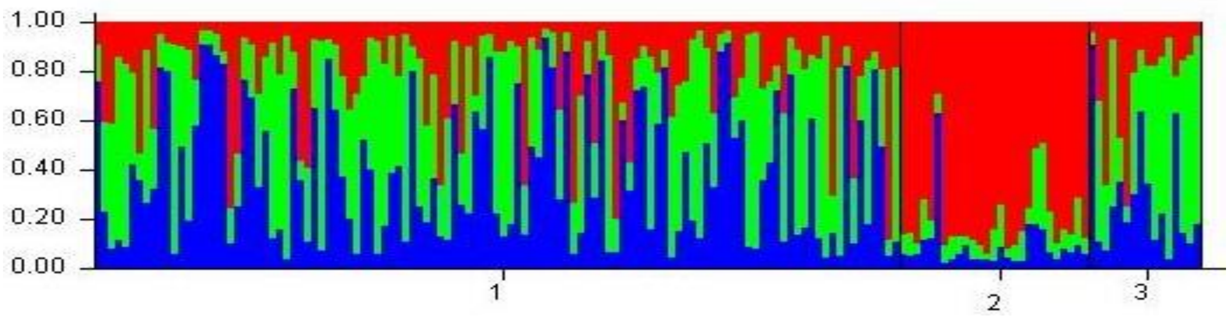

Figure 1: Genetic Structure. Each subpopulation was expressed with the numbers.1. Fleckvieh, 2. Montbeliarde, 3. Turkish Simmental.

Figure 1 represents the genetic structure of all three subpopulations. The effective k-value was determined as 3. According to the result of structure analysis, Montbeliarde subpopulation had more homogenity than Fleckvieh and Turkish Simmental subpopulations while Fleckvieh and Turkish Simmental subpopulations were not diverged from each other because of their similar heterogen pattern.

Conclusion

In this study, it was observed that the genetic structure of Montbeliarde subpopulation had more homogenity than Fleckvieh and Turkish Simmental subpopulations. Furthermore, it was observed that the genetic diversity values of TGLA126 locus was significantly decreased in Montbeliarde. Therefore, TGLA126 locus should be focused on in Montbeliarde subpopulation for further studies. On the other hand, the overall smaller genetic diversity of Montbeliarde subpopulation indicates that the breeding programs conducted for this breed should consider the management of the already decreased diversity with great care. Besides, the genetic structure of Turkish Simmental subpopulation was similar with Fleckvieh subpopulation. It is thought that the Turkish Simmental bulls were descended from Fleckvieh subpopulation because of predominant import of Fleckvieh's semen to Türkiye. Finally, it is important to emphasize that further studies with higher sample sizes and with other breeds are required to comprehensively evaluate the diversity parameters of those breeds under intensive selection.

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Conflict of Interest

The author of the study declares no conflicts of interest.

Author contribution

Ilke UNLUSOY has carried out data arrangement, statistical and bioinformatic analyses as well as writing of the manuscript.

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