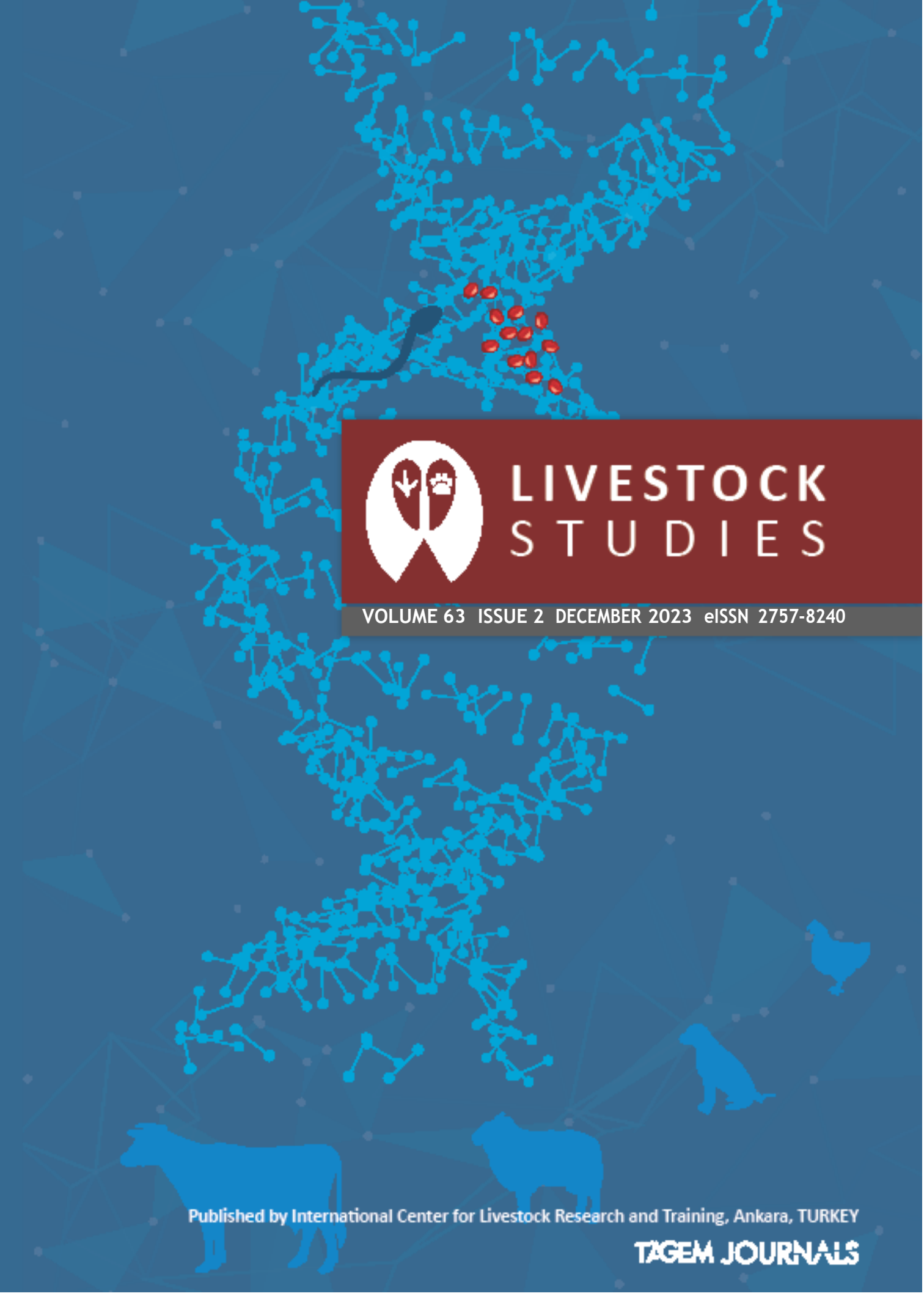

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LIVESTOCK STUDIES

RESEARCH PAPER

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.

	Fleckvieh			Montbeliarde			Turkish Simmental			Whole population		
Locus	**Ho**	**He**	**HW**	**Ho**	**He**	**HW**	**Ho**	**He**	**HW**	**Ho**	**He**	**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

	F_{IS}		
Locus	**Fleckvieh**	**Montbeliarde**	**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

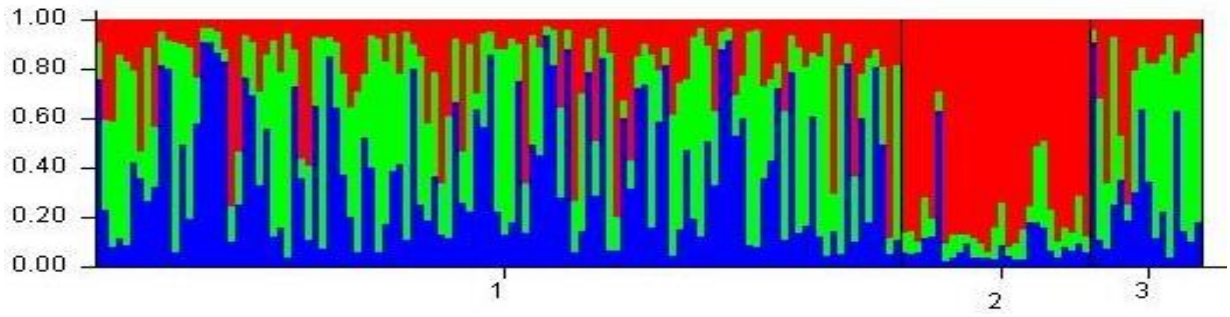

Figure 1: Genetic Structure. Each subpopulation was expressed with the numbers.1. Fleckvieh, 2. Montbeliarde, 3. Turkish Simmental.

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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LIVESTOCK STUDIES

RESEARCH PAPER

Investigation of Structural Characteristics of Central Anatolian Merino Sheep Farms and Effectiveness of the Breeding Project in Ankara Province

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Abstract

The aim of this study is to determine the general characteristics and management and feeding practices of 33 Central Anatolian Merino sheep farms in Ankara within the scope of the "National Project for Community-based Small Ruminant Breeding" coordinated by General Directorate of Agricultural Research and Policies. Within the scope of this purpose, a survey consisting of a total of 78 questions was conducted with the farmers. The questionnaire consists of questions about general information about the farmers and farms, herd management, determination of the care and feeding methods of the animals and the effectiveness of the breeding project. At the end of the study, it was determined that 60% of the breeders were between the ages of 41-50, 90.91% of them were primary and secondary school graduates, and all of them kept regular records for herd management. Sheep breeders interviewed that they do supplemental feeding (approximately 34%) before mating and that they are milking by hand. Sheep breeders stated that they gained the habit of keeping records thanks to the breeding project, lamb rearing and breeding selection were made more effectively, so they benefited positively from the project. Furthermore, it was determined that the breeders wanted to stay in the project and wished for the project to continue.

Introduction

Livestock farming, one of the important branches of agriculture, is a strategic and sustainable business line and is among the most important inventions of human history. The main factor that pushes people to this way is the nutrition they need to survive. Although plant-based foods are needed for nutritional purposes, animal proteins are indispensable for a balanced and adequate diet (Ordu and Zengin, 2020). In order to meet the need for animal protein in Türkiye, cattle, sheep, poultry and fish are raised.

In the 1980s, plans were made to build livestock farming on cattle and poultry breeding in Türkiye. During these periods, the number of goats was reduced on the grounds that they harmed the forest, and even crop production alternatives were offered to goat breeders. In addition, there were not made any saving on sheep breeding in the same time. During this period, the number of small ruminants decreased significantly, the number of sheep, which was approximately 40 million heads, decreased to 21 million heads, and the number of goats, which was 13 million heads, decreased to 5 million heads.

While determining development plan strategies in countries, agricultural production is often ignored or put in the background. However, the basis of the industry is based on agriculture. Considering agriculture and industry together in every future projection will be a right step in development moves. Türkiye has different cultures in terms of animal production. This diversity includes geographical structure, tradition in production and product richness.

Products obtained from sheep breeding, which is an important branch of animal husbandry, provide benefits at different points of life. Starting from this point, agricultural policies to be followed are also essential for sustainability in production. In order to achieve this, each region and even each province, if necessary, must be evaluated separately in terms of agricultural production and the general situation must be determined.

In terms of both the improvement of sheep breeds and the continuation of production in rural areas, the project named Central Anatolian Merino (CAM) Improvement in Ankara Province" belong to "National Project for Community-based Small Ruminant Breeding (HEKIP) is supported by

General Directorate of Agricultural Research and Policies. Within the scope of this project, data obtained from animals are evaluated and breeding is carried out. In this research, the general breeding conditions, care and feeding conditions of the breeders included in the project and the effect of the applied breeding project were revealed. In the face-to-face survey conducted in the farms within the scope of the study, the general conditions of the farmers such as age, educational level and experience in sheep breeding, as well as the breeding practices, management and feeding methods of the farms were examined.

The materials and method used in the study are explained in the next section. The findings were then discussed. Finally, the study has been concluded.

Materials and Methods

The material of research consisted of 33 CAM sheep farms and breeders in HEKIP carried out under the coordination of General Directorate of Agricultural Research and Policies. The sheep enterprises examined within the scope of the study are located in Polatlı, Haymana, Güdül, Sincan, Bala, Şereflikoçhisar, Kızılcahamam and Elmadağ districts of Ankara province. The CAM sheep breed raised in the study was developed as a result of the crossbreeding of German Meat Merino and Akkaraman sheep in order to produce high amounts of meat and fleece in the arid pasture conditions of Central Anatolia.

Survey questions, which was totally 4 main sections and 78 questions, were asked to breeders. The survey questions were created from the general characteristic farms in first part, the herd management in second part, the feeding methods in third part and the national project achievements in fourth part. Surveys were conducted face to face with breeders.

Results and Discussion

In this study we conducted for CAM breeders in Ankara, 18-40, 41-60 and 61 and over age distributions of breeder were determined respectively 24.24% (8 farmers), 57.58% (19 farmers) and 18.18% (6 farmers) (Table 1). When we look at the age groups, it is seen that young breeders are in the majority. This situation can be interpreted as the demand for sheep breeding in the region continues.

Table 1 Social Situation of CAM Breeders

Age	n	%	**Experience (years)**	n	%
18-40	8	24.24	1-10	2	6.06
41-60	19	57.58	11-20	3	9.09
61 and More	6	18.18	21-30	5	15.15
Educational Background			31 and More	23	69.70
Not Literate	0	0	**Land Adequacy**		
Primary school graduate	26	78.79	Sufficient	27	81.82
Secondary school graduate	4	12.12	Insufficient	6	18.18
High school graduate	2	6.06			
Undergraduate and above	1	3.03			

n: Number of CAM sheep breeders

Although there are no illiterate sheep breeders in this study, a significant proportion of them are determined primary school graduates (78.79%). Although the rate of secondary school and high school graduates is low (12.12% and 6.06%), the presence of breeders with a bachelor's degree can be considered a pleasing situation. Because, as in other fields, education in agricultural activities is very important to increase the quality and quantity in production. This will only be possible with education. Gül et al. (2022) in Aksaray province and Ceyhan et al. (2015) in Niğde province reported in their study that sheep breeders were generally primary school graduates. The study is similar to other studies in this aspect.

When the producers who participated in our study were asked about their experience, 23 farmers (69.70%) stated that they had been doing sheep breeding for more than 31 years. 81.82% of these farmers states additionally that they had sufficient land. In their study conducted in Mersin and Muğla regions, Tüney Bebek and Keskin (2018) and Aydın and Keskin (2018) stated that sheep breeders experience was respectively 25.9 and 27.6 years. The experience period of the sheep breeders in our study were found to be close to the experience period determined in previous studies in the literature (Gül et al., 2009; Gül and Örnek, 2018).

Breeders (27.28%) generally prefer between March and December throughout the year for grazing (Figure 1). Others graze their sheep on pastures between March-November (18.18%), April and November (15.15%), April-December (15.15%) and January-December (3.03%). Demir et al. (2015), in their study conducted in Eastern Anatolia Region, reported that sheep were grazed on pastures between April and December. The condition of pastures can be affected by climatic conditions. In addition, the amount and duration of annual rainfall are one of the most important factors. The difference between regions can be explained by climatic conditions and the status of the grass population in the pasture. Breeders who were within the scope of the project and participated in our survey were asked about their goals in sheep breeding and the answers received are given in Figure 2. It was determined in this study that some of them breed sheep in order to have milk (12.12%) and breeding stock (48.48%). Gül et al. (2022) announced that small sheep breeders in Aksaray province generally produce meat and milk. However, Aydın and Keskin (2018) stated that breeders in Muğla province mainly operate for the purpose of meat production.

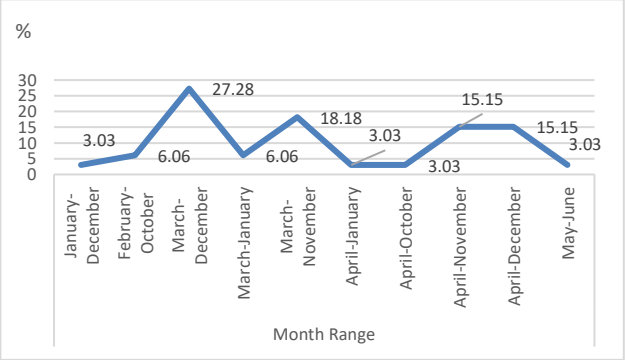

Figure 1 Grazing Duration and Pasture Periods (%)

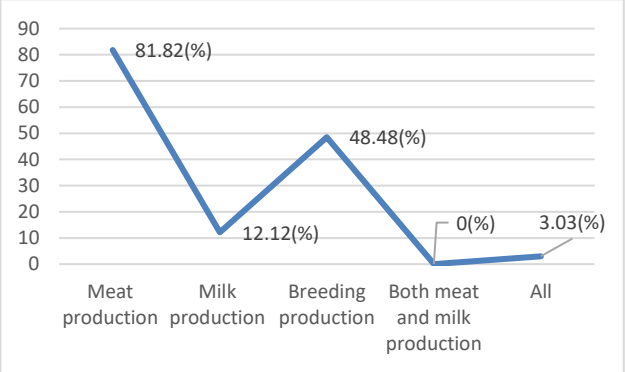

Figure 2 CAM Breeding Objectives (%)

The majority of CAM sheep breeders in our study state that male and female animals were used breeding age before the age of 18 months (78.79% and 84.85%) (Figure 3). In addition, some breeders stated to prefer that the first mating period of male and female yearlings is after 18 months of age. Gül et al. (2022) reported in Aksaray province that female yearling was used breeding at the age of 12 or 20 months. Ceyhan et al. (2015) stated that it was as 18.2 months for male yearlings. It is of great importance for male and female animals to be used for breeding to complete their biological and physiological development in terms of herd continuity and reproductive health.

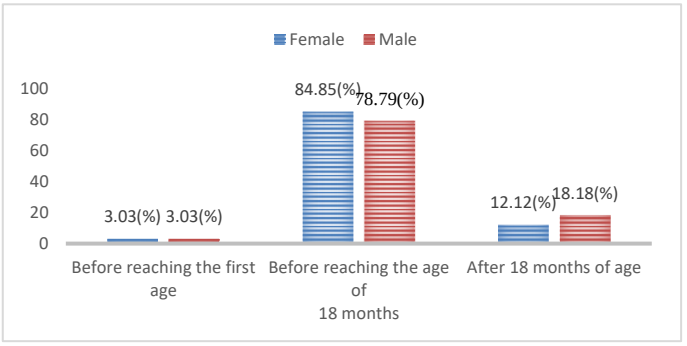

Figure 3 Age of First Breeding of Male and Female Yearlings

It has been determined that breeders generally make in a free mating (84.85%), however 15.15% of them use class mating (Table 2). It determineted in study that most farmers (87.88%) do not apply any hormones for oestrus synchronization, while 12.12% of them uses hormones. While breeders stated that they mostly kept their rams in the herd only during the mating period (96.97%), 1 breeder stated that they kept them in the herd all the time. Mating method of sheep in Türkiye is generally in the form of free mating. Lack of record keeping in herds, housing problems in breeders and lack of information of breeders can be considered among the biggest factors (Behrem and Keskin, 2013; Özyurek et al., 2018; Özsayın et al., 2019). It can be said that breeding technics with the free mating method continues to be widely used.

Table 2 Information about Mating Period

Hormone Use	**n**	**%**	**Mating type**	**n**	**%**
Yes	4	12.12	Free	28	84.85
No	29	87.88	Class	5	15.15
Flushing (female)			**Flushing (male)**		
Yes	16	48.49	Yes	11	33.33
No	17	51.51	No	22	66.67
Breeding period for sheep (years)			**Breeding period for ram (years)**		
2	1	3.03	4	3	9.09
3	5	15.15	6	13	39.4
4	11	33.33	7	11	33.33
5	4	12.12	10	6	18.18
6	9	27.28	**Number of sheep per ram (head)**		
7	3	9.09	20	9	27.28
Keeping Ram in Herd			25	12	36.36
Throughout the year	1	3.03	30	8	24.24
Mating season	32	96.97	35	0	0
			40	4	12.12

n: Number of CAM sheep breeders

It was determined that partial supplementary feeding was applied to rams and sheep before mating period. The rate of breeders giving additional feeding to rams was determined as 33.33% and sheep were 48.49%. Arıtunca and Karabacak (2019) reported in Konya province that they made additional feeding in 61.4% of the farms before mating season. In this study conducted in Ankara, it is thought that the difference in supplementary feeding of sheep and rams before mating is due to feed costs.

While the duration of using their animals for breeding varies in herd, it was determined that males were generally used as breeding for 4 and 6 years (33.33% and 27.28%), and females were used as breeding stock for 6 and 7 years (39.40% and 33.33%). In the study conducted by Tüfekci (2020) in Yozgat province and Gül et al. (2022) in Aksaray province, rams were respectively used for 2-3 and 2-4 years in herd. In addition, it was determined in study that the mating plan was generally calculated to include one ram for twenty five sheep (36.36%). This situation is similar to the study conducted by Aydın and Keskin (2018) in Muğla province.

Developmental characteristics of CAM lambs are given in Table 3. According to this chart, it was determined that the average lamb birth weight was between 3-4 kg (54.55%). In addition, while the rate of breeders with a birth weight between 2-3 kg was 39.39%, the birth weight of the lambs of 2 breeders was indicated between 4-5 kg in study.

Table 3 Information of Animals Growth Characteristics

Birth weight (kg)	**n**	**%**	**Suckling duration (month)**	**n**	**%**
2-3	13	39.39	2	3	9.09
3-4	18	54.55	3	30	90.91
4-5	2	6.06	**Feed practice time**		
90th day weight (kg)			1-14 days	6	15.15
30	7	21.21	15-30 days	22	66.67
35	15	45.46	30 days later	5	15.15
38	3	9.09			
39	1	3.03			
40	7	21.21			

n: Number of CAM sheep breeders

In addition, while the rate of breeders with a birth weight between 2-3 kg was 39.39%, the birth weight of the lambs of 2 breeders was indicated between 4-5 kg in study. It has been reported that the suckling time in the lambs was usually made for 3 months (90.91%). In the practice of eating habits, which is important for rumen development, a significant part of the breeders stated that they started giving forage and concentrated feed to the lambs from the age of 15 days (81.82%).

Table 4 shows information about ewe and lambs diet type. All of the breeders in study declared lamb fattening. The rate of those who fattened their lambs for 3 months was 33.33%, and the rate of those who fattened their lambs for 4 months was 54.55%. 72.73% of breeders said that they did not feed their animals additionally during the pasture period, the rate of those, gave additional feed depending on the condition of pasture, was calculated as 21.21%. Köseman et al. (2022) in Elazığ province reported that 55.4% of breeders did not give additional feed to their animals in pasture grazing period. Pastures are important feed sources in sheep breeding. It is thought that grazing time and seasonal conditions affect the grazing capacity and productivity of pastures.

Table 4 Lamb and Sheep Feeding Types (%)

Lamb fattening situation	**n**	**%**	**Pasture sufficiency status**	**n**	**%**
Yes	33	100	Sufficient	7	21.21
No	0	0	Insufficient	24	72.73
Lamb fattening duration (Months)			Partially sufficient	2	6.06
3	11	33.33	**Feeding method of sheep**		
4	18	54.55	Extensive	0	0
Others	3	12.12	Semi-intensive	33	100
Additional feeding during pasture period			Intensive	0	0
Yes	2	6.06	**Status of receiving support regarding animal nutrition**		
No	24	72.73	Yes	5	15.15
According to the grass condition in the pasture	7	21.21	No	28	84.85
Period of giving concentrated feed to sheep					
Before birth	29	87.88			
After birth	15	45.45			
Mating season	9	27.27			

n: Number of CAM sheep breeders

When asked about the pasture situation, it reported that pastures were inadequate (72.73%) for grazing. Seven breeders (21.21%) stated in study that pastures were sufficient. All of sheep breeders prefer semi-intensive feding. In addition, the number of breeders, receive support from experts about animal feeding topics, is very low (28 people - 84.85%). It was determined that the majority of breeders in study (87.88%) fed their animals before birth. Ceyhan et al. (2015) in their study in Niğde province,

reported that 89.6% of breeders gave supplementary feeding to their animals during gestation. It can be assumed that breeders are aware of the positive effects of feeding before birth on mother's milk, offspring survival and birth weight.

In the scope of study, Table 5 shows about information on feed sources, used in lamb and sheep feeding, in farms. It is seen that the majority of breeders (19 people) prefer alfalfa hay as a roughage source for lambs. All of breeders in study stated that they buy concentrate feed from factory. However, they also said to use grain feed such as wheat, oats and corn from time to time. It is thought that the differences in the feeding practices of farms originate from the current business situation, economic conditions and raw material supply.

Table 5 Raw Feeds Used for Sheep and Lamb Feeding

	Feeds		n	%
Lamb	**Roughage**	Alfalfa hay	19	57.58
		Meadow Grass	1	3.03
		Straw	5	15.15
		Vetch Grass	8	24.24
	Concentrated feed	Barley	7	21.21
		Concentrate	33	100
		Wheat	2	6.06
		Oat	4	12.12
		Corn	2	6.06
Sheep	**Roughage**	Alfalfa hay	22	66.67
		Meadow Grass	12	36.36
		Straw	18	54.54
		Silage	6	18.18
		Sainfoin	2	6.06
	Concentrated feed	Barley	29	87.87
		Corn	3	9.09
		Concentrate	21	63.63
		Wheat	17	51.51
		Oat	10	30.3
		Sugar Beet Pulp	3	9.09

n: Number of CAM sheep breeders

Breeders (22 people) in study preferred alfalfa hay as a roughage source in feeding their sheep, as in the case of lambs. In addition, they stated that they use wheat straw, vetch grass, silage and sainfoin as a source of roughage in feeding. The most of breeders buy concentrated feed from the factory to feed sheep. They use additionally barley (29 people), wheat (17 people), oats (10 people), corn (3 people) and sugar beet pulp in sheep feeding. Tüfekçi (2020) reported in Yozgat province that breeders used factory feed-barley-wheat as concentrated feed and also barley-wheat-lentils-chickpea straw-vetch grass as roughage. In different studies conducted on sheep farms, it was stated that the rate of using concentrated feed was 43.84% and the rate of using to own rations was as 32.88% (Gül and Örnek, 2019; Dellal et al., 2022). It is thought that the feed resources used by breeders are shaped according to climate, changes in crop production, animal breeding culture differences and feed prices.

96.97% of breeders in our survey have been involved in "National Project for Community-based Small Ruminant Breeding" for more than five years (Figure 4).

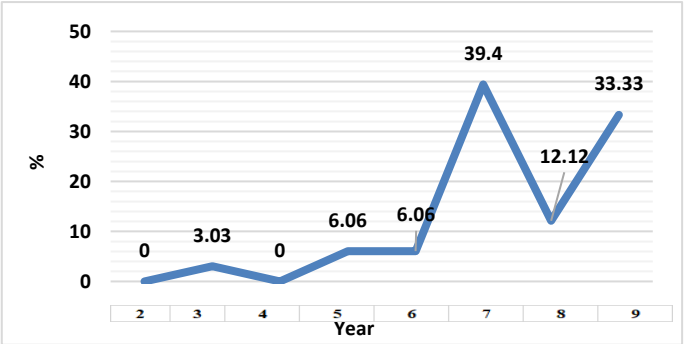

Figure 4 Duration of Farms in Breeding Projects (%)

The sheep breeders involved in study were asked thoughts on the breeding project and the answers received are given in Figure 5.

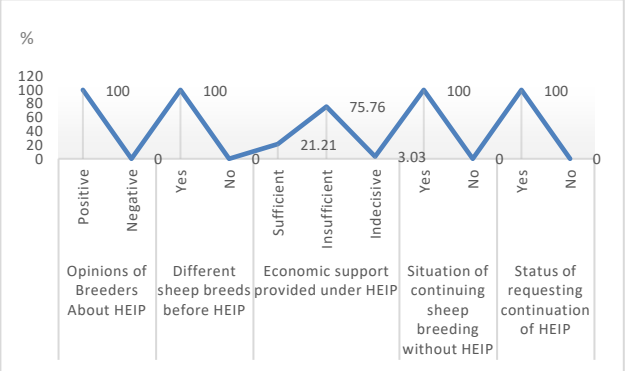

Figure 5 National Project for Community-based Small Ruminant Breeding (HEKIP)

According to the evaluations, all of the farmers (33 people - 100%) stated that they were satisfied with the project. However, they underlined also that the financial support provided within the scope of the project was insufficient (75.76%). While breeders stated that they would continue to breed sheep even if there was no project, they wanted to request the continuity of the project. It was said by the breeders that there were different sheep breeds in own herds before they were involved in the breeding project. However, they stated, removed different sheep breeds from the herd with breeding project. Information about these sheep is given in Figure 6.

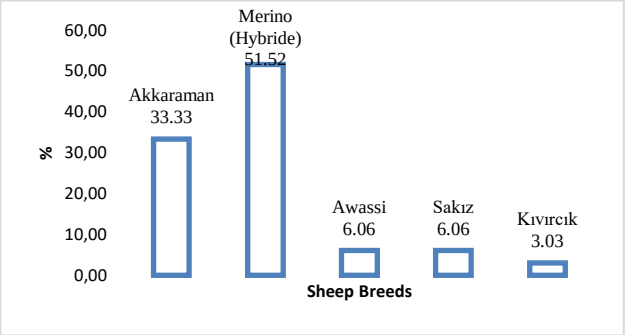

Figure 6 Different Sheep Breeds in Farms Before HEKIP (%)

As seen in Figure 6, there were mostly Merino hybrid (51.52%) and Akkaraman (33.33%) before entering the breeding project in farms.

In the survey, questions were asked about whether the breeding project had positive or negative any contribution to farms (Table 6). All of sheep breeders (100%) in study stated that there were positive changes in the infrastructure of farms. These effects include lamb deaths, birth and 90th day weights, multiple births, hygiene and care-feeding.

Conclusion

National breeding project carried out in different provinces in Türkiye are successfully applied. There are important positive outcomes for the breeders and country's economy in this project. In this context, the breeding project carried out for CAM breed was evaluated and the results were presented.

It has been observed that breeders, whose are breeding CAM sheep in Ankara region, have high experience in this field. However, they have low level of education. Therefore, it would be beneficial to increase education levels about modern animal husbandry. For this purpose, it will be useful to organize courses on the subject, issue certificates and give extra incentives to breeders with certificates. Insufficiency of pastures is one of the main problems. Improving pastures will have a positive impact on reducing production costs. Increasing the financial support provided to the project and purchasing the livestock produced by the breeders within the scope of the project at a value price will be an important step in terms of the effectiveness and sustainability of the project. As a result, it has been determined that all breeders within the project are extremely satisfied with the breeding project, and that thanks to the project, significant developments have occurred in them both financially and scientifically.

The limitations of the study are that farms operating in some districts of only one province of Turkey were selected. Additionally, these farms are included in the breeding project carried out in Ankara province. The contribution of this study to future studies may shed light on the examination of the feeding, care and cultivation methods of existing farms and the effects of the applied breeding project on the farms.

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REVIEW ARTICLE

LIVESTOCK STUDIES

Circadian Genes and Economic Traits in Livestock Animals

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livestock

Abstract

Circadian rhythms are oscillators of endogenous autonomic activity in all living organisms and regulate economic traits such as reproduction, milk characteristics and growth performance in farm animals. These rhythms are directly or indirectly controlled by the circadian clock in a 24-hour cycle. It has evolved as an adaptive system for living organisms in a cyclical natural environment. A regular circadian rhythm can be associated with good health, well-being, strong immunity, and high economic characteristics. The interaction between circadian rhythmicity and the physiology of farm animals is becoming an important focus of animal science. Understanding the circadian genes (*CLOCK, BMAL, PER1, PER2, CRY1, CRY2*) and actions regulating circadian oscillation in farm animals is important to improve management and increase economic traits. The study aimed to summarise the research on the effects of circadian rhythm genes on productivity and to highlight the importance of this topic.

Introduction

Circadian rhythms are vital to the existence of all life forms, directing sleep/wake cycles and metabolic processes (Rhodes *et al*., 2022). In mammals, the primary internal clock is located in the hypothalamic suprachiasmatic nucleus (SCN). The circadian rhythms of mammals are primarily synchronized with the natural light-dark cycle (Takahashi *et al*., 2008). Circadian mechanisms are essential for regulating the timing of daily processes and annual physiological and behavioral variations that are controlled and synchronized by day length (Li *et al*., 2021). Key clock genes driving circadian rhythms consist of positive and negative components that together form an autoregulatory feedback loop (Plaut and Casey, 2012). Circadian rhythms operate at the post-transcriptional and post-translational levels, but the clock still requires high-quality translation to function properly (Ferreira and Takahashi, 2019). Fundamental clock genes are well preserved across mammalian species. In their study of 11 circadian genes, Derdante *et al*. (2009) found that 10 of these clock genes are highly conserved in sheep compared to other animals. Brain and Muscle ARNT-Like 1 *(BMAL1)*, Circadian Locomotor Output Cycle Kaput Protein Hood *(CLOCK)* and Neuronal PAS Domain Protein 2 *(NPAS2)* genes regulate positive circadian rhythm control, whereas the Period *(Per)* and Cryptochrome *(Cry)* genes are involved in negative control (Plaut and Casey, 2012). The circadian timing system in livestock is hierarchically organized and comprises central and peripheral clocks. The master clock directs and synchronizes the peripheral clocks in every tissue of the body. This indicates that each tissue has its clock, and the circadian expression of tissue-specific transcriptomes near to them assists in the digestion system and physiology of the organism (Trujillo and Casey, 2016).

Economic Treat Related Circadian Studies in Livestock

Studies have focused on investigating the effect of circadian rhythm genes on animal performance, primarily those related to ovarian activity, milk yield and reproduction. Many functional activities in farm animals, such as reproduction, are closely related to circadian rhythm. Many studies in male and female animals have shown that circadian rhythm is a regulator of reproductive biology (Han *et al*., 2021). In cattle and mammals, the estrus cycle, LH levels, ovulation, production, sperm maturation, fertilization, artificial insemination, and litter size have also been reported to be related to circadian clock activity (Han *et al*., 2021). However, any disruption in circadian rhythm has been reported to have negative effects on the ovulation cycle, fertility rate, miscarriage rates and fetal development (Gotlieb, 2020). Long-term effects of circadian rhythm disruption include both physical and mental health. These effects are even recognized as triggers of diseases such as cancer and depression. In animals, disruption of the circadian rhythm increases the effects of disease and stress, while stress and disease also disrupt the circadian rhythm, creating a vicious negative cycle (Wagner *et al*., 2021).

Estrus Cycle-Ovulation Studies

Reproductive cycles in many animal species are seasonally regulated in a daylight-dependent manner. Murphy *et al.* (2015) have demonstrated that sheep ovaries exhibit rhythmic expression of molecular clock genes (*Clock, ARNT1, CRY1, CRY2, PER1, PER2*). Consequently, ovarian tissue, like peripheral tissue, has a time-sensitive function and a reproductive clock cycle. The researchers analyzed the genetic factors that affect the rate of ovulation and litter size in ewes. It was noted that specific single gene mutations can impact these parameters, and it may be crucial to extensively identify these mutations, including period genes, to compare their reproductive effects across seasons. However, there is a dearth of comprehensive research on this topic, as observed by Notter (2008). Furthermore, the location of timer cells responsible for seasonal control of the gonadal axis is not thought to be in the pituitary gland in the brain. The evidence suggests that reproduction is seasonally controlled by light and melatonin activity. However, the subsequent regulation occurs through rhythmic circadian genes expressed in tissues instead of the suprachiasmatic nucleus. Long-term regulation of these genes also controls appetite, food intake, and body weight in sheep, with the *Per/Cry* complex being especially potent (Lincoln *et al*., 2003). Circadian regulation is closely linked to metabolism (Takahashi, 2017). Han *et al.* (2021) examined the possible involvement of *BMAL1, PER1-2, and CRY1-2* genes in the ovine ovarian tissue clock between the luteal and follicular phases. They found that these genes are differently expressed in various phases of reproductive and non-reproductive tissues, demonstrating that the estrus cycle affects clock gene expression.

Mammary Gland and Milk Yield Studies

Lactation is a physiological process subject to numerous factors and is crucial for milk production in animals bred for this purpose. Mammary tissue expresses vital clock genes (*BMAL1 and CLOCK*), and their role in mammary gland development and lactation is currently under investigation. The expression of fundamental circadian clock genes has varied significantly across several lactation stages within the mammary gland, as shown by a study conducted by Casey *et al.* (2014). Transcriptome analysis revealed that 7% of genes communicated within the lactating tissue display circadian rhythms, based on research conducted by Maningat *et al.* (2009). Key circadian clock genes contribute to cell improvement, development, and multiplication, apoptosis and intracellular signaling cascade, as demonstrated by Trujillo and Casey (2016). The mammary clock also regulates the expression of genes responsible for milk synthesis, milk fat and lactose (Casey *et al*., 2014).

Chronic exposure of non-pregnant, non-lactating dairy animals to day-night shifts during late pregnancy had a detrimental effect on circadian rhythms, insulin and glucose homeostasis, and negatively affected udder development. Introduction of pregnant dairy cattle to day-night stage shifts for 14 days led to changes in the liver and mammary transcriptomes. Chronic disruption of the circadian rhythm can have negative implications for the well-being, welfare, and milk production capacity of cattle. Analysis of the transcript profile of udder tissue suggests that circadian disruption reduces udder remodeling and lipid transport, and adversely affects liver lipid metabolism (Casey *et al*., 2021).

Conclusion

Circadian rhythm is closely related to biological rhythms in animal production, which play a crucial role in traits like lactation yield, reproduction, and ovulation. The above summary depicts the relationship between circadian rhythm genes and yield traits in biologically rhythmic animals. The association of these genes with yield traits in seasonally rhythmic animals is apparent but requires further in-depth research to clarify.

Author Contributions

First Author: Conceptualization, Writing -review and editing; Second Author: Investigation, Visualization and Writing -original draft.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article. We

have no financial or personal relationships with individuals or organizations that could inappropriately influence our work. Furthermore, we confirm that the content presented in this manuscript is original and has not been previously published elsewhere.

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RESEARCH ARTICLE

LIVESTOCK STUDIES

Possibilities of Using "BUGA" Named Software for Breeding Value Estimation of Anatolian Water Buffalo Population of Istanbul

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Abstract

In this study, variance components and breeding value estimations of Anatolian water buffaloes were estimated from the data obtained within the scope of community based water buffalo improvement program of Anatolian water buffalo population of İstanbul. The "BUGA" named software, which was started to be developed with the support of Harran University Scientific Research Project Unit (HUBAP) for use in the estimation of (co)variance components and breeding value were used in this study. The BUGA software used in the research can predict genetic and environmental effects together with variance components as Best Linear Unbiased Prediction (BLUP) with the Restricted Maximum Log likelihood (REML) method. Unlike its counterparts of another software of foreign origin, it has a visual interface. The language of use is Turkish. The dataset includes records such as city, district, village, genealogy information, date of birth, gender, calving date, lactation parity, number of test day milk yield, lactation milk yield and previous calving date of 442 Anatolian buffaloes raised in this population. The records were analysed using the repeatability model with BUGA software and the additive genetic and permanent environmental effects of all animals in the dataset were estimated. Random environment variance, genotypic variance and permanent environment variance values were estimated as 107112.60 kg^2, 20045.22 kg^2 and 1259.50 kg^2, respectively, while heritability (h^2) and degree of repeatability (r) were determined as 0.1561 and 0.1659, respectively. The results obtained as an example of case study applied for the possibility of usage the BUGA software in the estimation of genetic parameters and breeding values showed that BUGA software can be easily used in farm recording conditions with the advantage of friendly user features.

Introduction

The identification and utilization of genetically superior individuals in animal breeding depend on the reliable determination of various genetic parameters, such as variance components, heritability, and breeding value, given the genotypic perspective (Kumlu, 2003). Since this is a highly complex process, more than twenty specialized computer software programs have been developed for this purpose since the 1960s to facilitate fast and easy calculations in this field. Some of these software programs have become obsolete as they are no longer being developed. The most commonly used software programs today include MTDFREML (Boldmon *et al*., 1995), ASREML (Gilmour *et al*., 1998), BLUPF90 (Anonymous, 2023a), WOMBAT (Meyer, 2007), and MIXBLUP (Anonymous, 2023b). A common feature of these software programs is that they are written in Fortran 77/90/95, C, and Pascal languages and operate through a command system.

There are a number of steps involved in the selection of animals to maximize the response achieved. These include: (i) managing the animals as equally as possible to make it easier to disentangle genetics and environment; (ii) adjusting records of performance for known environmental effects, and then (iii) predicting the breeding values of individual animals by the most appropriate method. Modern (BLUP) methods of genetic evaluation achieve the second and third of these steps simultaneously (Simm *et al*., 2022).

Predicting an animal's breeding value is a bit like completing a large, complicated jigsaw puzzle, where each piece of the puzzle is a record of performance from the animal itself or one of its relatives. More recently, the genomic information on the animal adds a further piece of the puzzle. Generally, the higher the proportion of genes in common between the animal and a given relative, the more useful the record of performance from that relative. But, records from progeny are of most value. As the number of records on progeny

increases, the correlation between predicted and true breeding values approaches one. With other classes of relatives the accuracy of prediction never reaches one, and for all classes of relatives there are diminishing returns in accuracy as the number of records increases (Simm *et al*., 2022).

Estimated bleeding values (EBVs) can have positive or negative values or be equal to zero. The sign indicates whether they are expected to be genetically above (+) or below (−) the average of the group of animals on which the calculations were performed, or some other defined group of animals whose predicted breeding values (PBVs) are set to average zero (the base). PBVs are expressed (at least initially) in the same units as the record of performance (e.g. kg of live weight, litres of milk, mm of fat). The PBVs of animals can only be compared within contemporary groups, herds or flocks unless there are genetic links between these groups, and the PBVs were from across-herd or across-flock BLUP evaluations (Mrode, 2005; Simm *et al*., 2022).

In the simplest case, when we have a single record of performance on the animal itself, the predicted or estimated breeding value (PBV or EBV) is the deviation in performance from con- temporaries, multiplied by the heritability of the trait concerned. The deviation in performance is calculated after adjusting the performance records for environmental effects. PBVs calculated from a single record of performance span a narrower range than the deviations in performance; the higher the heritability, the lower the proportion of non-genetic variation, and so the less severe the shrinking.

To run them, a recoded pedigree file and complex parameter files must be prepared. These files should specify which model will be used, which fixed and random effects will be included in the model, the initial values of the variance components to be estimated, and the number of iterations, among other parameters, all coded with great care. Almost all of these programs cannot directly read data organized in MS Excel. Data prepared in MS Excel must be converted to a different format called a "data file." Some of these programs are commercial (ASREML and MIXBLUP), while others are free (MTDFREML, WOMBAT, and BLUPF90) or partially free. These software programs lack user-friendly interfaces, require advanced knowledge for operation, suffer from a lack of Turkish resources explaining how to use them, and, in some cases, are paid. As a result, they are not effectively utilized in the livestock sector in our country.

To address these issues, there is a need for the development of a program that is user-friendly, does not require expertise or complex parameter file preparation, and has a Turkish user guide and interface specifically designed for the livestock sector and researchers. As a result of a project supported by Harran University Scientific Research Project Unit (HUBAP) (project no: 22035), a software program named BUGA has been developed. The aim of this study is to demonstrate the usability of the BUGA software in field conditions.

Material and Methods

Data

The data used in the research was obtained from a private enterprise affiliated with the Breeding Buffalo Farmers Union in Istanbul. The dataset includes records for 442 healthy Anatolian buffaloes, such as the city, district, village, pedigree information, date of birth, gender, calving date, lactation order, number of test days, lactation milk yield, and previous calving date.

Statistical Model:

For the determination of variance components, the following model (Equation 1) was preferred for the repeated observations used in the study (Mrode, 2005). The effects of environmental factors, such as birth year and month, lactation order, calving year and month, and lactation duration, were determined using variance analysis through the SAS software (2000), with the results showing that the effects of lactation order, calving month, and lactation duration were significant ($P<0.05$), while the effects of other factors were deemed non-significant ($P>0.05$). The significant environmental factors were used as fixed-effect environmental factors in the animal model containing repeated observations.

$$y = Xb + Za + Wpe + e \quad [1]$$

where:

y : vector of observations,

b : vector of fixed effects,

a : vector of random animal effects,

pe : vector of random permanent environmental effects and nonadditive genetic effects, and

e : vector of random residual effect.

X, Z, and W are incidence matrices relating records to fixed animal and permanent environmental effects, respectively.

Software and Analysis Application:

The BUGA software used in the research is programmed in PYTHON 3x (Anonymous, 2023c) programming language, with some parts also utilizing the C language. It is currently in an alpha version and is continuously being developed.

The BUGA software can estimate genetic and environmental effects, along with variance components, using the Restricted Maximum Log likelihood (REML) method and Best Linear Unbiased Prediction (BLUP). It employs two algorithms, Average Information (AI) and Expectation-Maximization (EM). Depending on the selected model, it can estimate phenotypic variance components (genetic, permanent environmental, and random environmental), as well as additive genetic and permanent environmental effects for animals.

The software, as seen in Figure 1, has a visual interface. In the top-left corner of the interface, there

are buttons for opening files, selecting the folder for saving analysis results, running the program, clearing the screen, and closing the program. On the side, there is a combo box that lists the models the user can choose, allowing them to select a model without the need to prepare a parameter file. On the left side of the interface, options for solving the relationship matrix (algebraic or Henderson methods), the output of this matrix, and the algorithm to be used in the solution (AIREML or EMREML) are provided as optional choices for the user.

The user can also optionally specify convergence criteria.

On the fixed effects page, the fixed effects in the model are coded and their names are entered in the header row. In the observation page, the repeated observations obtained for each animal are entered based on the animal number, and the name of the observation is written in the header row (In this study, as milk yield is considered, it is simply labeled as " süt_ver; milk_yield"). Finally, in the starting values page, initial values for genetic variance, permanent environmental variance, and random environmental variance are entered in cells B2, B3, and B4 (Figure 2).

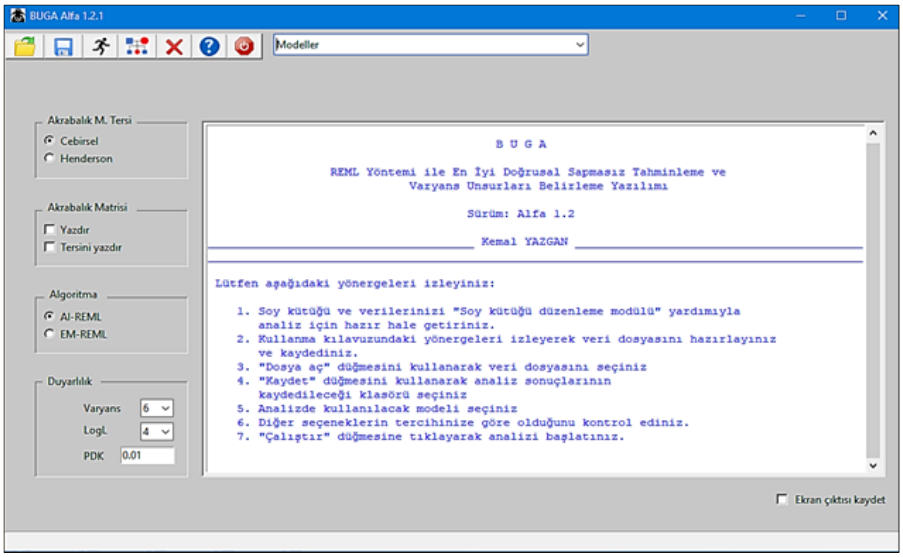


Figure 1. Interface of BUGA software

Default values are shown in the bottom-left corner of Figure 1 (Duyarlılık; Sensitivity). In addition, the software has its own screen for tracking the operations performed during runtime. Users can monitor these operations from the start of the program until completion, and they can choose to save them by checking the "Save Screen Output (Ekran çıktısı kaydet)" box in the bottom-right corner of the interface.

The software requires only one MSExcel file for input (Figure 2). The pages of this file (for the model used in this study) should be named in the following order: pedigree, permanent environmental, fixed effects, observation, and starting values. The first rows on these pages are header rows. Subsequently, on the pedigree page, animal sire and dam numbers should be entered in sequence, starting from 1, with the header rows labelled as animal, sire, and dam, respectively.

Similarly, on the permanent environmental page, the name of the permanent environmental effect (In this study, a permanent environmental page has been created since repeated observations are used. This is not mandatory for a simple animal model) can be written in the first row. The permanent environmental effect contains the animal number for each animal as many times as it has repeated observations. For example, if animal number 25 has three observations, number 25 is written three times in succession.

After these steps, the data file is closed, and the user selects the location of this file and the folder where the output files will be saved using the relevant buttons on the interface. Afterward, the user selects the appropriate model (Equation 1), as shown in Figure 3, and clicks on the "running man" icon to execute the program.

After completing the software process, it displays the message "PROGRAM SONLANDI; PROGRAM ENDED" on its dedicated screen, indicating that the program has successfully concluded. If the user has made errors in preparing the data file or selected the wrong model, the software will identify where the error occurred and notify the user with an error code on its own screen. After completing its operation, the software generates five output files for this model, including two MSExcel files and three graphs (Figures 4 - 8).

These files are named as follows: Pedigree_Solution.xlsx; Soy_Kütüğü_Çözümü.xlsx, Results.xlsx; Sonuçlar.xlsx, genetic_effects.jpg; genetik_etkiler.jpg, Permanent_Environmental_Effects.jpg; Kalıcı çevre etkileri.jpg and Kalıntılar.jpg; Residuals.jpg. In the Pedigree_Solution.xlsx output file, various statistical information related to the pedigree is provided (Figure 4). Additionally, if the user has requested the

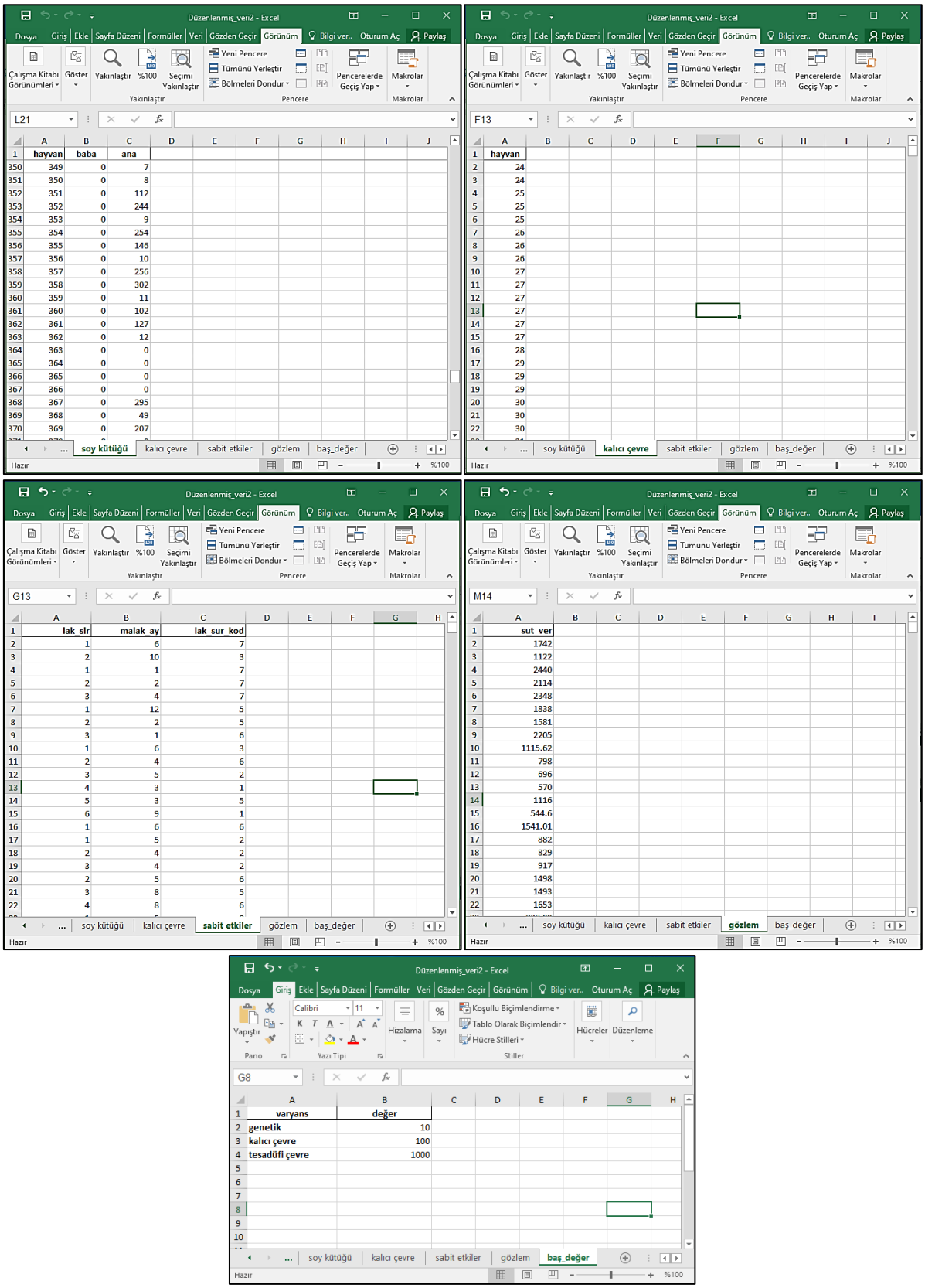


Figure 2: Data file and sections prepared with MSEXcel for BUGA software

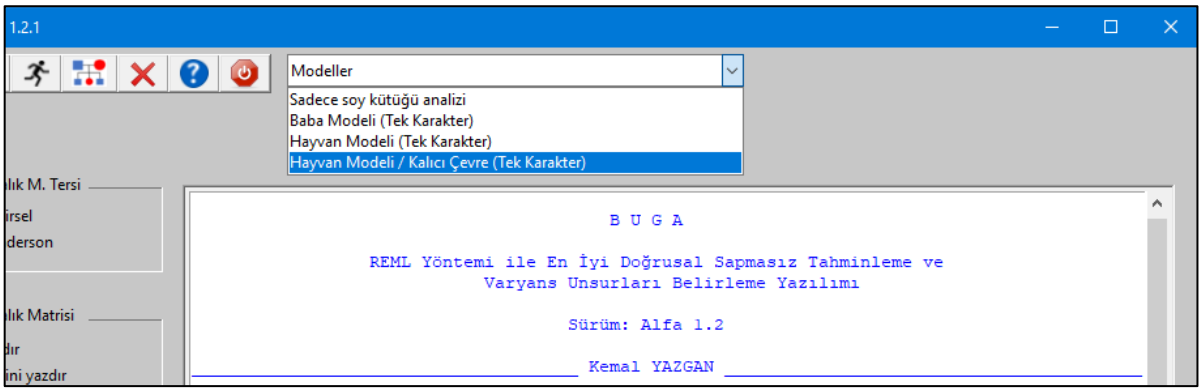
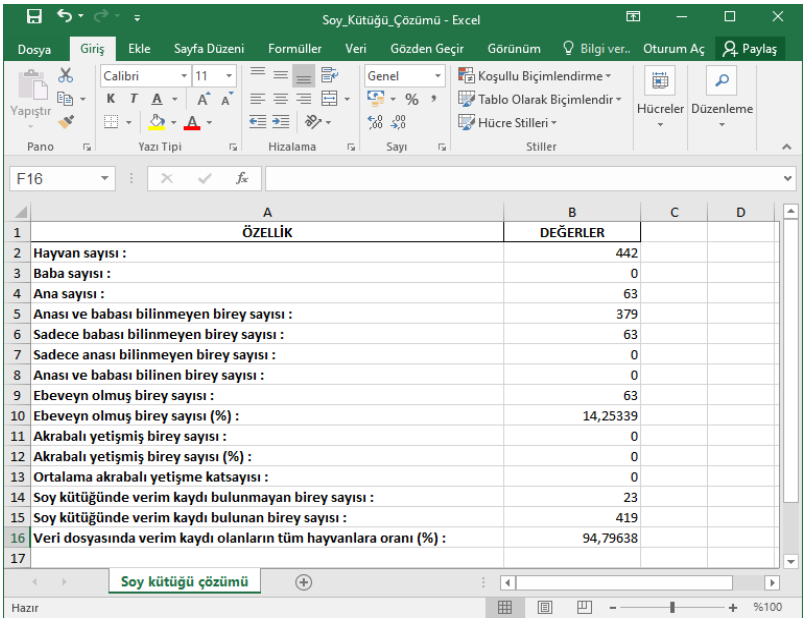


Figure 3. Model selection.



	A	B	C	D
1	ÖZELLİK	DEĞERLER		
2	Hayvan sayısı :	442		
3	Baba sayısı :	0		
4	Ana sayısı :	63		
5	Anası ve babası bilinmeyen birey sayısı :	379		
6	Sadece babası bilinmeyen birey sayısı :	63		
7	Sadece anası bilinmeyen birey sayısı :	0		
8	Anası ve babası bilinen birey sayısı :	0		
9	Ebeveyn olmuş birey sayısı :	63		
10	Ebeveyn olmuş birey sayısı (%) :	14,25339		
11	Akrabalı yetişmiş birey sayısı :	0		
12	Akrabalı yetişmiş birey sayısı (%) :	0		
13	Ortalama akrabalı yetişme katsayısı :	0		
14	Soy kütüğünde verim kaydı bulunmayan birey sayısı :	23		
15	Soy kütüğünde verim kaydı bulunan birey sayısı :	419		
16	Veri dosyasında verim kaydı olanların tüm hayvanlara oranı (%) :	94,79638		
17				

Figure 4. Soy_Kütüğü_Çözümü.xlsx (Pedigree_Solution.xlsx) file and its contents.

relationship matrix and/or its inverse, these are also included in different pages within the same file. The Results.xlsx file consists of an analysis report, fixed effect solutions, genetic effect solutions, permanent environmental solutions, genetic parameters, log-likelihood changes, and residuals pages (Figure 5).

In the genetic effect page, the estimated additive genetic effect for each animal is listed, along with standard errors for each estimate and the correlation values between the true and estimated values. These values can be considered as the breeding values of the animals. In addition, values related to inbreeding coefficients for each animal are provided. As shown in Figure 5, the standard errors of the estimated variance components are also given.

Result and Discussion

Upon completion of the program's operation, as can be seen from the output files it generated (Figure 5), the values for residual variance, genotypic variance, and permanent environmental variance were estimated as 107112.60 kg^2, 20045.22 kg^2, and 1259.50 kg^2, respectively. The heritability (h^2) and repeatability (r) were determined as 0.1561 and 0.1659, respectively. Furthermore, it was observed that the additive genetic effects for the 442 animals ranged from +235.76 to -246.60 kg. In other words, the animal with the highest additive genetic effect produced 235.76 kg more milk than the population average. This value is solely attributable to the genetic effect of the animal, independent of environmental factors. Therefore, the additive genetic effect values for animals can be used as selection criteria.

When compared to other software programs, this software offers advantages such as the ability for the user to directly select the model they want to work with, the absence of the need to prepare parameter files, the ability to prepare data files in MSExcel format and obtain result files in the same format, and the overall ease of use. This makes it possible for the software to become widespread and effectively used in field conditions.

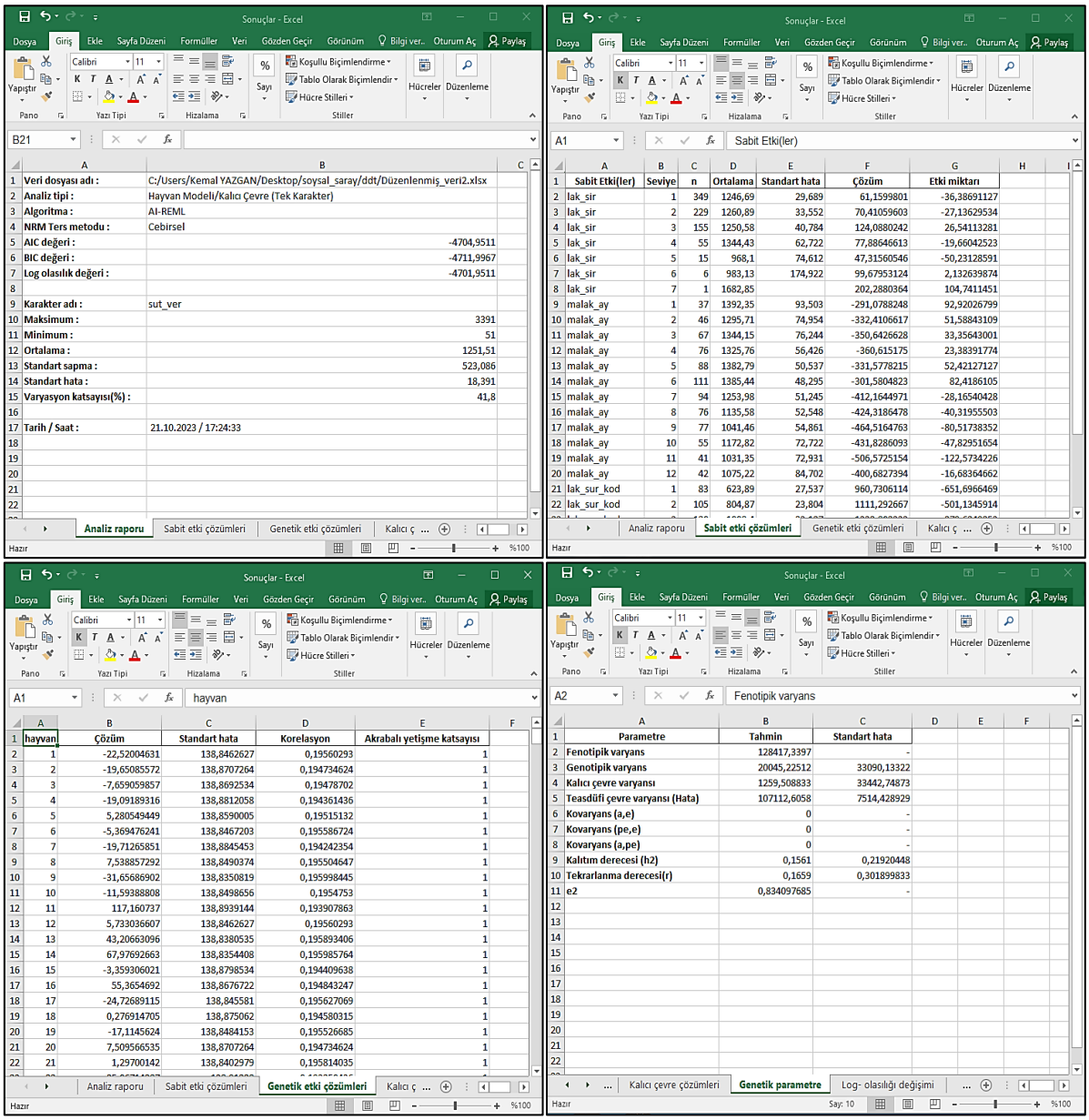


Figure 5. Sonuçlar.xlsx (Results.xlsx) file and its contents.

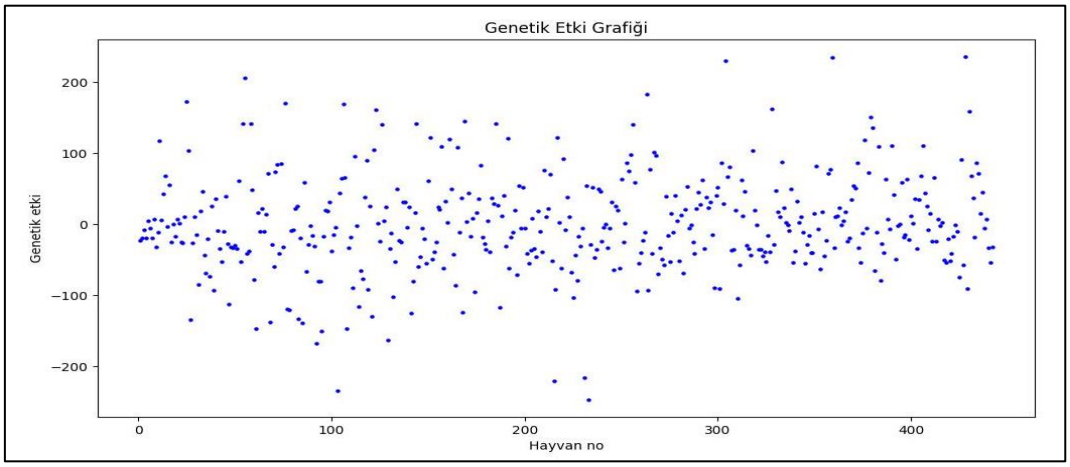


Figure 6. Scatter diagram of additive genetic effect plot.

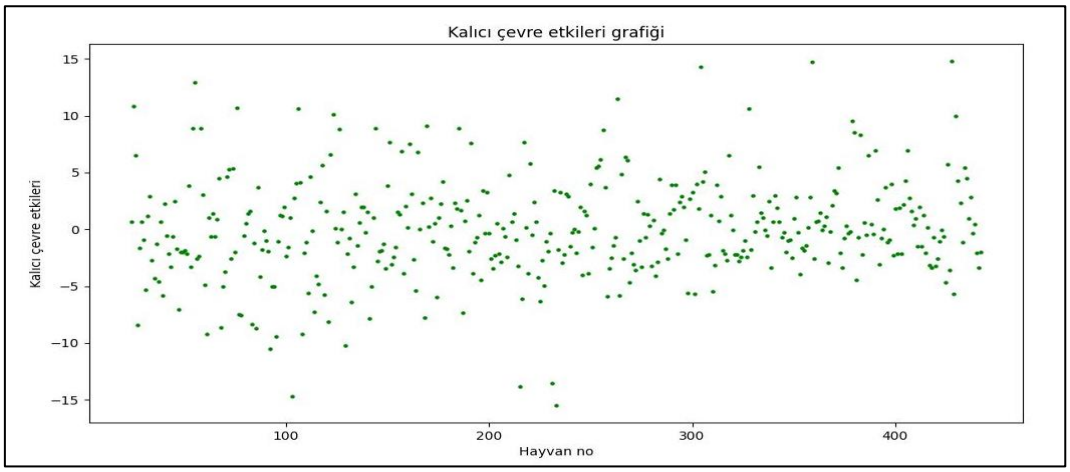


Figure 7. Scatter diagram of permanent environmental impacts plot**.**

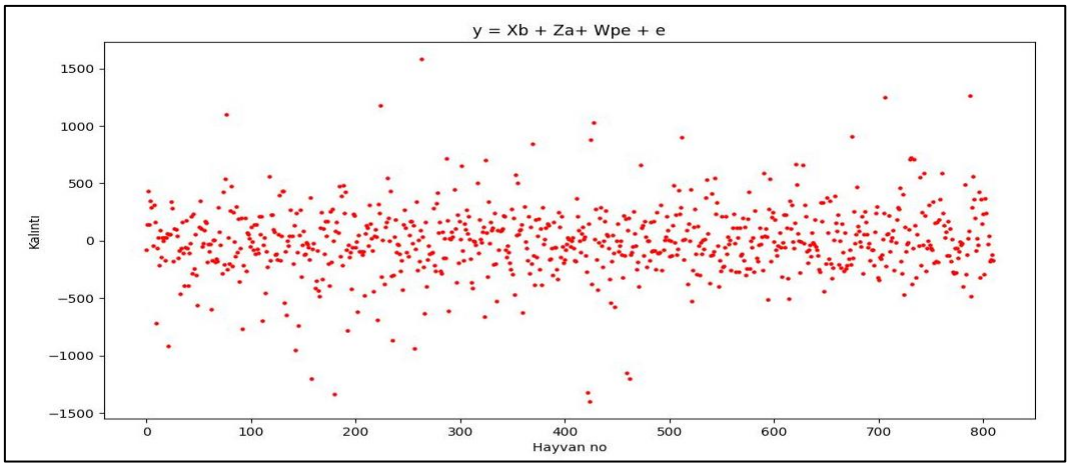


Figure 8. Scatter diagram of residuals (kalıntılar) plot**.**

Conclusion

The results obtained from this case study demonstrate that the BUGA software can be easily employed in farm recording conditions due to its user-friendly features. Furthermore, if the usage of this software with its unique user-friendly features is promoted, it is expected to make a significant contribution to the efficiency of breeding programs. This will increase the accuracy of selection studies in breeders' enterprises with a regular registration system. The software is continually being enhanced and configured to handle more complex models in the future.

Conflicts of Interest

There is no conflict of interest between the authors.

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LIVESTOCK STUDIES

RESEARCH PAPER

Investigation of Some Autosomal Recessive Inherited Diseases (BLAD, DUMPS, CVM, and FXID) in Holstein Cattle

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Abstract

Genetic disorders are often transmitted via autosomal recessive inheritance, which negatively affect health, welfare, and yield traits in farm animals such as cattle. In this study, a total of 80 animals belonging to Holstein Friesian (HF) reared in the dairy farm of Akdeniz University were investigated in terms of Bovine Leukocyte Adhesion Deficieny (BLAD), Deficiency of Uridine Monophosphate Synthase (DUMPS), Factor XI Deficiency (FXID), and Complex Vertebral Malformation (CVM) via three molecular genotyping methods such as Polymerase Chain Reaction (PCR), PCR-Restriction Fragment Length Polymorphism (PCR-RFLP), and Allele-Sprcific PCR (AS-PCR). BLAD and DUMPS were analysed by PCR-RFLP, while PCR and AS-PCR were utilized to investigate FXID and CVM disorders, respectively. Based on fragment patterns on agarose gel electrophoresis, animals were genotyped as normal, mutant, and carrier. In this study, no carrier or mutant animals were detected for BLAD, CVM, DUMPS, and FXID diseases in HF cattle, since these animals were previously imported from disease-free dairy farms located in different provinces of Türkiye. Nevertheless, this population should be periodically checked for autosomal genetic disorders, since inherited diseases may be observed in the next generations due to causative mutations in the related genomic regions.

Introduction

Being able to survive in almost all climatic zones of the world, cattle may convert some crops and industrial products, which cannot be directly utilized for human nutrition, into animal-derived protein resources. As reported by the Food and Agriculture Organisation of the United Nations (FAO), 84% and 30% of the world's milk and beef production are met exclusively by cattle (FAO 2020). In Türkiye, cattle play a more important role in agriculture, since according to Turkish Statistical Institute (TUIK), a higher part of the milk (92.1%) and beef (74.8%) production are obtained from cattle farming (TUIK 2022a; 2022b). Hence, cattle are of great importance for human nutrition and the economies of developing countries such as Türkiye. It is known that a large part of the cattle population are exotic breeds (59.4%) and their crosses (42.8%), whereas 7.8% of the total population is predicted to be represented by native Turkish cattle breeds. Among exotic cattle populations, Holstein Friesian (HF) is the most reared breed in Türkiye (Demir et al., 2023).

It is essential to increase the quantity and quality of economically important traits via selection, while health problems significantly decrease yield traits in livestock species. Therefore, the selection of healthy and tolerant animals is required to maintain the economic production systems. Inherited diseases negatively affect health and yield traits causing physical and functional anomalies in farm animals (Citek et al., 2006; 2007). Besides, as reported by Demir et al., (2021), genetic disorders are of negative effects on animal welfare.

Genetic disorders are mainly transmitted to the next generations via autosomal recessive inheritance in cattle (Windsor and Agerholm 2009). Using bulls with a high breeding value in artificial insemination increases the spreading risks of known and unknown genetic diseases in cattle breeding (Gentile and Testoni 2006; Citek and Blahova 2004). One of the most effective approaches to eliminating genetic diseases in a certain cattle population is to detect disease-carrier animals. However, due to the nature of the autosomal recessive inheritance, heterozygous animals, responsible for the occurrence of the diseases, show normal phenotype. This phenomenon requires accurate methods to detect

disease-carrier heterozygous animals. Thanks to molecular diagnosis methods, disease-carrier heterozygous animals could be detected even before birth in terms of numerous genetic disorders (Citek et al., 2006; 2007).

Bovine Leukocyte Adhesion Deficiency (BLAD), Deficiency of Uridine Monophosphate Synthase (DUMPS), Complex Vertebral Malformation (CVM), and Factor XI Deficiency (FXID) are frequently observed genetic disorders in HF breed (Meydan et al., 2010). BLAD disease is caused by a point mutation (A→G) (Shuster et al., 1992), at position 383 of the gene encoding the CD18 glycoprotein on the bovine chromosome 1 (Nagahata et al., 2004) and DUMPS disease is caused by another point mutation (C→T transition at codon 405) occurring in the UMPS gene on the same chromosome (Avanus ve Altınel 2017). The molecular basis of CVM disease is the result of a point mutation in nucleotide position 559 of the SLC35A3 gene located on bovine chromosome 3, which causes guanine to turn into thymine (Eren et al., 2019). FXID is caused by a 76 bp insertion in exon 12 of the Factor XI gene (Ghanem et al., 2005). Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) may detect BLAD and DUMPS diseases, while FXID and CVM could be assessed via PCR and Allele -Specific PCR (AS-PCR), respectively (Karslı et al 2011; Şahin et al., 2013). Hence, this study aims to screen 80 animals belonging to HF breed reared in the dairy farm of Akdeniz University in terms of some genetic disorders.

Materials and Methods

Ethics Statement

This research was approved by the Akdeniz University Animal Experiments Ethics Committee, Antalya, Türkiye (Protocol No: 1393/2022.01.005)

Sample Collection and DNA Extraction

In 2022, a total of 80 animals belonging to HF breed were randomly chosen from the dairy farm of the Akdeniz University, Faculty of Agriculture located in Antalya province of Türkiye. Blood samples were taken from the jugular vein into vacuum tubes with K3EDTA as a coagulant. Blood samples were stored at -20°C until DNA extraction was performed. DNA was extracted via a salting-out method reported by Miller et al., (1988) following a spectrophotometer and 1% agarose gel electrophoresis in order to assess concentration and purity parameters.

Detection of BLAD, DUMPS, CVM, and FXID Genotypes

A total of three molecular methods such as PCR-RFLP (BLAD and DUMPS), AS-PCR (CVM), and traditional PCR (FXID) were utilized to genotype animals in terms of four genetic disorders. The methodology including oligonucleotide sequences as well as expected band sizes for normal, mutant, and carrier animals in terms of four genetic disorders was summarised in Table 1. Amplification was done in PCR reaction including 50 ng template DNA, 1.2 µL HQ buffer-GeneAll, 2 µL 10X buffer-GeneAll, 2.5 mM dNTPs, 10 pM primer, 2.5 U Taq DNA Polymerase, and 11.4 µL H2O with the following protocol: 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 52-62 °C for 45 seconds, and extension at 72 °C for 45 seconds, with an initial denaturation step at 95 °C for 5 minutes and a final extension step at 72 °C for 10 minutes. The annealing temperature was optimized at 57 ºC, 52 ºC, 55 ºC, and 62 ºC for BLAD, DUMPS, FXID, and CVM diseases, respectively. Since only one nucleotide differs in the primer sequence of the AS-PCR process, the annealing temperature was kept as high as possible to eliminate non-specific amplifications.

Results and Discussion

A 343 bp length PCR product cut by *Taq*I enzyme revealed two bands of 152 and 191 bp across all genotyped animals (Figure 1). No mutant or carrier was detected in the studied population in terms of BLAD disorder.

A total of three bands (53, 36, and 19 bp length) were observed in all animals in terms of DUMPS disease

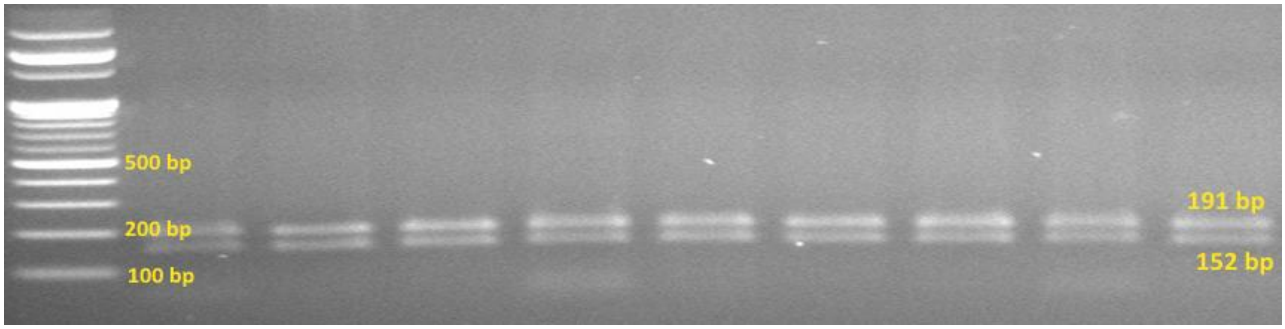

Figure 1. Image of a 2% agarose gel showing the band lengths obtained after cutting PCR products for BLAD disease via the TaqI restriction enzyme. (DNA Ladder: 100 bp, Thermo, Catalog number: 15628050)

Figure 2. 3% agarose gel image of the band sizes obtained as a result of PCR-RFLP with *AvaI* restriction enzyme of PCR products for DUMPS disease (DNA Ladder: 100 bp, Thermo, Catalog number: 10416014)

(Figure 2). Due to failure in the discrimination power of agarose gel electrophoresis, the band of 19 bp is not clearly observable by bare eyes in Figure 2.

In terms of CVM disorder, PCR products were successfully amplificated in all animals using normal primers, whereas no amplification was observed by using the mutant primer (Figure 3). This indicates that no CVM disease carrier is present in the studied cattle population.

All animals yielded a 244 bp length band in terms of FXID disorder, while the 230 bp length band was absent across the population. This finding indicates that there is no carrier or mutant animal in the studied cattle population.

Numerous studies showed that heterozygous carriers of the BLAD, DUMPS, CVM, and FXID disorders were present in HF breeds raised in different countries such as China (Zhang et al., 2010; Wang et al., 2011; Sun et al., 2011), Mexica (Virgen-Méndez et al., 2019), India (Patel et al., 2014), Poland (Gozdek et al., 2020). Similarly, Meydan et al., (2010) investigated the BLAD, DUMPS, CVM, Bovine Citrulinaemia (BC), and FXID diseases in 350 HF cattle reared in different regions of Türkiye in which a total of 14 and 12 animals were reported to be carriers of CVM and FXID disorders, respectively. On the contrary, no CVM carriers were reported in 150 heads of HF population raised in Kayseri province of Türkiye (Kulaklı and Akyüz 2011). Karsli et al., (2011) reported a low prevalence of FXID (0.4%), while no DUMPS carriers were present in a total of 504 animals belonging to HF breed reared in different districts of Antalya province of Türkiye. However, BLAD carriers were detected at the frequency of 2% and 4.8% in HF populations reared in Antalya and Kayseri provinces, respectively (Şahin et al., 2013; Akyüz et al., 2015). DUMPS and BC diseases were not reported in 219 Holstein cattle bred in Eskişehir, whereas two FXID and three BLAD carriers were present (Kaya et al., 2016). By using AS-PCR, Eren et al., (2019) confirmed that 7 out of 200 individuals analyzed were CVM carriers in HF population reared in Antalya. Screening a total of 48 animals in terms of BLAD and FXID disorders, Aksel et al., (2021) detected only one carrier animal for BLAD disorder.

Previous studies conducted on HF populations reared in Antalya province showed that the frequency of FXID carriers was 0.4%, while it was 2% for BLAD carriers, and 3.5% for CVM carriers (Karslı et al., 2011; Şahin et al., 2013). The frequency of FXID carriers in Holstein cattle reared in Burdur province was reported as 1.8% and the frequency of BLAD carriers as 2% (Ağaoğlu et al., 2015). Unlike previous researches, no carrier animals in terms of BLAD, FXID, and CVM disorders were detected in this study. It is thought that the main reason underlying this difference is the routine screening of artificial insemination bulls for these

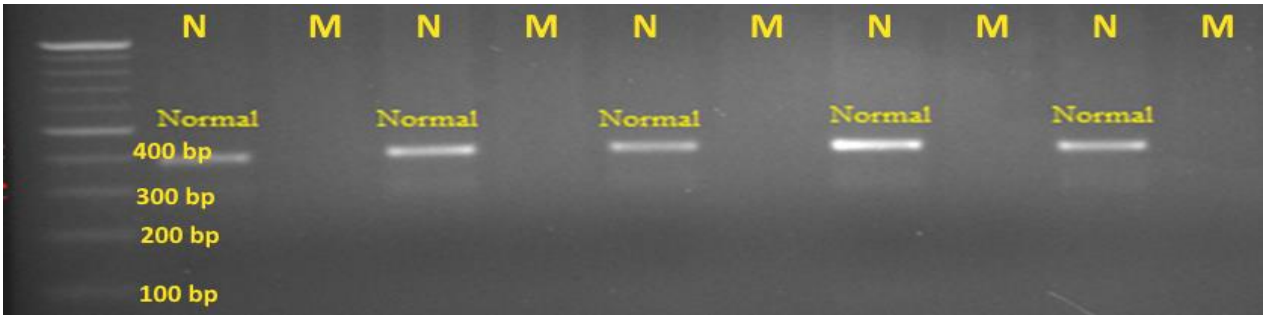

Figure 3. Image of band sizes obtained in AS-PCR for CVM disease on a 2% agarose gel. (DNA Ladder: 100 bp, Thermo, Catalog number: 15628050)

Table 1. The method used to identify the relevant mutations and some descriptive information about genotyping

Diseases		BLAD	DUMPS	CVM	FXID
Method		PCR-RFLP		AS-PCR	PCR
Primer Sequence	Forward	CCTGCATCATATCCACAG	GCAAATGGCTGAAGAACATTCTG	CACAATTTGTAGGTCTCATGGCG	CCCACTGGCTAGGAATCGTT
	Reverse	GTTTCAGGGGAAGATGGAG	GCTTCTAACTGAACTCCTCGAGT	GTTATACTACAGGAGTCACCTCT	CAAGGCAATGTCATATCCAC
	Mutant	-	-	CACAATTTGTAGGTCTCATGCAT	-
PCR lengths		343	108	395	244 or 320
Restriction enzymes		*Taq*I	*Ava*I	-	-
Genotypes	Mutant	343	89 and 19	395	320
	Carrier	191, 152, and 343	89, 53, 36, and 19	395	244 and 320
	Normal	152 and 191	53, 36, and 19	395	244
References		Şahin et al., (2013)	Karslı et al., (2011)	Eren et al., (2019)	Karslı et al., (2011)

diseases for a long time, from which semen is obtained both abroad and locally. Another reason may be considered to be that the sampling was made from a single enterprise. However, as reported by Demir and Balcioğlu (2019), HF population of the dairy farm of the Akdeniz University was re-established in 2017 by importing animals from several disease-free commercial enterprises located in ten different provinces of Türkiye. Therefore, the current population is thought to be a representative herd.

Conclusion

Genetic disorders in cattle, which are mostly transmitted through autosomal recessive inheritance, have negative impacts on animal health, welfare, and productivity. Molecular diagnosis methods are effective in detecting these disorders in carrier heterozygous animals, even before birth. In this study, no carrier animals in terms of BLAD, DUMPS, CVM, and FXID disorder were detected in HF population raised on the farm of the Akdeniz University due to the fact that these animals were imported from disease-free farms localized in ten provinces in Türkiye. This information can be used to improve breeding strategies, including the selection of animals with high breeding values and the prevention of the spreading of known and unknown genetic diseases in cattle breeding. Further studies are recommended to extend the screening to other cattle populations in different regions of Turkey with larger sample sizes to understand the distribution of these genetic disorders and develop strategies for the prevention and control of these diseases.

Acknowledgments

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Conflict of Interest

The authors declare no conflicts of interest.

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LIVESTOCK STUDIES

RESEARCH PAPER

Growth and Immune Responses, Oxidative Stress Biomarkers, and Antioxidative Enzymes of Broilers Fed with Supplementation of *Chenopodium ambrosioides L.* and *Crassocephalum crepidioides* leaf meals

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Leaf meal
Supplement
Stress biomarkers

Abstract

This study assessed the effect of dietary supplementation of Crassocephalum crepidioides (CCLM) and Chenopodium ambrosioides L. (CALM) leaf meals on relative growth rate, oxidative stress biomarkers, immune response, serum and meat antioxidative enzymes of broilers. A total of 120 one-day-old Cobb 500 chicks were randomly assigned to four dietary treatments (three replicates of 10 birds/treatment) as follows, Diet 1 (basal/control diet), Diet 2 (basal +200mg/kgVitC), Diet 3 (basal +2500mg/kgCCLM) and Diet 4 (basal +2500mg/kgCALM) for 42 days. The result revealed that supplementation of CCLM and CALM significantly reduced ($P < 0.05$) the concentration of heat shock protein and hydroxy-2-deoxyguanosine in broilers compared to the control group. Broilers fed diet containing CCLM and CALM exhibited higher ($P < 0.05$) serum glutathione peroxidase content compared to the control group. The level of immunoglobulins were significantly higher in birds fed diet containing CCLM, CALM and Vit C compared to control group. However, supplementation of CCLM and CALM did not influence ($P > 0.05$) the relative growth rate and antioxidant content of breast muscle compared to control group. It's concluded that CCLM and CALM could be used as natural additive to alleviate oxidative damage, improve immune system and serum antioxidant content of broilers.

Introduction

Commercial poultry is often subject to a range of stressors, from environmental to nutritional factors, that could impair performance during growing period, despite advances in management, genetic selection, and biosecurity [1]. In terms of nutrition, poultry production requires a balanced diet to support the immune system and inhibit cell damage. However, stresses that occur due to nutritional imbalances or deficiency may induce the production of free radicals, which in turn could weaken the immune system, create health problems, and result in significant production-related financial losses [1-3]. In order to avoid this, the inclusion of natural antioxidants in diets has been encouraged [4-5]. This is because natural antioxidants contain inherent bioactive compounds that can activate the production of immunoglobulin [6], increase endogenous antioxidants [7], and inhibit the oxidative process that is caused by an imbalance between the production of reactive oxygen species (ROS) and endogenous antioxidant defense mechanisms in the body [4,8]. In addition, studies have shown that natural antioxidants can be used to improve feed intake, enhance body weight gain, and improve meat quality and the overall performance of poultry during production [7, 9, 10].

Interestingly, most natural antioxidants are obtained from plant materials, such as herbs (flowers, leave, fruits, seed, stem, root, etc.), vegetables, and spices, as well as their extracts such as essential oils [4, 11]. Its utilization has been encouraged in animal diets because they are easily affordable, accessible and safe when used at low concentrations compared to

antibiotic growth promoters [12]. Natural antioxidants possessed enormous phytochemicals such as phenols, flavonoid, alkaloid, tannin, saponin, and other bioactive compounds. Chenopodium ambrosioides L. and Crassocephalum crepidioides plants are among the natural antioxidants with a lot of potential that can be utilized as feed additives in poultry production.

Chenopodium ambrosioides L. is an aromatic herbaceous medicinal/vegetable plant that belongs to the family of Chenopodiaceae and the genus Chenopodium. The plant is extensively found in West Africa, particularly in Nigeria, and is generally known as Mexican tea [13]. In traditional medicine, the leaf of the plant is used to treat uterine fibroids as well as bacterial, fungal, parasitic, and viral diseases [14-15]. According to research by Maldonado-Garcia *et al.* [16] and Reyes-Becerril *et al*. [17], fish fed a diet supplemented with C. ambrosioides L. at 0.5, 1.0, and 2.0% (w/w) had stronger immune cell and antioxidant enzyme activities than fish without the supplements. Crassocephalum crepidioides is a succulent annual leafy vegetable and herb that belongs to the family Asteraceae (Compositae). It is widely distributed in tropical and subtropical regions of the world, including Africa, Asia and Australia [18]. Every part of a plant is known to have abundant secondary metabolites with the ability to elicit antibacterial, hypoglycemic, and antioxidant actions [18-19]. According to a preliminary investigation by Falowo *et al*. [19], both Chenopodium ambrosioides L. and Crassocephalum crepidioides plants are readily available and their leaves possessed moderate nutritional content, high bioactive compounds and antioxidant properties, which qualifies them as potential natural additives. In fact, the dietary supplementation of leaf meal of Crassocephalum crepidioides plant has been reported to improve the performance, immune systems and meat quality of broiler chicken [20]. However, to our knowledge the dietary utilization of Chenopodium ambrosioides L. leaf meal as natural additives on boiler performance has not been studied. Therefore, this study was designed to examine the effect of Chenopodium ambrosioides L. and Crassocephalum crepidioides leaf meals on oxidative stress biomarkers, immune response, serum and meat antioxidative enzymes of broiler chickens.

Materials and Methods

Plant collection and extract preparation

Freshly harvested leaves of Chenopodium ambrosioides L. and Crassocephalum crepidioides were purchased from the commercial markets in southwestern Nigeria. The leaves were cleaned and air-dried in an open shade. The dried leaves were ground using an electric blending machine and the powdered samples were packed into a black polyethylene bag for further analysis.

Experimental site

The study was conducted at the Poultry Unit of the Teaching and Research Farm, Federal College of Agriculture, Akure, Nigeria. The experimental site is located at 7°25' N and 5°19' E with average annual temperature and annual rainfall of 25.3°C and 1455 mm, respectively. The entire study was carried out for six weeks following the research ethics and guidelines of the Animal Health and Prodution Technology Department of the institution.

Experimental diets and animals

Two basal diets [starter (0-21 days) and finisher phase (22-42days)] were formulated to meet the broiler's nutritional requirement as recommended by NRC [21] (Table 1). At each phase, the experimental diets were divided into four treatments and designated as Diet 1 (basal/control diet), Diet 2 (basal + 200mg/kg Vitamin C as positive control), Diet 3 (basal +. 2500mg/kg CALM) and Diet 4 (basal + 2500mg/kg CCLM). One hundred and twenty 1-day-old Cobb 500 unsexed broiler chicks were randomly distributed to four dietary treatments. Each treatment was replicated three times. Thirty birds were assigned to each treatment (10 birds/replicate) in a completely

Table 1. The Experimental Basal Diets' Composition.

Ingredients	Starter feed	Finisher diet
Maize	52.33	59.32
Maize bran	7.02	0.00
Rice bran	0.00	6.03
Fish meal	3.00	3
Soybean meal	30	24
Premix	0.30	0.30
Bone meal	3.00	3.00
Soy oil	3.00	3.00
Methionine	0.30	0.30
Limestone	0.50	0.50
Salt	0.30	0.30
Lysine	0.25	0.25
Analyzed composition (g/kg)		
Crude fibre	3.55	3.63
Crude fat	4.47	3.94
Crude protein	22.19	20.09
Calculated composition (g/kg)		
Calcium	1.02	0.97
Available phosphorus	0.44	0.41
Methionine	0.68	0.65
Lysine	1.36	1.24
Metabolizable energy (Kcal/kg)	3018.93	3108.16

randomized design (CRD). The birds were housed in their respective pen (200 x 100 cm) with the floor covered with wood shavings. The temperature of the house was maintained within 31oC ± 2 for the first 7 days and reduced by 2°C after each consecutive 7 days until the house temperature was 26 ± 2 oC. The feed and water were provided ad libitum throughout the six weeks feeding trial. The lights were left on for 24 hours on the first day, and then reduced by 1 hour until 7th days, and later reduced to 20 hours per day til the end of the feeding trial.

Growth, Oxidative Stress Biomarkers, Immune and antioxidant enzymes analysis

The body weights of broilers were measured every seven days. The relative growth rate (RGR) was calculated using the following formula [22]:

RGR= [(w2-w1)/ ((w1+w2))/2] *100

Where w1= Bodyweight when the trial began, and w2= Bodyweight at the conclusion of the study

At 42 weeks of age, three birds per treatment were randomly selected and humanely slaughtered. Before slaughter, feeds were withdrawn from the birds overnight. During slaughtering, blood samples were obtained from jugular veins into a plain blood sample bottle for antioxidant enzymes (catalase and glutathione peroxidase). The blood sample in each of the plain bottles was centrifuged for 10 min at 3000 rpm to obtain clean supernatant serum. The harvested supernatant serum was kept at -20 °C before analysis. The serum enzymatic activities of the catalase (CAT) and glutathione peroxidase (GPx) activities were determined according to the method of Aebi [21,3] and Rotruck *et al.* [22,4], respectively. Also, the fresh centrifuged serum was used to determine the concentration of immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin E (IgE) of the broiler chickens. The concentrations of IgE, IgM, and IgA were determined by mixing serum with buffer solution and antibody reagents to form an antigen/antibody complexes which, following agglutination, were measured turbidimetrically, using commercial ELISA quantitation kits (Fortress Diagnostics Limited, United Kingdom): IgG (Catalog No BXC0721A), IgM (Cat No BXC0731A), IgE (Cat No BXCF751A) and IgA (Cat No BXC0701A).

The levels of oxidative stress biomarkers were analyzed in the collected serum. The concentration of 8-hydroxydeoxyguanosine (8-OHdG) was determined in the blood serum using a commercial ELISA Kit (Elabscience Biotechnology Inc, USA) with catalog No E-EL-0028 96T, based on the manufacturer's instructions. The level of heat shock protein (HSP)/heat shock factor (HSP) was also determined using ELISA kit, according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanghai, China). For antioxidant meat enzyme analysis, about 100g of fresh meat samples were excised from the breast muscle of broiler chicken after slaughter and evisceration for determination of catalase activity and superoxide dismutase, by the methods described by Hadwan and Khabt [23, 5] and Marhlund and Marklund [24, 6]

Statistical analysis

Data obtained on growth and immune responses, oxidative stress biomarkers and antioxidative enzymes of broilers were analyzed using PROC GLM procedures of Statistical Analysis System (SAS, version 9.1.3 of 2007). Differences in mean values were computed using Duncan's Multiple Range Test for multiple comparisons. For all statistical tests, significance was determined at $P < 0.05$.

Results

The result of the effect of CCLM and CALM supplements on serum oxidative biomarkers of broilers is presented in Table 2. The result revealed significant ($P < 0.05$) differences in heat shock protein (HSP) and 8-Hydroxy-2-deoxyguanosine (8OHDG) content of broilers among the treatments. Broilers fed the diet supplemented with CCLM (0.50pg/mL), CALM (0.46pg/mL) and Vit C (0.48 pg/mL) recorded lower heat shock protein content compared to the control group (0.65 pg/mL). Similarly, the 8-Hydroxy-2-deoxyguanosine content was significantly lowered in broiler chicken fed diet supplemented with CCLM (95.06 ng/mL), CALM (83.01 ng/mL) and Vit C (81.82 ng/mL) compared to the control group (122.06 ng/mL).

Table 3 shows the result of the serum antioxidant enzymes of broiler chickens fed diet supplemented with CCLM and CALM. The analysis revealed that broiler chickens fed diet containing CCLM (45.79 mg/ml), CLAM (32.20 mg/ml) and Vit C (46.01 mg/ml) had a higher concentration of glutathione peroxidase

Table 2: Serum oxidative stress biomarkers of broiler chicken fed diets supplemented with *Chenopodium ambrosioides L. and Crassocephalum crepidioides leaf meal*.

Parameters	Diet 1 Control	Diet 2 200mg/kg Vit C	Diet 3 2500mg/kg CCLM	Diet 3 2500mg/kg CALM	SEM	*P value*
HSP (pg/mL)	0.65^{a}	0.48^{b}	0.50^{b}	0.46^{b}	0.02	0.00
8OHDG (ng/mL)	122.06^{a}	81.82^{b}	95.06^{ab}	83.01^{b}	9.01	0..03

Means within a row with different letters and significantly different ($P < 0.05$). SEM Standard error. CALM = *Chenopodium ambrosioides* leaf meal. HSP = Heat shock protein. 8OHDG= 8-Hydroxy-2-deoxyguanosine

Table 3: Serum antioxidant enzymes of broiler chicken fed diets supplemented with *Chenopodium ambrosioides L. and Crassocephalum crepidioides leaf meal*

Parameters	Diet 1 Control	Diet 2 200mg/kg Vit C	Diet 3 2500mg/kg CCLM	Diet 3 2500mg/kg CALM	SEM	*P value*
GPX (mg/ml)	29.72[b]	46.01[a]	45.79[a]	32.20[b]	2.13	0.01
Catalase (mg/g)	135.01	160.78	160.94	170.53	9.27	0.13

Means within a row with different letters and significantly different (*P* < 0.05). SEM Standard error. CALM = *Chenopodium ambrosioides* leaf meal, GPX = Glutathione peroxidase, LDH = Lactate dehydrogenase

compared to the control group (29.72 mg/ml) (P < 0.05). The concentration of lactate dehydrogenase was significantly higher (P < 0.05) in broiler chickens fed diet containing CALM compared to other treatments. However, there was no significant influence (p > 0.05) of supplemented diets (CCLM and CALM) on the catalase content of the broiler chicken compared to the control group.

Table 4 shows the result of the immune response of broiler chicken fed the diet supplemented with CCLM and CALM. The results revealed significant differences (P< 0.05) in the value of immunoglobulin A (IgA) and M (IgM) across the experimental treatments. Broiler chicken fed diet supplemented with CCLM (277.55 mg/dl), CALM (259.65 mg/dl) and Vit C (253.79 mg/dl) had higher IgA content compared to the control group (209.58). Similarly, broiler chicken fed a diet containing CCLM, CALM and Vit C had higher IgM content at 382.92, 456.41 and 365.12 mg/dl respectively, compared to the control group (357.51 mg/dl). However, the result did not show any significant effect of supplemented diets (CCLM and CALM) on immunoglobulin E (IgE) and G (IgG) of the broiler chicken across the experimental treatments.

As presented in Table 5, supplementation of CCLM and CALM did not significantly influence the meat antioxidant content (catalase and superoxide dismutase) of the broiler chicken (P > 0.05) across treatments. The result of the effect of CCLM and CALM supplements on relative growth rate of the broilers is shownin Figure 1. The result did not show any significant (P > 0.05) effect of CCLM and CALM supplements on relative growth rate of the broilers compared to control group in both the starter and finisher phases of the feeding trial.

Discussion

Oxidative stress biomarkers

Natural antioxidants are commonly used in animal nutrition to promote growth, enhance endogenous antioxidants, inhibit oxidative stress, and boost the immune response of broilers [4, 6]. In this study, supplementation of CCLM and CALM significantly decreased the expression of heat shock protein in the broilers compared to the control. HSPs are specific proteins that are produced by cells in response to a variety of oxidative stressors such as oxidants, toxins, heavy metals, free radicals, and microbes [27]. They are produced as molecular chaperones to ameliorate the adverse effect of oxidative stress during animal production [28]. HSps have been implicated in a diverse range of cellular activities, including senescence, endoplasmic stress, cell death signaling and inflammation [29]. The result of this study is suggesting that CCLM and CALM possessed the ability to attenuate the production of HSPs and alleviate oxidative damage in the cell. Different studies have shown that the dietary supplementation of natural antioxidants that is rich in phytochemicals can be used to modulate or inhibit the expression of heat shock protein in broilers under heat stress [30-32]. Conversely, the observed reduction in serum concentration of 8-Hydroxy-2-deoxyguanosine (8OHDG) of broiler chicken fed the diet supplemented with CCLM and CALM is further buttressing the ability of the CCLM and CALM to inhibit oxidative damages in poultry during production. Many 8-Hydroxy-2'-deoxyguanosine (8-OhdG) are produced in the cells as a product of DNA damage or oxidation, which is caused

Table 4: Immune response of broiler chicken fed diets supplemented with *Chenopodium ambrosioides L. and Crassocephalum crepidioides leaf meal.*

Parameters (mg/dl)	Diet 1 Control	Diet 2 200mg/kg Vit C	Diet 3 2500mg/kg CCLM	Diet 3 2500mg/kg CALM	SEM	*P value*
IgA	209.58[b]	253.79[a]	277.55[a]	259.65[a]	11.70	0.001
IgE	1082.15	1075.88	1077.37	1100.25	25.67	0.88
IgG	303.24	318.50	344.67	318.58	20.91	0.73
IgM	357.51[b]	365.12[b]	382.92[b]	456.41[a]	9.57	0.00

Means within a row with different letters and significantly different (*P* < 0.05). SEM Standard error. CALM = *Chenopodium ambrosioides* leaf meal, IgA =Immunoglobulin A, IgE= =Immunoglobulin E, IgG =Immunoglobulin G, IgM =Immunoglobulin M

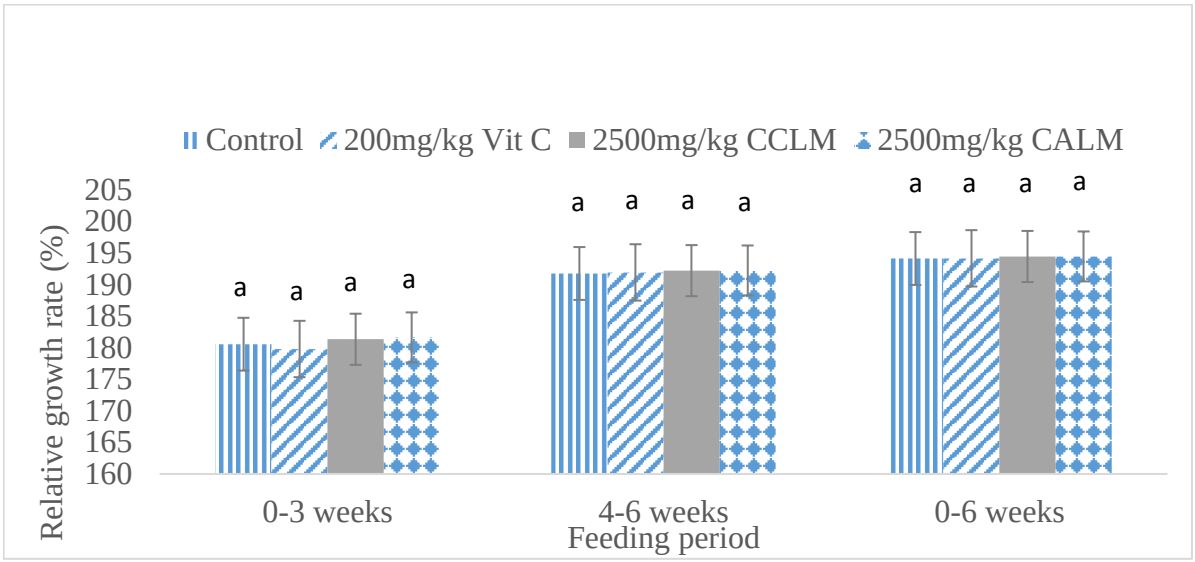

Figure 1: The effects of *Chenopodium ambrosioides L. and Crassocephalum crepidioides leaf meal* on the relative growth rate of broilers.

by hydroxyl radical, superoxide, hydrogen peroxide, singlet oxygen or direct photodynamic action [33]. This result is similar to the findings of Prima *et al*. [34] who found that broiler chickens fed the diet supplemented with olive leaves and marigold petal extract significantly reduce the plasma concentration of 8-Hydroxy-2-deoxyguanosine compared to the control. Liu *et al.* [35] in their study also found that the dietary inclusion of Macleaya cordata extracts significantly reduced hepatic contents of 8-hydroxy-2'-deoxyguanosine in broiler chickens compared to the control group.

Antioxidant enzymes

The result of serum antioxidant enzymes revealed that supplementation of CCLM and CALM significantly increased the concentration of glutathione peroxidase compared to control. Glutathione peroxidase is one of the endogenous enzymatic antioxidants that help to protect the cell against oxidative damage by inactivating or scavenging or removing free radicals and other reactive oxygen species in cells [36]. This result is in agreement with the findings of Adeyeye *et al.[*36] who reported a significant increase in glutathione peroxidase content of broiler chickens fed the diet supplemented with wild sunflower and goat weed leaf meals composite-mix compared to the control group. The increase in GPX in diets supplemented with CCLM, CALM and Vit C is indicating that they could exert higher antioxidative activity against oxidative damage in broiler chicken compared to the control. However, the observed similarity in the value of catalase of the broiler chicken across experimental treatments is in contrast with the report of Adu *et al.* [7] who found a significant increase in catalase content of broiler chicken fed the diet supplemented with Syzygium aromaticum leaf meal compared to control.

Immune response

Besides the potential of plant leaf meal as a natural antioxidant to inhibit oxidative damage and increase endogenous antioxidants, several studies have reported their ability to induce or modulate the immune response and increase immunoglobulin secretion in broiler chicken during production 37-38]. Immunoglobulins (IgA, IgM, IgE and IgG) are produced by plasma cells as part of the body's adaptive humoral immune response against a foreign pathogen [39]. In this study, the observed increase in the immune responses of the broiler chickens has shown that supplementation of CCLM and CALM could be used to stimulate the production of immunoglobulin M and A

Table 5: Meat antioxidant content of broiler chickens fed diets supplemented with *Chenopodium ambrosioides L. and Crassocephalum crepidioides leaf meal.*

Parameters	Diet 1 Control	Diet 2 200mg/kg Vit C	Diet 3 2500mg/kg CCLM	Diet 3 2500mg/kg CALM	SEM	*P value*
Catalase (mM/ml/min)	48.53	48.58	46.91	46.17	0.57	0.15
Superoxide dismutase (%)	91.22	89.75	93.66	94.63	1.88	0.31

Means within a row with different letters and significantly different ($P < 0.05$). SEM Standard error.

during production. Immunoglobulin (Ig) M is known as the first antibody isotype that helps to protect the host against infections and regulate other immune responses and tolerance [39]. While IgA plays an important role in inhibiting macromolecule absorption or binding of allergens to mucosal target cells, neutralizing bacterial toxins and enhancing nonspecific defense mechanisms in animals [41-42]. The result is similar to the findings of Osman *et al.* [43] and Cheng *et al.* [44] who found that boiler chickens fed the diet supplemented with turmeric powder and lotus leaf extract as natural antioxidants, respectively, had higher IgA and IgM concentrations compared to control. On the contrary, this result agreed with the report of Adeyemi *et al.* [20] who found no significant difference in the value of serum IgM of broiler chicken supplement with Crassocephalum crepidioides leaf meat at 1000 and 2000 ppm compared to the control. However, the observed similarity in concentration of IgE and IgG is indicating that the dietary inclusion of CCLM and CALM at 2500mg/kg could not improve their content compared to the control. This result is in contrast with the findings of Osman *et al.* [43] and Yao *et al.* [45] who reported a significant improvement in Immunoglobulins (IgA, IgM, IgE and IgG of chicken supplemented with turmeric powder and sea buckthorn extract as a natural antioxidant, respectively, compared to control.

Meat antioxidant content

The observed similarity in the meat catalase and superoxide dimutase across experimental treatments is indicating that the inability of dietary inclusion of CCLM and CALM at 2500mg/kg to meat antioxidant content of the broiler chicken during production. This result is in line with the report of Adu *et al.* [7] and Gbore *et al.* [46] who found that dietary supplementation of Syzygium aromaticum and moringa leaf meal at 2500mg/kg each, respectively did not increase the catalase content of breast meat of broiler chicken compared to control.

Relative growth rate

The observed similarity in the relative growth rate between the broilers fed leaf meals and control diet is indicating that the utilization of CCLM and CALM at 2500mg/kg could not improve the growth performance of broilers during production. This result is in contrast with report of Oloruntola *et al.* [22] and Adu *et al.* [7] who found that dietary inclusion of plant leaf meal as feed additives at 2500mg/kg significantly increased the growth performance (body weight) of broilers compared to control group.

Conclusion

The findings of the present study reveal the potential of Chenopodium ambrosioides L. and Crassocephalum crepidioides leaf meal to inhibit oxidative damage, enhance endogenous antioxidant enzymes, improve the immune system and maintain the meat quality of broiler chickens when used as a natural antioxidant in the diet. It was shown that the inclusion of CCLM and CALM at 2500mg/kg reduced the production of heat shock protein and Hydroxy-2-deoxyguanosine content, increased the level of serum glutathione peroxidase and catalase content, enhance the production of immunoglobulin IgA and IgM, and maintain the breast meat antioxidant of broiler chicken during production. Further research is required to assess CCLM and CALM effects on the meat fatty acid profile of broiler chickens.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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