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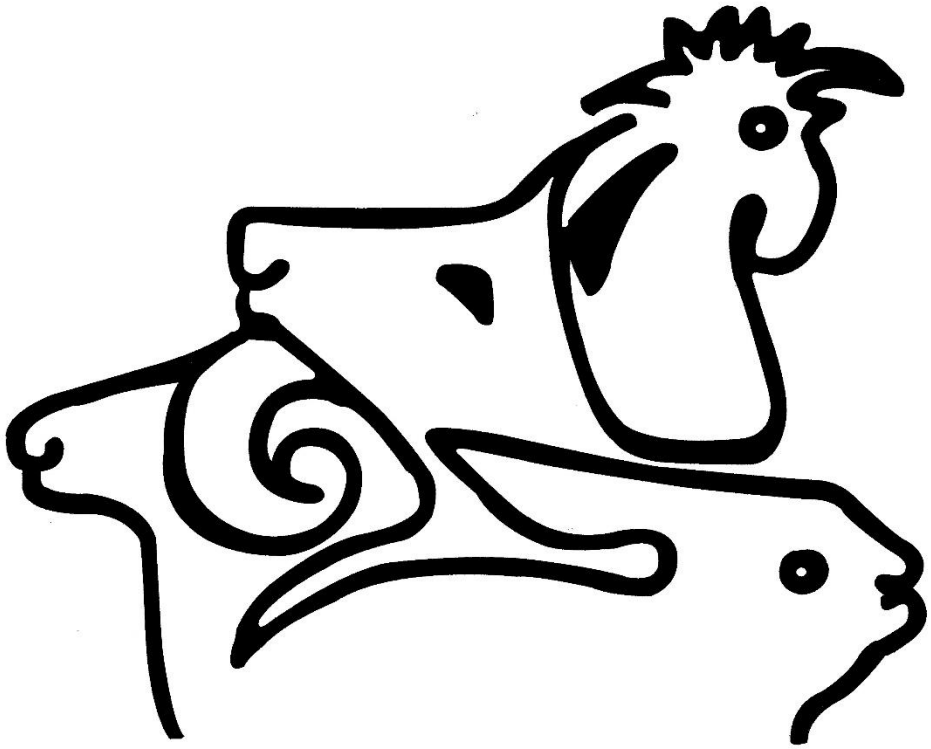

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Impact of Management Practices on Calf Mortality Rates in Dairy Farms: A Study in the Gaziantep Region of Türkiye

ABSTRACT

Objective: This study assesses calf-rearing practices and their effects on calf mortality in the TRC1 region of Southeastern Anatolia, Türkiye. By classifying farms into low, medium, and high mortality groups, it identifies key factors impacting calf survival and offers insights to enhance calf health, reduce mortality, and improve dairy farm sustainability.

Material and Methods: The study was conducted in Türkiye's TRC1 region, home to around 388,000 dairy cattle. Data were collected from 145 dairy farms during the 2019–2020 period using stratified random sampling. Farms were grouped by mortality rate using K-means clustering. Statistical tests (ANOVA, Kruskal-Wallis, t-test, Mann-Whitney U test) and multiple regression analysis assessed factors such as colostrum intake, milk feeding frequency, and weaning age on calf mortality.

Results: Significant variability in calf-rearing practices was observed. The average colostrum intake was 2.64 liters initially and 3.15 liters for the second feeding, with wide ranges. Milk was fed 2.08 times per day, and weaning ages spanned 30-180 days. Low-mortality farms had higher colostrum intake and consistent feeding schedules. Regression analysis identified colostrum intake, milk feeding frequency, and weaning age as significant calf mortality predictors.

Conclusion: The study underscores the importance of early calf management on mortality rates. Improved colostrum intake, feeding frequency, and appropriate weaning age can enhance calf survival and farm profitability, offering valuable guidance for dairy farmers.

Keywords: Calf feeding practices, calf health, calf mortality, colostrum intake, dairy farm management, farm sustainability.

Yönetim Uygulamalarının Süt Çiftliklerinde Buzagı Ölüm Oranları Üzerindeki Etkisi: Türkiye'nin Gaziantep Bölgesinde Bir Çalısma

ÖZ

Amaç: Bu çalışma, Türkiye'nin Güneydoğu Anadolu Bölgesi olan TRC1 bölgesinde buzağı yetiştirme uygulamalarını ve bunların buzağı ölümleri üzerine etkilerini değerlendirmektedir. Bu çalışma çiftlikleri düşük, orta ve yüksek ölüm oranlarına göre sınıflandırarak buzağıların hayatta kalmasını etkileyen temel faktörleri tanımlamakta ayrıca buzağı sağlığını iyileştirmeye, ölümleri azaltmaya ve süt çiftliği sürdürülebilirliğini geliştirmeye yönelik bilgiler sunmaktadır.

Materyal ve Metot: Araştırma, Türkiye'nin yaklaşık 388.000 süt sığırına ev sahipliği yapan TRC1 bölgesinde gerçekleştirilmiştir. Veriler 2019-2020 üretim döneminde 145 süt çiftliğinden tabakalı tesadüfi örnekleme yöntemi ile toplanmıştır. Örneklenen çiftlikler, K-ortalama kümeleme yöntemi kullanılarak ölüm oranlarına göre grublandırılmıştır. İstatistiksel testler (ANOVA, Kruskal-Wallis, t-testi, Mann-Whitney U testi) ve çoklu regresyon analizi ile kolostrum alımı, süt besleme sıklığı ve sütten kesim yaşı gibi buzağı ölümleri üzerine etkili faktörler değerlendirilmiştir.

Bulgular: Buzağı yetiştirme uygulamalarında önemli farklılıklar gözlenmiştir. Ortalama ilk kolostrum alımı 2,64 litre, ikinci beslemede ise geniş aralıklarda 3,15 litre olarak gerçekleşmiştir. Günde 2,08 kez süt beslemesi yapılmış ve sütten kesim yaşları 30-180 gün arasında olduğu tespit edilmiştir. Ölüm oranının düşük olduğu çiftliklerde daha yüksek kolostrum alımı ve tutarlı beslenme programları gerçekleştirilmiştir. Regresyon analizi ile kolostrum alımını, süt besleme sıklığını ve sütten kesme yaşının buzağı ölümlerinin önemli belirleyicileri olduğu tespit edilmiştir.

Sonuç: Bu çalışma buzağılarda erken dönem yönetim uygulamalarının ölüm oranları üzerindeki önemini vurgulamaktadır. Kolostrum alımının iyileştirilmesi, süt besleme sıklığı ve uygun sütten kesme yaşının tespiti buzağıların hayatta kalmasını ve çiftliklerin kârlılığını arttırarak hayvancılık işletmelerine değerli bir katkı sağlayacaktır.

Anahtar Kelime: Buzağı besleme uygulamaları, buzağı sağlığı, buzağı ölümü, kolostrum alımı, süt çiftliği yönetimi, çiftlik sürdürülebilirliği

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INTRODUCTION

Calf mortality presents a significant challenge for dairy farmers, directly influencing herd productivity, profitability, and the future health of the farm. The early life stages of a calf are especially critical; mortality during this period results in immediate economic losses and compromises long-term herd performance, as calves represent the next generation of cattle whose successful development is essential for the farm's sustainability (Avcioğlu et al., 2024; Hoischen-Taubner et al., 2021; Lancaster & Larson, 2022; Lovarelli et al., 2020; Pulina et al., 2021). Key management practices—such as ensuring adequate colostrum intake, optimizing milk feeding frequency, determining the right weaning age, and providing consistent access to clean water and starter—play pivotal roles in improving calf survival and promoting healthy growth (Lorenz, 2021; Machado & Ballou, 2022; Shiasi Sardoabi et al., 2021). Effective early-life interventions, including precise feeding protocols and environmental management, are vital in reducing mortality rates and enhancing overall farm efficiency (Hyde et al., 2022; Lancaster & Larson, 2022; Machado & Ballou, 2022). Understanding the impact of these practices during the vulnerable calf-rearing phase is crucial for improving the economic viability and resilience of dairy farming operations. Previous studies have highlighted the importance of factors like colostrum management, feeding routines, and housing conditions in reducing calf mortality (Barry et al., 2020; Johnsen et al., 2021; Machado & Ballou, 2022); however, there remains a need to systematically analyse how these variables contribute to mortality outcomes across different farm settings. By identifying management practices associated with high, medium or low mortality rates, dairy farmers can adopt evidence-based interventions to reduce losses and improve herd performance.

Considering the food crisis that began due to COVID-19 and has continued to escalate recently (Khan et al., 2020; Rahimi et al., 2022), calf losses on farms pose a significant risk worldwide (Hashem et al., 2020). In terms of feeding, treatment, and labor costs in calf rearing, each calf lost incurs substantial damage to the economy (Dubrovsky et al., 2020; Han et al., 2020). However, calf losses also have serious implications for the sustainability of farms and the dairy industry (Avcioğlu et al., 2024; Hoischen-Taubner et al., 2021; Vaarst et al., 2020). Calf losses are most commonly observed during the milk-feeding period (Machado & Ballou, 2022; Schild et al., 2020). Therefore, management practices related to calf care, feeding management, colostrum management, and the prevention of digestive and respiratory diseases are crucial (Lorenz, 2021; Machado & Ballou, 2022; Shiasi Sardoabi et al., 2021). Studies on calf rearing demonstrate that management practices significantly impact calf losses (Hayer et al., 2021; Kantwa et al., 2023). A recent study indicated that 54% of animals that died in the United Kingdom over seven years were less than 24 months of age. Moreover, calves under the age of three months accounted for 25% of the total death rate in the country (Hyde et al., 2020). Additionally, research conducted in Türkiye found the calf mortality rate on a specific farm to be 13.3% (Küçükoflaz & Sarıözkan, 2023). However, for calves aged 2 to 3 months, the acceptable mortality rate should not exceed 2% (Cornell, 2003). These losses clearly highlight the impact of management practices on calf rearing.

Another study investigated the effect of the time between birth and the first colostrum given on mortality rates. It reported that administering colostrum to calves immediately after birth significantly reduced mortality (Barry et al., 2019). Additionally, the amount of colostrum given right after birth has a significant effect on mortality rates (Lombard et al., 2020). A study on daily milk allowance in calves reported that feeding frequency, as a management practice, was not significant (Jensen et al., 2020). In another study conducted in Portugal, calf losses due to diarrhea, respiratory problems, and sudden death were reported at 78.8%, 60.7%, and 82.1%, respectively (Santos et al., 2019). One contributing factor to calf deaths from diarrhea during the milk-feeding period is the temperature of the milk provided (Schinwald et al., 2022). Ultimately, calves in dairy farms directly affect sustainability and profitability and constitute the future of the farm (Avcioğlu et al., 2024; Hoischen-Taubner et al., 2021; Lancaster & Larson, 2022; Lovarelli et al., 2020; Pulina et al., 2021). The accuracy of management practices in calf rearing minimizes mortality and enables calves to become healthy and highly productive animals in the future (Schnyder et al., 2019). This study aimed to investigate the impact of various calf-rearing practices on calf mortality rates. Through a robust methodological approach, the research classified farms into low, medium, and high mortality rate groups, allowing for a comprehensive comparison of management practices across the Gaziantep region (TRC1) of Turkiye. By identifying the critical factors contributing to calf mortality, this study offers actionable insights that can improve calf health, reduce mortality, and enhance overall dairy farm efficiency. The findings provide valuable guidance for producers seeking to optimize their calf-rearing strategies and strengthen the long-term sustainability of their operations.

MATERIAL and METHODS

The research was carried out in the TRC1 region, which is recognized as the largest agricultural production zone in Southeastern Anatolia, Türkiye (Figures 1 and 2). This region is home to approximately 388,000 cattle, representing about 3% of the total dairy cattle population in Türkiye (TUİK, 2020). Data for the study were gathered through surveys conducted during the 2019-2020 production cycle. Additionally, information from prior research and records obtained from various institutions and organizations was incorporated. The study's methodologies are divided into three main categories: (i) data collection for research, (ii) classification of calf mortality rates across dairy farms, and (iii) statistical analyses.

Figure 1: Location of the research area within Türkiye and in a global context

Figure 1: Araştırma alanının Türkiye ve küresel ölçekteki konumu

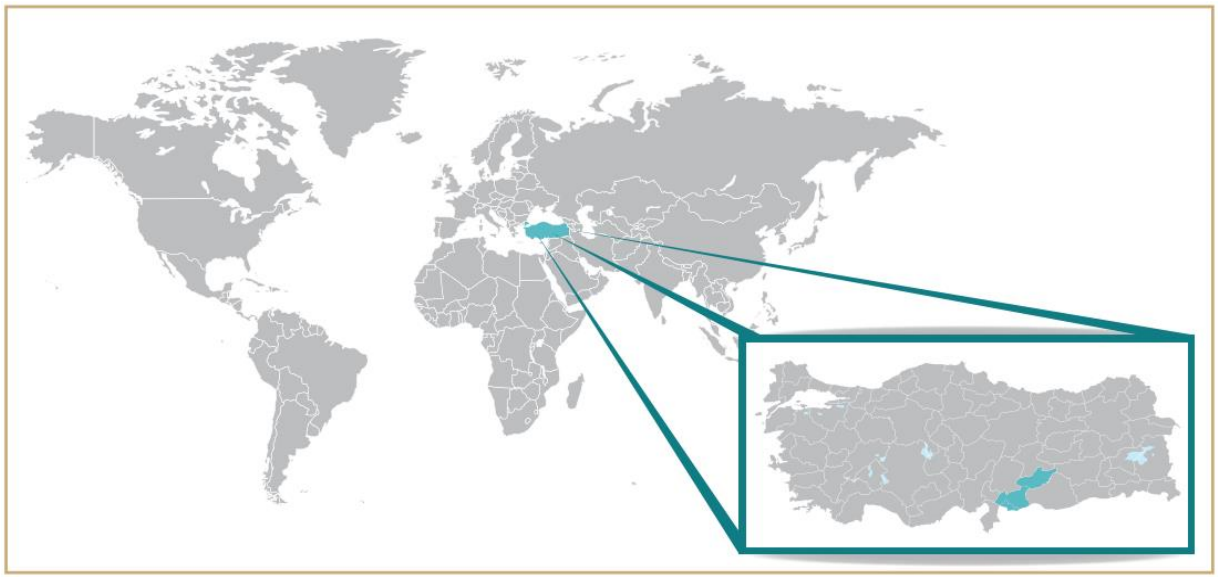

Figure 2: Map of the research area (Gaziantep, Kilis, and Adıyaman).

Figure 2: Araştırma alanının haritası (Gaziantep, Kilis ve Adıyaman).

Collecting Research Data

A stratified random sampling technique, known as the Neyman method, was employed to determine the number of dairy farms to be surveyed in the study area. The selection of farms was based on the number of animals owned, which served as the primary sampling criterion. Initially, a total of 791 farms were identified as the sampling frame. To ensure statistical reliability, the sampling process adhered to a 5% margin of error and a 99% confidence level. Further details about the surveyed farms can be found in Tables 1 and 2.

Table 1. Stratified allocation of dairy farms for the survey.

Tablo 1. *Anket için süt çiftliklerinin tabakalı dağılımı*

Stratums (Animal no)	Population	Mean	Standard Error	Sample Size
First stratum (01 - 30)	370	23.69	2.87	68
Second stratum (31 - 100)	307	47.06	17.30	56
Second stratum (101 – 101+)	114	305.18	219.11	21
Total	791	73.33	127.15	145

Based on the sampling formula provided by Yamane (2001), it was determined that 145 dairy farms should be surveyed:

$$n = \frac{(\sum N_h S_h)^2}{N^2 D^2 + \sum N_h S_h^2}$$

In this equation, n represents the number of farms to be surveyed, N stands for the total population of farms, N_h is the number of farms in each stratum, S_h denotes the standard deviation within each stratum, and D indicates the allowable margin of error.

To allocate the sample size proportionally across strata, the standard deviation of each stratum was used as the basis, calculated as follows:

$$n_h = \frac{N_h S_h}{\sum N_h S_h} \times n$$

In this formula, n refers to the optimal sample size, N_h represents the number of samples within stratum h, N_h indicates the total number of farms in stratum h, and S_h stands for the standard deviation within stratum h.

Table 2. Provincial distribution of surveyed dairy farming enterprises.

Tablo 2. *Ankete katılan süt hayvancılığı işletmelerinin illere göre dağılımı*

Stratums (Animal no)	Gaziantep	Adıyaman	Kilis	TRC1
First stratum (01 - 30)	51	16	1	68
Second stratum (31 - 100)	34	18	4	56
Second stratum (101 – 101+)	15	6	0	21
Total	100	40	5	145

Grouping of Farms

The dairy farms included in the study were clustered based on calf mortality rates, and the resulting clusters were labelled as low, medium, and high mortality rates farms according to the average mortality rate within each cluster, ordered from lowest to highest. This clustering was performed using K-means analysis, and the formula used in this method is described as follows (MacQueen, 1967):

$$J = \sum_{j=1}^{k} \sum_{i=1}^{n} \|xi\ (j) - cj\|2$$

In this equation, J represents the objective function, k denotes the number of clusters, n indicates the total number of observations, xi is the ith observation, and cj signifies the centroid of the Jth cluster. The term ‖xi (j) - cj‖ denotes the distance function. The efficacy of the K-means clustering method was assessed using the Error Sum of Squares (SSE), defined as follows (Tan et al., 2006):

$$SSE = \sum_{i=1}^{K} \sum_{x \in C_i} dist^2 \langle m_i, x \rangle$$

In this context, “dist” represents the standard Euclidean distance, where x refers to a dairy farm situated within a cluster C_i, and m_i denotes the centroid of a cluster C_i. The K-means clustering analysis described operates based on the Euclidean distance criterion and continues iterating until no observations migrate between clusters. The formula for the Euclidean distance is given by:

$$d(x_i, x_j) = \sqrt{\sum_{k=1}^{p} (x_{ik} - x_{jk})^2}$$

Statistical analyses

A one-way ANOVA was conducted to compare clusters of farms based on mortality rates, using the Benjamini–Hochberg method to adjust for multiple comparisons (Benjamini & Hochberg, 1995). The statistical model applied is as follows:

$$Yi = \mu + Grpi + Cov + ei$$

where Yi represents the dependent variable, μ is the overall mean, Grpi is the group effect based on mortality rates, Cov is the covariate, and ei is the random error term.

For non-normally distributed variables, the Kruskal-Wallis H test was applied, and the Chi-Square test was used for categorical variables. Continuous variables that met normality assumptions were compared using the t-test, while the Mann-Whitney U test was used for nominal and ordinal data.

A multiple regression analysis was performed to examine the combined influence of various factors on calf mortality rates. The dependent variable was the calf mortality rate, and the independent variables included:

X1: Amount of colostrum given at the first feeding

X2: Amount of colostrum given at the second feeding

X3: Daily milk feeding frequency

X4: Daily total milk feeding amount

X5: Age at weaning

X6: Age at first water intake

X7: Age at first starter feeding

The regression model is represented as:

$$Y = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 4X4 + \beta 5X5 + \beta 6X6 + \beta 57X7 + \varepsilon$$

where Y is the calf mortality rate, β0 is the intercept, β1, β2, ..., β7 are the regression coefficients, and ε is the error term.

Correlation coefficients were computed to explore the relationships among variables. The Pearson correlation coefficient is calculated as follows:

$$r = \frac{\Sigma(Xi - X)(Yi - Y)}{\sqrt{\Sigma(Xi - \overline{X})^2 . \Sigma(Yi - \overline{Y})^2}}$$

where r is the correlation coefficient, Xi and Yi are individual data points, and $\overline{X}$ and $\overline{Y}$ are the means of the respective variables.

All statistical analyses were performed using IBM SPSS Statistics Version 20 (SPSS Inc., Chicago, IL, USA).

RESULTS

The research findings have been presented under three subheadings: (i) General characteristics and descriptive statistics of dairy farms, (ii) comparative analysis of clusters for calf mortality rate, and (iii) regression analysis and correlation results.

General Characteristics and Descriptive Statistics of Dairy Farms

Table 3 provides information on the general characteristics and descriptive statistics of dairy farms in the studied region. The average first colostrum intake (within 2 hours of birth) and second colostrum intake (within 12 hours of birth) for calves were 2.64 liters and 3.15 liters, respectively, with ranges of 1–10 liters for the first intake and 1–15 liters for the second intake. Milk feeding frequency averaged 2.08 times per day, with a range of 1 to 5 feedings, while the average duration of milk intake was 4.77 days, ranging from 2 to 10 days. The average weaning age was 85.21 days, with values ranging from 30 to 180 days. Water and starter feed intake began at average ages of 18.79 and 25.59 days, respectively, with ranges from 1 to 120 days for both. The average calf mortality rate was 7.81%, with a range from 1% to 30%.

Table 3. General characteristics and descriptive statistics of dairy farms.

Table 3. Süt işletmelerinin genel özellikleri ve tanımlayıcı istatistikleri.

Variables	Min	Max	Mean±Std.Dev.
1st Colostrum (Lt)	1	10	2.64±2.06
2nd Colostrum (Lt)	1	15	3.15±2.47
Milk feed frequancy (d)	1	5	2.08±0.48
Milk intake (d)	2	10	4.77±1.02
Weaning age (d)	30	180	85.21±29.39
1st Water intake (age/d)	1	120	18.79±23.19
1st Starter intake (age/d)	1	120	25.59±29.47
Mortality (%)	1	30	7.81±7.30

Table 4 provides insights into the general characteristics of dairy farms clustered by calf mortality rates—low, medium, and high. The mean calf mortality rates for the low, medium, and high mortality clusters were 3.25%, 5.05%, and 8.99%, respectively. In terms of colostrum intake, no statistically significant differences were observed between the clusters, although the medium mortality group had slightly higher means for both first and second colostrum intakes. Milk feeding frequency was consistent across all groups, averaging 2.00 times per day, while milk intake duration showed slight variation, with the medium mortality group having the highest mean duration. Regarding weaning age, the medium mortality group weaned calves earlier, with a mean age of 70.00 days, compared to the other clusters. For water and starter feed intake, the high mortality group exhibited much greater variability, particularly in the age at which starter feed intake began.

Table 4. General characteristics of dairy farms clustered by calf mortality rates.

Table 4. Buzağı ölüm oranlarına göre gruplanan süt çiftliklerinin genel özellikleri.

	Low mortality (N:18)			Medium mortality (N:17)			High mortality (N:110)		
Variables	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**
Mortality (%)	1	8	3.25±2.26^{a}	2	15	5.05±4.04^{ab}	1	30	8.99±7.83^{b}
1st Colostrum (Lt)	1	6	2.38±1.30	2	10	3.82±3.08	1	10	2.50±1.94
2nd Colostrum (Lt)	2	10	3.33±2.30	2	10	3.09±2.33	1	15	3.13±2.54
Milk feed frequancy (d)	2	2	2.00±0.00	2	2	2.00±0.00	1	5	2.11±0.55
Milk intake (d)	3	6	4.83±0.83	3.5	7	5.32±0.90	2	10	4.67±1.05
Weaning age (d)	60	180	87.50±35.96	60	90	70.00±11.62	30	180	87.15±29.73
1st Water intake (age/d)	1	21	9.67±7.39	1	15	5.91±3.70	1	120	22.278±25.48
1st Starter intake (age/d)	3	30	12.50±9.05^{ab}	1	30	8.00±8.19^{a}	1	120	30.46±32.04^{b}

a.b.c Values with different superscript letters within a row are statistically significant at the 0.05 level

Comparative Analysis of Clusters for Calf Mortality Rate

Table 5 provides a cluster-based analysis of factors affecting calf mortality rates, examining differences between the low, medium, and high mortality clusters. The analysis reveals that certain management practices show statistically significant variations across clusters, which may contribute to the observed differences in calf mortality rates.

The comparisons between clusters for first and second colostrum intake indicate no statistically significant differences (P>0.05). Milk feeding frequency also displayed no significant variation across clusters, as the feeding frequencies were consistently similar, with negligible mean differences.

For milk intake duration, slight differences were noted between clusters, though none reached statistical significance (P>0.05). However, weaning age demonstrated some variability, with the low mortality group averaging 17.50 days later than the medium mortality group, though this difference was not statistically significant (P>0.05). The age of first water intake also showed a greater difference between clusters, particularly between the low and high mortality groups, although these differences were not statistically significant.

The timing of the first starter intake displayed a significant difference (P<0.05) between the medium and high mortality groups, with the high mortality cluster showing a considerably later starter intake (22.46 days on average) compared to the medium mortality cluster (P<0.05).

Regression Analysis and Correlation Results

Table 6 presents the results of a regression analysis aimed at identifying factors influencing calf mortality rates. The analysis showed no statistically significant results, as indicated by the wide confidence interval (-8.53 to 15.78). For the variables related to colostrum intake, the coefficients for first and second colostrum volumes were -0.25 and -0.03, respectively, both of which were not statistically significant (P>0.05).

Milk feeding frequency (days) showed a positive coefficient of 0.71, but this was not statistically significant (P = 0.68). Similarly, milk intake per day had a negligible effect on mortality rates (β = -0.06, P = 0.94), with no significant association between milk intake and calf mortality.

Weaning age showed a positive association with calf mortality, though this was not statistically significant (P = 0.18). While the result was not significant, it suggests that a higher weaning age may be marginally associated with increased calf mortality. The variables for first water intake age (P = 0.28) and first starter intake age (P = 0.12) also demonstrated non-significant relationships with calf mortality rates. Overall, none of the variables in this model displayed statistically significant associations with calf mortality rates (P>0.05).

Table 5. Cluster-based analysis of factors affecting calf mortality rates.

Table 5. Buzağı ölüm oranlarını etkileyen faktörlerin kümeleme temelli analizi.

Variables	Cluster (I)	Cluster (J)	Mean Diff. (I-J)	Std. Err.	P-value *	95% CI	
						Low. Bound	Upp. Bound
1st Colostrum (Lt)	Low	Medium	-1.44	0.85	0.25	-3.51	0.62
		High	-0.13	0.63	1.00	-1.67	1.42
	Medium	Low	1.44	0.85	0.25	-0.62	3.51
		High	1.32	0.66	0.14	-0.28	2.92
	High	Low	0.13	0.63	1.00	-1.42	1.67
		Medium	-1.32	0.66	0.14	-2.92	0.28
2nd Colostrum (Lt)	Low	Medium	0.24	1.04	0.99	-2.29	2.77
		High	0.21	0.78	0.99	-1.68	2.10
	Medium	Low	-0.24	1.04	0.99	-2.77	2.29
		High	-0.03	0.81	1.00	-1.99	1.93
	High	Low	-0.21	0.78	0.99	-2.10	1.68
		Medium	0.03	0.81	1.00	-1.93	1.99
Milk feed frequancy (d)	Low	Medium	0.00	0.20	1.00	-0.49	0.49
		High	-0.11	0.15	0.84	-0.47	0.25
	Medium	Low	0.00	0.20	1.00	-0.49	0.49
		High	-0.11	0.16	0.85	-0.49	0.27
	High	Low	0.11	0.15	0.84	-0.25	0.47
		Medium	0.11	0.16	0.85	-0.27	0.49
Milk intake (d)	Low	Medium	-0.48	0.42	0.58	-1.51	0.54
		High	0.16	0.32	0.94	-0.61	0.93
	Medium	Low	0.48	0.42	0.58	-0.54	1.51
		High	0.64	0.33	0.15	-0.15	1.44
	High	Low	-0.16	0.32	0.94	-0.93	0.61
		Medium	-0.64	0.33	0.15	-1.44	0.15
Weaning age (d)	Low	Medium	17.50	12.18	0.39	-12.10	47.10
		High	0.35	9.10	1.00	-21.76	22.46
	Medium	Low	-17.50	12.18	0.39	-47.10	12.10
		High	-17.15	9.45	0.20	-40.11	5.80
	High	Low	-0.35	9.10	1.00	-22.46	21.76
		Medium	17.15	9.45	0.20	-5.80	40.11
1st Water intake (age/d)	Low	Medium	3.76	9.42	0.97	-19.14	26.65
		High	-12.61	7.04	0.21	-29.71	4.49
	Medium	Low	-3.76	9.42	0.97	-26.65	19.14
		High	-16.37	7.31	0.08	-34.12	1.39
	High	Low	12.61	7.04	0.21	-4.49	29.71
		Medium	16.37	7.31	0.08	-1.39	34.12
1st Starter intake (age/d)	Low	Medium	4.50	11.88	0.97	-24.36	33.36
		High	-17.96	8.87	0.13	-39.52	3.60
	Medium	Low	-4.50	11.88	0.97	-33.36	24.36
		High	-22.46*	9.21	0.05	-44.84	-0.08
	High	Low	17.96	8.87	0.13	-3.60	39.52
		Medium	22.46*	9.21	0.05	0.08	44.84

* The mean difference is significant at the 0.05 level.

Table 6. Regression analysis of factors influencing calf mortality rates.

Table 6. Buzağı ölüm oranlarını etkileyen faktörlerin regresyon analizi.

Variables	Coefficients		T-values	P-values *	95% CI	
	β	Std. Error			Lower	Upper
Intercept	3.63	6.12	0.59	0.55	-8.53	15.78
1st Colostrum (Lt)	-0.25	0.44	-0.57	0.57	-1.12	0.62
2nd Colostrum (Lt)	-0.03	0.37	-0.08	0.94	-0.77	0.71
Milk feed frequancy (d)	0.71	1.73	0.41	0.68	-2.72	4.15
Milk intake (d)	-0.06	0.78	-0.08	0.94	-1.61	1.49
Weaning age (d)	0.04	0.03	1.35	0.18	-0.02	0.09
1st Water intake (age/d)	-0.05	0.04	-1.09	0.28	-0.13	0.04
1st Starter intake (age/d)	0.05	0.03	1.59	0.12	-0.01	0.12

* The mean difference is significant at the 0.05 level.

The Pearson correlation matrix presented in Table 7 examines the relationships among various factors influencing calf mortality rates. Notably, the mortality rate showed a positive correlation with both weaning age (r = 0.18) and the age at first starter intake (r = 0.19). A moderate correlation was observed between first and second colostrum intake (r = 0.47), indicating a positive association between these variables. Additionally, a strong positive correlation was found between the age at first water intake and the age at first starter intake (r = 0.64), suggesting a concurrent progression in dietary development stages.

The correlations between milk feeding frequency and other factors, such as milk intake (r = -0.07) and weaning age (r = 0.09), were generally weak, indicating minimal direct associations. The correlation between milk intake and weaning age was negative (r = -0.27).

Overall, the matrix reveals a complex interplay of factors, where dietary transitions—represented by water and starter intake—are more strongly associated with calf development timelines than with direct influences on mortality rates.

Table 7. Pearson correlation matrix of factors influencing calf mortality rates.

Table 7. Buzağı ölüm oranlarını etkileyen faktörlerin Pearson korelasyon matrisi.

Pearson Correlations	Mortality (%)	1st Colostrum (Lt)	2nd Colostrum (Lt)	Milk feed frequancy (d)	Milk intake (d)	Weaning age (d)	1st Water intake (age/d)	1st Starter intake (age/d)
Mortality (%)	1.00	-0.09	-0.01	0.08	-0.06	0.18	0.05	0.19
1st Colostrum (Lt)	-0.09	1.00	0.47	-0.06	0.05	0.01	-0.17	-0.17
2nd Colostrum (Lt)	-0.01	0.47	1.00	0.30	-0.01	0.06	-0.05	0.00
Milk feed frequancy (d)	0.08	-0.06	0.30	1.00	-0.07	0.09	-0.06	0.04
Milk intake (d)	-0.06	0.05	-0.01	-0.07	1.00	-0.27	-0.24	-0.19
Weaning age (d)	0.18	0.01	0.06	0.09	-0.27	1.00	0.32	0.34
1st Water intake (age/d)	0.05	-0.17	-0.05	-0.06	-0.24	0.32	1.00	0.64
1st Starter intake (age/d)	0.19	-0.17	0.00	0.04	-0.19	0.34	0.64	1.00

DISCUSSION and CONCLUSION

The findings of this study highlight the complex relationship between management practices and calf mortality rates in dairy farms across the Gaziantep region of Türkiye. Given the significance of early-life calf management in determining long-term productivity and sustainability in dairy farming, it is critical to explore how specific practices can mitigate mortality risk.

The statistics revealed notable variability in several key management areas, which may significantly influence calf health and mortality outcomes. This variability suggests differing approaches to colostrum feeding that could affect passive immunity transfer. However, despite the established importance of colostrum in promoting calf health, our analysis revealed no statistically significant differences in mortality outcomes based on colostrum intake among the various clusters in the studied region. It is important to note that, in all three enterprise groups (low, medium, and high mortality), the first and second colostrum intakes were within the recommended ranges for calf health, suggesting that colostrum intake alone is unlikely to explain the variation in mortality rates. This finding aligns with the previous studies, which suggested that while timely and adequate colostrum feeding is essential, other factors may also contribute to the observed mortality rates (Abuelo et al., 2021; Abuelo et al., 2019; Barry et al., 2019). Although calves receiving higher volumes of colostrum demonstrated improved passive immunity, the absence of a significant impact on mortality in these studies suggests that additional interventions may be necessary to enhance calf survival. These interventions could include vaccination against septicemia and pneumonia, two of the primary causes of calf mortality. While vaccination is an important preventative measure, it is clear that other factors, beyond colostrum intake and vaccination, contribute to mortality outcomes. Some of these factors may include environmental conditions such as temperature fluctuations, humidity, and the cleanliness of the barns. Previous research has shown that poor hygiene and inadequate environmental conditions can exacerbate disease transmission and increase the likelihood of calf infections (Khan et al., 2020). Furthermore, stress from handling, transportation, or overcrowding could compromise the immune system, making calves more susceptible to disease, even with adequate vaccination.

The data indicated a uniform average milk feeding frequency across clusters, suggesting that other management aspects, such as the quality of milk and the feeding environment, may play a more critical role in calf health. The lack of significant variation in milk intake duration further underscores the need for dairy producers to focus on the broader context of feeding management, including the nutritional quality of milk and adherence to feeding protocols. Previous studies have pointed out that while feeding frequency might not directly correlate with mortality, it is essential for optimizing growth and development during this critical period (Johnson et al., 2021; Mohammed et al., 2020; Zhao et al., 2021). This suggests a potential area for future research to explore the qualitative aspects of feeding alongside quantitative measures.

Weaning age emerged as a noteworthy factor, particularly in the comparative cluster analysis. Although the differences were not statistically significant, earlier weaning in the medium mortality group suggests the need for a more cautious approach to weaning practices. The data revealed differences in weaning strategies across farms, highlighting the potential for earlier weaning practices to contribute to calf stress and health challenges. Previous literature has highlighted the stress associated with early weaning, which can negatively affect calf health and subsequent productivity (Bittar et al., 2020; Mikuš & Mikuš, 2020; Nicolao et al., 2022; Wenker et al., 2022). The data also suggest that calves weaned earlier may have higher mortality rates, although these correlations were relatively weak. Future investigations could benefit from a more in-depth examination of the physiological and behavioral implications of weaning strategies, potentially guiding farmers toward practices that support healthier transitions.

Our findings also suggest a possible link between the timing of water and starter feed intake and calf mortality rates. The positive correlation between the age of first starter intake and mortality points to the potential benefits of earlier introductions of solid feed, which could foster better rumen development and overall health, consistent with previous studies (Arshad et al., 2021; Lorenz, 2021; Palczynski et al., 2020; Shiasi Sardoabi et al., 2021). Additionally, the findings emphasize differences in early feeding practices across farms, which could influence hydration, rumen development, and overall calf growth. The wide variability in the timing of starter intake across farms suggests an opportunity for standardizing feeding protocols to enhance consistency in calf

growth and health outcomes. This could be crucial, as delayed starter intake might be associated with higher mortality rates, potentially impacting rumen development and overall calf growth.

This study stresses the necessity for dairy farmers to adopt a comprehensive approach to calf management that integrates feeding practices, weaning age strategies, and environmental considerations. Implementing evidence-based interventions, such as standardizing colostrum management and optimizing weaning practices, may significantly reduce calf mortality rates. Additionally, educating farmers about the critical role of timely starter feed introduction and access to clean water could further bolster calf health. While this study provides valuable insights, it is essential to acknowledge its limitations, including the relatively small sample size and the potential for unmeasured confounding factors. Future research should aim to include a larger and more diverse set of dairy farms to enhance the generalizability of the findings. Longitudinal studies could also provide a deeper understanding of the causal relationships between management practices and calf mortality outcomes.

In conclusion, the interplay of management practices significantly influences calf mortality rates in dairy farming. By focusing on standardized, evidence-based practices, dairy producers can enhance calf survival, improve overall herd health, and contribute to the sustainability of dairy farming operations. The findings from this study serve as a foundation for further research and practical applications aimed at minimizing calf losses and optimizing dairy farm productivity.

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A Study on Milk Quality Characteristics of Simmental (Fleckvieh) Cows Reared Karacabey District of Bursa Province

ABSTRACT

Objective: The aim of this study was to determine the milk quality characteristics of Simmental (SIM) (Fleckvieh) cattle originating from Austria and Germany.

Materials and Methods: a total of 1928 milk samples taken during morning milking in different seasons between 2018 and 2022 from cattle raised in a disease-free private dairy farm in Karacabey District of Bursa Province were evaluated. Milk quality characteristics include fat (MFC), protein (MPC), lactose (MLC), total dry matter content (TDMC), milk urea nitrogen (MUN) amount and Log10SCC were determined.

Results: Parity effects on MLC (P<0.01) and TDMC (P<0.01), calving year effects on all traits (P<0.05) except MFC, lactation month effects on MPC (P<0.01), MLC (P<0.01), MUN (P<0.01) and Log10SCC (P<0.01), and calving season effects on MPC (P<0.01), MLC (P<0.01) and Log10SCC (P<0.01) were determined to be statistically significant. The averages of MFC, MPC, MLC, TDMC, MUN and Log10SCC were 3.83±0.02%, 3.42±0.01%, 4.83±0.01%, 12.78±0.03%, 16.49±0.09 mg/dl and 4.646±0.014 (44.274 cells/mL), respectively.

Conclusion: Although the MFC of SIM cattle originating from Austria and Germany was found to be slightly lower than those reported in the literature, the low SCC content indicates that the prevalence of mastitis in this genotype is quite low

Keywords: Dual purpose cattle, mik fat content, milk protein content, milk urea nitrogen, somatic cell count

Bursa Ili Karacabey Ilçesinde Yetistirilen Simmental (Fleckvieh) Irkı Sıgırların Süt Kalite Özellikleri Üzerine Bir Arastırma

ÖZ

Amaç: Bu çalışmanın amacı Avusturya ve Almanya kökenli Simental (SIM) (Fleckvieh) sığırların süt kalite özelliklerinin belirlemesidir.

Materyal ve Method: Bursa İli Karacabey İlçesi'nde hastalıktan ari özel bir işletmede yetiştirilen sığırlarından 2018-2023 yılları arasında farklı mevsimlerde sabah sağımında alınan toplam 1928 süt örneği değerlendirilmiştir. Süt kalite özellikleri olarak yağ oranı (SYO), protein oranı (SPO), laktoz oranı (SLO), toplam kuru madde oranı (TKMO), süt üre azot miktarı (SÜA) ve Log10SHS özellikleri belirlenmiştir.

Bulgular: SLO (P<0.01) ve TKMO (P<0.01) üzerine parite etkisi, SYO dışındaki tüm özellikler üzerine buzağılama yılı etkisi (P<0.05), SPO (P<0.01), SLO (P<0.01), SÜA (P<0.01) ve Log10SHS (P<0.01) üzerine laktasyon ayı etkisi ve SPO (P<0.01), SLO (P<0.01) ve Log10SHS (P<0.01) üzerine de buzağılama mevsimi etkilerinin istatistiksel olarak önemli olduğu tespit edilmiştir. SYO, SPO, SLO, TKMO, SÜA ve Log10SHS özelliklerine ait ortalamalar sırasıyla %3.83±0.02%, 3.42±0.01%, 4.83±0.01%, 12.78±0.03%, 16.49±0.09 mg/dl ve 4.646±0.014 (44.274 hücre/ml) dir.

Sonuç: Avusturya ve Almanya kökenli SIM sığırların SYO'su literatürde bildirilenlerden biraz daha düşük bulunmasına rağmen, SHS içeriğinin düşük olması bu genotipte mastitis prevalansının oldukça düşük olduğunu göstermektedir

Anahtar Kelime: Kombine verimli sığır, süt yağı oranı, süt protein oranı, süt üre azotu, somatik hücre sayısı

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#This article is summarized from the first author's doctoral thesis.

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INTRODUCTION

Milk, which is the basic nutrient for the growth and development of mammals in the first period of their lives, also contains animal protein, fat, lactose, mineral substances, etc., which are the basic nutrients needed for adequate and balanced nutrition of humans. Milk yield and composition in cattle, which provide a large portion of the world's milk production, are affected primarily by genetics and also to many environmental factors (Koç, 2007a; Özkan, 2017).

Milk quality in cattle is generally examined in two main groups: the composition of milk (fat, protein, lactose, minerals, non-fat solids, total dry matter, casein, etc.) and hygienic properties such as total bacterial count, somatic cell number and antibiotic residue (Koç and Öner, 2023). The composition of milk varies according to many factors such as the ration composition given to the animal, the milk production level of the cow, the order and period of lactation and the hygiene quality of milk, the hygienic quality of milk is an indicator of the cow's udder health, milking hygiene and storage and transportation conditions in the process until the milk is processed into final products (Koç and Öner, 2023). The total bacterial count is significantly affected by udder health, milking hygiene, milking system and storage conditions of milk until processing, somatic cell count (SCC), which causes significant changes in milk yield and composition, is a milk quality criterion that provides important information about udder health and raw milk quality. In bovine milk, a level of 200,000 cells/ml is accepted as the threshold value, and if the SCC level is above this value, it is accepted that the udder of the cow from which that milk is milked has mastitis (Dohoo and Leslie, 1991).

In studies conducted on the milk components of Simmental (SIM) breed, which is a dual purpose breed, the milk fat content (MFC, %) of the breed is between 3.32% and 4.32%, the milk protein content (MPC, %) is between 3.02% and 3.9%, the milk lactose content (MLC, %) is between 4.19% and 4.96%, total dry matter ratio (TDMC, %) is between 11.23% and 12.6%, SCC is between 15.848 and 128.825 cells/mL, milk urea nitrogen (MUN) is between 12.28 mg/dL and 25.75 mg/dL (Akbulut, 1998; Bendelja et al., 2011; Budimir et al., 2011; Litwińczuk et al., 2011; Pantelić et al., 2013; Önal et al., 2014; Cioch et al., 2015; Cziszter et al., 2016; Nistor et al., 2017; Wei et al., 2021 ; Erdem and Okuyucu, 2023; Franzoi et al., 2023; Koç and Öner, 2023; Buonaiuto et al., 2024;). There are also many studies conducted to determine milk quality characteristics and SCC level in different cattle breeds (Koç, 2006; 2007b; 2011; 2015; Özdede, 2009; Yılmaz, 2010; Kaya et al., 2014; Okuyucu and Erdem, 2017; Koç and Erdem, 2017; Koç and Arı, 2020; Koç and Gürses, 2020).

Although there are many studies on milk yield, fertility, fattening performance and carcass characteristics of Swiss origin SIM cattle, which have been bred in Turkey for many years, it has been emphasized that the number of studies on the milk quality and SCC of the breed is limited (Koç, 2016). On the other hand, it has been noted that the number of studies on the performance of SIM breed (Fleckvieh) of Austrian and German origin, with increased milk yield, which has attracted great attention from breeders in Turkey in recent years, is almost non-existent in our country's conditions. Starting from this point, this study aimed to determine the milk quality characteristics of Austrian and German origin SIM cattle (Fleckvieh).

MATERIAL and METHODS

This study was carried out on SIM breed cattle of Austrian and German origin, raised in a disease-free private farm in Karacabey district of Bursa province, Türkiye. The farm is established on an area of 29700 m2 and the cattle are housed in a barn with a free stall. The shelter has an automatic waterer, automatic hydraulic and chain manure scrapers, a feed through with a lock system, a feed road, cooling fans and shower system, and rubber bedding. Cattle raised in the farm are grouped according to their productivity levels, and Total Mix Ration (TMR) is given three times a day after milking, in the amount and form appropriate to their needs. Lactating cattle are housed in 6 separate paddocks according to their productivity levels, and animals grouped according to milk production levels and lactation periods are milked three times a day. While the roughage used to feed the cattle in the farm is provided from the agricultural lands belonging to the farm and the rented agricultural fields, concentrated feeds and feed additives (premix) are supplied from various feed dealers.

The milk components and SCC level of the breed were determined from a total of 1928 milk samples taken during morning milking in different seasons in the enterprise between 2017 and 2023. Approximately 50 mL of milk samples were taken into sample containers to represent milking, and the samples were kept in the cold chain until analyzed. Milk samples were analyzed using Bentley–Merkim (2021) brand SomaCount FC and

DairySpec FT devices in the milk analysis laboratory operating within the Bursa Provincial Cattle Breeders Association, Türkiye. In the raw milk analysis, the levels of MFC, MPC, MLC, TDMC, MUN and SCC in milk were determined.

Statistical analysis

Statistical analysis of the data was done by using SAS 9.4 package program and the differences between the groups were determined according to the Tukey ($P<0.05$) multiple comparison test results. The cows subject to this study were grouped into 5 groups according to the lactation number, and the cows in the 5th and above lactation numbers were included in the 5+ lactation number. The 5-year group between 2018 and 2022 was taken into account as the calving year, and due to the low number of data in 2017 and 2023, the data for these years were included in the closest year groups. The first 11 lactation months were taken into consideration as lactation months, and lactation months longer than 11 were included in the 11th lactation month group. Four seasonal groups have been accepted as calving seasons, March-May as the 1st season (spring), June-August as the 2nd season (summer), September-November as the 3rd season (autumn) and December-February as the 4th season (winter). The statistical model used in the analysis of the data is as follows:

$$Yijklm = \mu + ai + bj + ck + dl + eijklm$$

Yijklm is the observatipon of MFC, MPC, MLC, TDMC, MUN and Log10SCC,

μ is oveall mean,

ai is calving year effects (i=2018, 2019,. ….. 2022),

bj is calving season effects (j=1 (spring), 2 (summer), 3 (autumn) and 4 (winter)),

ck is lactation number effects (k= 1, 2, 3, 4 and 5+),

dl is lactation month effects (l=1, 2, …… 11+)

eijklm is residual random error.

RESULTS and DISCUSSION

The averages and standard errors of MFC, MPC, MLC, TDMC, MUN, Log10SCC traits of SIM (Fleckvieh) cattle of Austrian and German origin are given in Table 1.

The average MFC was calculated as 3.83±0.02%, and the effects of lactation number, calving year, lactation month and calving season on this trait were all insignificant ($P>0.05$). The MFC average obtained in this study for SIM cattle is similar to Kučević et al. (2005) and Nistor et al. (2014) who reported on the same breed as 3.81±0.09% and 3.82±0.378%, respectively. However, Okuyucu and Erdem (2017), Erdem and Okuyucu (2019), Önal et al. (2021) and Kaygısız and Şahin (2023) reported lower means for SIM breed (3.49%, 3.38%, 3.72±0.03%, and 3.68±0.031% respectively) than the mean obtained in this study. On the other hand, in the literature higher means (between 3.84% and 4.32%) reported for MFC of SIM breed (Akbulut, 1998, Petrović et al., 2006; Pantelić et al., 2008; Bendelja et al., 2011; Nikšić et al., 2011; Pantelić et al., 2013; Pantelić et al., 2014; Cziszter et al., 2016 and Litwińczuk, 2016; Franzoi et al., 2020; Wei et al., 2021; Falta et al., 2023; Koç and Öner, 2023; Vrhel et al., 2024; Buonaiuto et al., 2024;. The means of Budimir et al. (2011) for the first 3 lactation numbers for SIM breed were higher than the mean detected in this study. Budimir et al. (2011)'s means were 3.83±0.03%, 3.84±0.01% and 3.86±0.02%, respectively, and Cioch et al. (2015)'s means were 3.99±0.40%, 4.04±0.49% and 3.85±0.44%, respectively.

It is well known that there is an inverse relationship between milk yield and MFC in cattle, and the MFC decreases due to the increase in milk yield. In this study, it can be said that in this farm, where the SIM breed with increased milk yield was raised, a decrease was observed in the MFC due to the roughage/concentrate ratio in the ration being kept in favor of concentrated feed and the forage particle length being kept a little short in order to meet the nutrients needed for increased milk yield. The fact that the MFC obtained in this study was generally slightly lower than previous studies is due to the differences in management and feeding conditions applied in the farm where this study was conducted, as well as the fact that, except for some studies in recent years, the SIM genotype used in previous studies was reported to be of Swiss origin SIM cattle, which have lower milk yield.

Table 1. Means and standard errors of milk quality characteristics of Simmental (Fleckvieh) cattle
Tablo 1. Simmental (Fleckvieh) sığırlarının süt kalite özelliklerinin ortalamaları ve standart hataları

	MFC (%)		MPC (%)		MLC (%)		TDMC (%)		MUN (mg/dl)		Log_{10}SCC	
Factor	n	$\overline{X} \pm S_{\overline{X}}$	n	$\overline{X} \pm S_{\overline{X}}$	n	$\overline{X} \pm S_{\overline{X}}$	n	$\overline{X} \pm S_{\overline{X}}$	n	$\overline{X} \pm S_{\overline{X}}$	n	$\overline{X} \pm S_{\overline{X}}$
Parity		NS		NS		**		**		NS		NS
1	415	3.85±0.06	418	3.43±0.02	237	4.91±0.02^{a}	236	12.92±0.08^{a}	217	16.64±0.33	400	4.565±0.035
2	493	3.92±0.05	506	3.44±0.02	503	4.85±0.02^{ab}	500	12.79±0.06^{ab}	277	16.59±0.29	492	4.611±0.031
3	375	3.76±0.06	380	3.37±0.02	377	4.81±0.02^{bc}	378	12.73±0.07^{ab}	371	16.62±0.27	357	4.648±0.038
4	312	3.81±0.07	321	3.42±0.02	314	4.81±0.02^{bc}	315	12.69±0.08^{ab}	319	16.04±0.30	300	4.693±0.039
5+	301	3.79±0.07	303	3.39±0.03	301	4.77±0.02^{c}	301	12.67±0.08^{b}	303	16.57±0.32	278	4.703±0.043
Calving year		NS		**		**		*		**		**
2018	234	3.94±0.08	234	3.37±0.03^{ab}	53	4.80±0.05^{ab}	54	13.03±0.17^{a}	--	----	230	4.486±0.050^{a}
2019	227	3.86±0.08	227	3.40±0.03^{a}	224	4.85±0.02^{a}	224	12.98±0.09^{a}	22	17.66±0.83^{a}	219	4.486±0.048^{a}
2020	283	3.85±0.07	287	3.30±0.02^{b}	284	4.84±0.02^{ab}	284	12.86±0.07^{a}	284	16.91±0.25^{a}	276	4.667±0.041^{b}
2021	549	3.75±0.05	571	3.44±0.02^{a}	565	4.89±0.01^{a}	565	12.42±0.05^{b}	571	16.53±0.17^{a}	539	4.711±0.028^{b}
2022	603	3.73±0.05	610	3.52±0.02^{c}	606	4.77±0.01^{b}	603	12.50±0.05^{b}	610	14.87±0.17^{b}	563	4.869±0.028^{c}
Lac. Month		NS		**		**		NS		**		**
1	153	3.91±0.09	159	3.42±0.03^{ad}	147	4.88±0.03^{ab}	146	12.84±0.10	115	15.43±0.41^{ac}	150	4.628±0.051^{ab}
2	210	3.79±0.07	215	3.23±0.03^{b}	187	4.88±0.03^{ab}	185	12.62±0.09	161	16.92±0.36^{b}	207	4.573±0.043^{ab}
3	182	3.73±0.08	186	3.28±0.03^{bc}	169	4.90±0.03^{a}	167	12.60±0.9	162	16.29±0.36^{abc}	175	4.702±0.047^{ab}
4	167	3.79±0.08	170	3.35±0.03^{ac}	164	4.84±0.03^{ab}	162	12.75±0.09	144	17.07±0.38^{b}	166	4.690±0.048^{ab}
5	148	3.85±0.09	150	3.44±0.03^{ad}	149	4.84±0.03^{ab}	149	12.83±0.10	137	17.44±0.39^{b}	141	4.512±0.052^{a}
6	148	3.81±0.09	153	3.41±0.03^{acd}	152	4.78±0.03^{b}	151	12.73±0.09	144	16.75±0.37^{ab}	143	4.546±0.052^{ab}
7	142	3.75±0.09	143	3.42±0.03^{ad}	134	4.83±0.03^{ab}	135	12.72±0.10	132	16.94±0.39^{b}	135	4.529±0.053^{a}
8	161	3.86±0.08	163	3.50±0.03^{d}	128	4.78±0.03^{b}	128	12.88±0.10	111	16.34±0.41^{abc}	157	4.689±0.049^{ab}
9	165	3.94±0.08	166	3.49±0.03^{d}	128	4.78±0.03^{b}	130	12.94±0.10	109	16.47±0.41^{abc}	156	4.726±0.050^{ab}
10	163	3.82±0.08	166	3.50±0.03^{d}	129	4.81±0.03^{ab}	130	12.71±0.10	91	16.48±0.44^{abc}	154	4.735±0.051^{ab}
11+	257	3.83±0.07	257	3.46±0.02^{ad}	245	4.81±0.02^{ab}	247	12.74±0.07	181	15.27±0.30^{c}	243	4.750±0.041^{b}
Calving Season		NS		**		**		NS		NS		**
1 (spring)	274	3.81±0.06	277	3.32±0.02^{a}	250	4.84±0.02^{ab}	251	12.63±0.08	219	16.48±0.33	251	4.617±0.040^{ab}
2 (summer)	484	3.80±0.05	494	3.40±0.02^{b}	441	4.85±0.02^{a}	442	12.77±0.06	405	16.23±0.28	470	4.696±0.030^{a}
3 (autumn)	571	3.85±0.05	585	3.49±0.02^{c}	562	4.78±0.02^{b}	559	12.85±0.06	479	16.47±0.26	562	4.697±0.027^{a}
4 (winter)	567	3.85±0.05	572	3.42±0.02^{b}	479	4.84±0.02^{a}	478	12.78±0.06	384	16.79±0.28	544	4.564±0.030^{b}
Overall	**1896**	**3.83±0.02**	**1921**	**3.42±0.01**	**1732**	**4.83±0.01**	**1730**	**12.78±0.03**	**1487**	**15.92±0.09**	**1827**	**4.646±0.014 (44.274 cell/mL)**

In this study, the average MPC of SIM cows was determined as 3.42±0.01%. While the MPC showed a significant changes according to calving year (P<0.01), lactation month (P<0.01) and calving seasons (P<0.01), the effect of lactation number on it was insignificant (P>0.05). According to calving years, the highest MFC average was calculated for 2022 (3.52±0.02), while this year was found to be different from all other years (P<0.05), 2021 is also different from 2020 (P<0.05), other differences between the years are insignificant (P>0.05).

The average MPC, which was 3.42±0.03% in the first month of lactation, decreased to 3.23±0.03%, which was the lowest level in the second month of lactation, when the peak lactation milk yield was observed in cattle, as expected, and increased in the following months and reached around 3.5% at the end of lactation (Table 1). MPC, which showed significant changes according to the calving season, reached its lowest value of 3.32±0.02% in the spring season when milk yield was high, and the average MPC, which increased in summer and autumn, decreased again in winter and reached 3.42±0.02%. While summer and winter months are similar (P>0.05), these two seasons are also different from the other two seasons (spring and autumn), which are different from each other (P<0.05). In addition to feed additives adding to the ration against heat stress the cooling fan and shower system in the barn where the cows were raised protects the cows more or less from heat stress in hot summer months especially those who calved in this months and the cows at the beginning of lactation.

The MPC average obtained in this study for SIM cattle (3.42±0.01%) could be compared with Koç and Öner (2023), Koç and Arı (2020), Önal et al. (2021) and Cioch et al. (2015). The means of MPC reported in those studies were 3.45±0.01%, 3.43±0.01%, 3.40±0.015% and 3.44±0.19%, respectively. In the literature for the same breed, Bendelja et al. (2011), Nistor et al. (2014), Cziszter et al. (2016), Litwińczuk (2016), Okuyucu and Erdem (2017), Erdem and Okuyucu (2019) and Wei et al. (2021) reorted lowewr values (3.36±0.016%, 3.12±0.358%, 3.25±0.02%, 3.10±0.30%, 3.02%, 3.07%, and 3.33±0.43% respectively), but Franzoi et al. (2020), Falta et al. (2023), Kaygısız and Şahin (2023), Wrhel et al. (2024) and Buonaiuto et al. (2024) reported higher values (3.52±0.005%, 3.60±0.350%, 3.52±0.18%, 3.54±0.20% and 3.53±0.22% respectively). Cioch et al. (2015) also reported higher MPC values for the SIM cattle at first three lactations as between 3.49±0.19% and 3.55±0.012%.

The average MLC, another component found in milk, was determined as 4.83±0.01% for SIM cattle in this study. The effect of all factors on MLC is significant at the P<0.01 level. The MLC level, which was obtained as 4.91±0.02% in animals in the first lactation, decreased according to the lactation number and became 4.77±0.02% in animals in 5+ lactations. While the difference between these two lactation numbers was statistically significant (P<0.05), the first lactation number was similar to the second lactation number (P>0.05) but different from other lactation numbers (P<0.05). Additionally, 5+ lactation number is also different from the second lactation number (P<0.05) and other differences between lactation numbers were insignificant (P>0.05). The lowest MLC average according to calving years was calculated as 4.77±0.01% for 2022 and it was determined that this year was different (P<0.05) from 2019 (4.85±0.02%) and 2021 that has the highest MLC average as 4.89±0.01%. Other differences between the years were insignificant (P>0.05). The MLC average showed significant differences according to the lactation months and the highest MLC mean was obtained in the 3rd lactation month (3.90±0.03%). While this month was different from the 6th, 8th and 9th lactation months (P<0.05), it is similar to the other lactation months (P>0.05). The MLC average, which varies significantly according to calving seasons, was highest in cows calving in summer (4.85±0.02%) and lowest in autumn calving cows (4.78±0.02%). These two seasons were also different from each other (P<0.05). Unlike MFC and MPC, the MLC level had the lowest average in cows calving in autumn and the highest average in cows calving in summer.

While the average MLC (4.86±0.01%) obtained for SIM cattle in this study is similar to the 4.81±0.019% value reported by Koç and Arı (2020), who studied the same breed, Litwińczuk (2016), Falta et al. (2023) and Vrhel et al. (2024) reported higher MLC for the same breed (4.94±0.32%, 4.94±0.233% and 4.96±0.15% respectively). On the other hand, Bendelja et al. (2011), Okuyucu and Erdem (2017), Erdem and Okuyucu (2019), Franzoi et al. (2020), Koç and Öner (2023), Wei et al. (2021), Önal et al. (2021) and Kaygısız and Şahin (2023) reported values (4.55±0.01%, 4.19%, 4.27%, 4.77±0.003%, 4.24±0.02%, 4.75±0.36%, 4.74±0.01% and 4.73±0.011%, respectively) lower than the mean obtained in this study for MLC.

In this study, the TDMC average of SIM cattle was calculated as 12.78±0.03%. While the effects of lactation number (P<0.01) and calving year (P<0.05) were found to be significant on this trait, the effects of lactation month and calving season were insignificant (P>0.05). According to the lactation number, the highest TDMC average was obtained in cows in the first lactation, as the lactation number increased, the TDMC average

decreased, and the lowest average was calculated as 4.77±0.02% for cows in 5+ lactations. The difference between the TDMC averages of cows in the first and 5+ lactations was also found to be statistically significant (P<0.05), while other differences between lactations were insignificant (P>0.05). It is expected that TDMC will decrease as the lactation number increases, because there is a negative correlation between milk yield and TDMC, and it is thought that as the lactation number increases in dairy cattle, the milk TDMC level decreases due to the increase in milk yield.

According to calving years, the highest TDMC average was calculated as 13.03±0.17% in 2018, and the lowest TDMC level, which decreased in the following years, was calculated as 12.41±0.05% in 2021. While the years 2018-2020 are similar to each other in terms of TDMC (P>0.05), these years are different from the years 2021 and 2022, which are also similar to each other (P<0.05). It is thought that the significant difference in TDMC level according to lactation number and calving years is due to the high number of animals in the first and second lactation periods, whose milk yield was lower in the first years of the farm that started operating recently compared to other lactation numbers, thus the TDMC level is found to be higher in the first years than in the following years.

While the TDMC average ((12.78±0.03%) calculated for SIM cattle in this study is close to the value (12.72±0.035%) reported by Kaygısız & Şahin (2023) , however, in some studies (Litwińczuk et al., 2016; Okuyucu and Erdem, 2017; Erdem and Okuyucu, 2019; Koç and Arı, 2020; Wei vd., 2021; Falta vd., 2023; Koç and Öner, 2023) lower values ranging from 11.23% to 12.60% were reported for the same breed.

Another trait emphasized in this study is MUN level in milk is a parameter used to evaluate the protein and energy status of dairy cattle, as it is a trait closely related to the protein level taken in feed. It is considered normal for the MUN level to be between 10-16 mg/dl, and a MUN level higher than 16 mg/dl means the ration protein level is high. Breeders are trying to increase ration protein levels to increase the milk yield of animals. However, high amounts of dietary protein levels are broken down into amino acids in the rumen, and urea is produced from ammonia derived as a result of the normal daily amino acid metabolism of the liver and the breakdown of body proteins in the rumen. If rumen bacteria cannot convert ammonia into microbial protein, this excess ammonia is absorbed by the rumen wall and mixes with the blood. Since high blood ammonia levels are toxic, the liver converts this ammonia into urea and this urea is excreted through urine or milk. On the other hand, if the rumen ammonia level is not low, rumen microorganisms may reduce microbial protein production, causing milk yield and milk protein level to remain low. The increased amount of urea in milk is an indication that the blood ammonia level is high, and there is a decrease in the reproductive fertility of animals with high MUN levels (Anonymous, 2024).

In this study, the mean MUN for SIM cattle was calculated as 16.49±0.09 mg/dL, while the effects of calving year and lactation month were found to be significant (P<0.05) on this trait, the effects of lactation number and calving season were significant (P>0.05). It is seen that the average MUN according to calving years decreased as the years progressed and was realized within appropriate limits in 2022 (Table 1). In the first years after the establishment of the enterprise (here 2018), the MUN level in milk was not examined, but in the following years, it is seen that the enterprise attaches importance to determining the milk MUN level due to its relationship with nutrition. The MUN level was determined to be above the upper limit of MUN as 17.66±0.83 mg/mL in 2019, and it was determined that the MUN level gradually decreased in the following years. While the years 2019-2021 are similar to each other (P>0.05), these years are different from 2022 (P<0.05). According to the lactation months, the MUN average in the 3rd and 4th months of lactation, when feed consumption in cattle increases, was above 17 mg/dL, and the MUN average was above 16 mg/dL in all months except the first lactation month and the last lactation month.

In this study, it can be said that the average MUN determined in the milk of SIM cattle (16.49±0.09 mg/dL) is almost appropriate in terms of ration energy and protein balance. However, as can be seen from Table 1, it should be emphasized that the MUN average was within appropriate limits only in 2022, and was above the upper limit in other years. It is understood that the enterprise has been trying to keep the energy and protein balance in the animals' rations at appropriate levels, especially in recent years, both from the importance it attaches to determining the milk MUN level and from the fact that the MUN level is within appropriate limit in 2022.

The overall mean MUN obtained in this study (16.49±0.09 mg/dL) can be compared with the values reported by Bendelja et al (2011), Franzoi et al (2020), Koç and Arı (2020), Falta et al (2023) and Kaygısız and Şahin (2023) as 24.56±0.34 mg/dL, 20.44±0.11 mg/dL, 12.28±0.138 mg/dL, 25.75±7.419 mg/dL and 17.81±0.353 mg/dL, respectively for the SIM breed than those obtained in this study, while Koç and Arı (2020) reported a lower value (12.28±0.138 mg/dL) than the average obtained in this study for the same genotype.

In this study, the average Log10 SCC of SIM cattle was determined as 4.646±0.014 or 44.274 cells/mL. The effects of calving year, lactation month and calving season on this trait were significant (P>0.01), while the effect of parity was insignificant (P>0.01). It is expected that the SCC level in cattle will increase depending on the increase in the lactation order. This trend was also realized in this study, the average Log10SCC of cows in the first parity increased from 4.565±0.035 (36.728 cells/mL) to 4.703±0.043 (50.466 cells/mL) in animals in the 5+ parity, but, the difference of 13.738 cells/mL between these two parities was not found to be statistically significant (P>0.05).

The Log10SCC level showed significant changes according to the calving years, the lowest Log10SCC average was determined as 30.120 cells/mL in both 2018 and 2019, and the Log10SCC level, which increased regularly in the following years, reached its highest value of 4.703±0.043 (73.961 cells/mL) in 2022. The 2022 calving year was found to be different from all other years (P<0.05), and the years 2020 and 2021, which are similar to each other (P>0.05), are also different from other years (P<0.05). It is thought that the increase in SCC level over the years is due to the fact that the cows raised in the first years of the farm, have lower SCC levels due to their low parity. Because cows in their first lactation have lower milk yields, they are less likely to suffer from mastitis than animals in later lactations.

SCC level, which showed significant changes according to lactation months, decreased in the second month, then increased in the 3rd and 4th months and increased in the 5th-7th months. The SCC level, increased towards the end of lactation, as expected, and reached the highest level of 56.234 cells/mL in the 11+ lactation month. It is thought that the increase in SCC level towards the end of lactation is not due to the increased possibility of cows suffering from mastitis, but to the increase in the number of cells per unit volume due to the decrease in milk yield in the last months of lactation. In this study, while the 11+ lactation months were different from the 5th and 7th lactation months (P<0.05), other differences between the months were insignificant (P>0.05).

In this study, it was determined that the SCC level of SIM cattle showed significant changes according to the calving season (P<0.05). The lowest Log10SCC level was calculated in cows calving in winter (4.646±0.014 or 44.274 cells/mL). While this season is similar to spring (P>0.05), it is different from summer and autumn (P<0.05). The SCC levels of cows calving in summer and autumn were calculated to be 13.015 and 13.130 cells/mL higher than those of cows calving in winter, respectively (P<0.05). Although there are cooling systems in the farm, the higher SCC level found in the summer and autumn than in winter calving cows could be due to low body resistance because the cows calved in these season had high milk yield but, in negative energy balance and lose live weight, as a result of that SCC in milk was increased in these seasons.

In this study, the SCC level obtained for SIM cattle (4.699±0.014 or 50.004 cells/mL) was compared with other studies is lower than the values of Cziszter et al. (2016), Okuyucu and Erdem (2017), Koç and Arı (2020), Önal et al. (2021), Falta et al. (2023), Koç and Öner (2023) and Kaygısız and Şahin (2023) and who reported 233.800 cells/mL, 181.339 cells/mL, 251 768 cells/mL, 192.000±15.32 cells/mL, 109.647.82 cells/mL, 128.825 cells/mL and 178.220±14.532 cells/mL, respectively, while the mean SCC found in this study is higher than the values of 4.23±1.98 (16.982 cells/mL) reported by Wei et al. (2021).

CONCLUSIONS

In this study, important information was obtained about the milk quality characteristics of SIM cattle of Austrian and German origin, to which producers have shown great interest in recent years, but not much research has been done on their performance in our country's conditions. Since the type traits, fertility and milk yield of this genotype were evaluated as separate studies, only milk quality characteristics were evaluated in this study. According to the findings, while the MPC, MLC, TDMC traits of SIM cattle are generally similar to the reports in the literature, the fact that the MFC average is lower than most of the values reported in previous

studies is thought to be due to the difference in the SIM genotype grown in the farm where this study was conducted, as well as differences in management and feeding conditions.

The fact that the MUN average, which is an indicator of the ration protein/energy balance given to animals, decreased over the years and was within appropriate limits in 2022, the last year, shows that this is the result of the attention paid to the ration content given to animals in the farm.

The fact that the SCC average was determined at a very low level of 44.274 cells/mL in this farm and this low level maintained in different years can be considered as an indication that the necessary importance is given to the udder health of the cows raised in this disease-free farm and that milking hygiene rules are taken into consideration. Considering the SCC levels obtained in this study and those reported for SIM breed cattle in the literature, it can be seen that the SCC level of the breed is much lower than the Holstein-Friesian breed, which is widely used in milk production around the world and in our country, thus the prevalence of mastitis in this breed is higher than the Holstein-Friesian breed. It is thought that a study on the comparison of reproductive fertility, milk yield and milk quality characteristics in a farm that raises the Holstein-Friesian breed together with the SIM genpotype of Austrian and German origin, with increased milk yield, will provide important information on this subject. Another point that needs to be emphasized here is how correct it is to consider all of the SIM genotypes, which have many different genotypes and productivity characteristics, such as Black Simmental, which has been developed for meat production purposes in the USA, and SIM (fleckvieh) genotypes of German and Austrian origin, which have been developed for dairy purposes, within the scope of a single breed.

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Associations of *VLDLR* Gene Polymorphism (intron 11, 392C > T) with Egg Production and Weight in Japanese Quails (*Coturnix coturnix japonica*)

ABSTRACT

Objective: This study aimed to assess the association between the *VLDRLR* gene intron 11 (392 C>T) polymorphism and egg production and weight in quails.

Material and Methods: Egg yield and weight were recorded over 90 days for 191 Japanese quails, which were genotyped using the restriction fragment length polymorphism technique for the *VLDLR* gene variation.

Results: The *VLDLR* gene was polymorphic due to conserving all possible genotypes (TT, TC, and CC). The TT genotype was the most common with a frequency of 0.70, while the frequency of the TC and CC genotype were 0.16 and 0.14, respectively. The mean 90-day egg production was 78.31, 76.07, and 73.94 in TT, TC, and CC genotypes, respectively, while the mean egg weight ranged from 915.80 (CC genotype) to 939.19 (TT genotype). Association analysis revealed a significant relationship between the *VLDLR* genotypes and egg production traits (P<0.05), while no significant relationship was detected for egg weight.

Conclusion: This study showed that the TT genotype for *VLDLR* gene intron 11 (392 C>T) polymorphism can be used in marker-assisted selection studies in order to increase egg production in quails.

Keywords: Candidate genes, egg traits, genetic variation, MAS, PCR-RFLP

Japon Bıldırcınlarında (*Coturnix coturnix japonica*) *VLDLR* Gen Polimorfizminin (11 intron, 392C > T) Yumurta Üretimi ve Agırlıgı ile Iliskisi

ÖZ

Amaç: Bu çalışma, Japon bıldırcınlarında *VLDLR* geninin intron 11 (392 C>T) polimorfizmi ile yumurta üretimi ve ağırlığı arasındaki ilişkiyi değerlendirmeyi amaçlamıştır

Materyal ve Metot: *VLDLR g*en varyaysyonu için restriksiyon fragment uzuluğu polimorfizmi tekniğiyle gengenotiplendirilen 191 Japon bıldırcınında 90 günlük yumurta verimi ve ağırlığı verisi kaydedilmiştir.

Bulgular: Muhmetel bütün genotipleri (TT, TC ve CC) içerdiğinden dolayı *VLDLR* geninin polimorfik olduğu belirlenmiştir. En yaygın genotipin 0.70 frekans ile TT olduğu, TC ve CC genotip frekanslarının ise sırasıyla 0.16 ve 0.14 olduğu belirlenmiştir. 90 günlük yumurta üretimi ortalamasının TT, TC ve CC genotipleri için sırasıyla 78.31, 76.07 ve 73.94 olduğu, yumurta ağırlığı ortalamasının ise 915.80 (CC genotipi) ile 939.19 (TT genotipi) aralığında değiştiği gözlemlenmiştir. Yapılan ilişki analizi, *VLDLR* genotipleriyle yumurta üreteimi arasında önemli bir ilişkinin olduğunu (P<0.05), yurmurta ağırlığı açısından ise herhangi bir ilişkinin olmadığını göstermiştir.

Sonuç: Bu çalışma, *VLDLR* geninin intron 11 (392 C>T) polimorfizmini için TT genotipinin japon bldırcınlarında yumurta üretimini arttırmak için uygulanacak olan marker destekli seleksiyon çalışmalarında kullanılabileceğini göstermiştir.

Anahtar Kelime: Aday genler, yumurta özellikleri, genetik çeşitlilik, MDS, PZR-RFLP

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INTRODUCTION

The Japanese quails (*Coturnix coturnix japonica*) are globally recognized laboratory animal species and the smallest avian species bred for egg and meat production. They offer several species-specific advantages such as small body size, ease of breeding, short generation interval, high egg production, being more resistant to diseases than other poultry species and requiring less space for production making the quails a convenient model animal for poultry breeding (Alkan et al. 2010; Alkan et al. 2013). Furthermore, ongoing breeding studies have made quails one of the preferred poultry species for egg and meat traits. Indeed, approximately 250 eggs may be produced per year in quails by traditional breeding studies (Narinç et al. 2013; Akarikiya et al. 2022), which can be further improved by marker-assisted selection (MAS) studies in order to improve egg yield.

Molecular methods are now used in breeding studies to improve the quantity and quality of yields, which have economic importance in farm animals (Demir et al. 2023; Karsli et al. 2020). SNP or InDel mutations may be easily detected across genomes of poultry farm animals (Karsli et al. 2017; Demir et al. 2020a) like quail (Bozkaya et al. 2013; Ahmed and Al-Barzinji, 2020) thanks to the rapid advances in the area of molecular genetics. Associations analyses are available to reveal the relationships between various phenotypic traits (meat, egg production, and quality characteristics) and genomic variations allowing for using candidate genes in MAS (Balcıoglu et al. 2014; Raschia et al. 2018). Previous studies have revealed several candidate genes related to meat yield (Kadlec et al. 2011; Thu et al. 2020; Thu et al. 2021), egg yield, and quality (Karsli et al. 2017; Demir et al. 2020b; Roy et al. 2024) in poultry farm animals. Of these genes, Very Low-Density Lipoprotein Receptor (*VLDLR*) has been studied in several avian species such as chicken (Abdulwahid et al. 2019), duck (Pan et al. 2017) and quail (Wu et al. 2015) due to its regulation functions (lipid, triglycerides and cholesterol metabolism, cell proliferation and differentiation) related to economically important traits (Pan et al. 2017; Abdulwahid et al. 2019; Bello et al. 2022). In poultry, the process of egg production stimulated by estrogen results in notable increases in liver lipid production and leads to alterations in the diameter of assembled *VLDL* (very low-density lipoprotein) from a general *VLDL* (~70 nm in diameter) facilitating the transport of lipids to peripheral tissues, to yolk-targeted VLDL (*VLDLy*; ~30 nm) (Salvante et al. 2007). Yolk lipids are carried to the oocytes by VLDL yolk-targeted (VLDLy), which has a specific receptor *(VLDLR*) located on the surface of the ova (Al-Hassani et al. 2023). VLDLR, a transmembrane lipoprotein receptor within the low-density lipoprotein receptor family, is prevalent in skeletal muscle, adipose tissue, heart, and brain, while being notably lacking in the liver (Nimpf et al. 2000). *VLDLR* is also known as the vitellogenesis receptor or vitellogenin receptor, mediating the absorption of plasma very low-density lipoprotein and vitellogenin (Wang et al. 2011). Additionally, it functions as a part of triglyceride and cholesterol metabolism (Brown and Goldstein, 1986) and plays a role in numerous cellular processes such as cell proliferation, migration, and differentiation (Hussain, 2001). It is reported that *VLDLR* plays a significant function in avian reproduction by influencing oocyte development and yolk lipoprotein deposition (Wang et al. 2011; Abdulwahid et al. 2019). Therefore, this study aimed to i) identify *VLDRLR* gene polymorphism (intron 11, 392C > T), ii) assess relationships between genetic variations and phenotypic traits such as egg production and egg weight and iii) discuss the possibilities of using this gene region in MAS studies to improve egg production in Japanese quails.

MATERIAL and METHODS

Phenotypic Data Records

This study was designed to cover 250 samples. However, phenotypic records for 198 samples were obtained at the end of the experiment due to mortality and laying problems. Similarly, a total of 7 samples were excluded from the experiment due to unexpected laboratory practices (non-amplification in PCR or non-specific digestion in PRFLP). As a result, this study was carried out with 191 samples, which is still consistent with some previous studies in Japanese quails (Lan et al., 2017; Rifki et al., 2021). Two phenotypic data (egg yield and egg weight) belonging to the studied animals were recorded at Akdeniz University's Poultry Farm, Faculty of Agriculture, Department of Animal Science. Routinely, following the incubation period, chicks are kept in cages for the first 30 days and fed ad libitum with fodder containing 24% raw protein and 2800 kcal/kg metabolic energy for the first 5 weeks. At the end of the growing period, egg yield and egg weight were recorded for 250 female quails taken into individual cages (20x20x29 cm) for 90 days. During the laying period, the quails were fed with a ration consisting of 2800 kcal/kg ME and 21% HP as ad libitum and exposed to 16 h of light and 8 h of darkness. Daily egg collection and weighing procedures were carried out in the morning hours (08:00-10:00).

Collection of Feather Samples and DNA Isolation

The shed feathers inside the individual cages were collected and numbered according to the cages for DNA isolation. DNA was isolated from collected feathers following the protocol described by Bello et al. (2001). Agarose gel and spectrophotometer were used to determine DNA quality and quantity. Before the PCR process, the DNA concentration was adjusted to approximately 20 ng/µl.

Process of PCR-RFLP and Genotyping

VLDLR gene intron 11 (392C > T) variation was identified via PCR-RFLP method utilizing specific oligonucleotide primers (F: CCTCTATTGATACCCGTGAT and R: TTAGGCCATTGGATTTCTGT) reported by Wu et al. (2015). The 493 bp length of VLDLR intron 11 region was amplified by thermal cycler with initial denaturation at 94 ºC for 5 min, followed by 35 cycles consisting of denaturation at 94 ºC for 45 sec, annealing at 55 ºC for 45 sec, extension at 72 ºC for 45 sec, with final extension 72 ºC for 5 minutes. PCR reaction mixture consisted of 20 ng/µl template DNA, 1.2 µL HQ buffer (GeneAll), 2 µL 10X buffer (GeneAll), 2.5 mM dNTPs, 10 pM/µl primer, 2.5 U Taq DNA polymerase, and 11.4 µL H2O. In the RFLP process, the amplified PCR products were digest with *NlaIII* (isoschizomer of *FatI*) (Thermo Scientific, ER1831) restriction enzymes in which 5 µL of PCR product and digestion mixture (2 µL of buffer, 5 µL of H2O and 2.5 U cutting enzyme) were mixed and incubated at the suitable temperature and time recommended by the manufacturer.

Statistical Analysis

Allele and genotype frequency were calculated by the POPGENE 1.31 program (Yeh et al. 1997). Hardy-Weinberg equilibrium was checked by using the chi-square ($\chi 2$) statistic in the population (Hartl and Clark, 1989). The sample size calculation was performed by G*Power 3.1.9.2 (Faul et al. 2007) software with the options of linear multiple regression and a priori, while other input parameters were set to default setting (effect size=0.15, α =0.05, and power=0.95). The phenotypic traits were compared among the genotypes. The genotypes obtained in the relevant gene regions and the model used to determine the 90-day egg yield ad egg weight aregiven below.

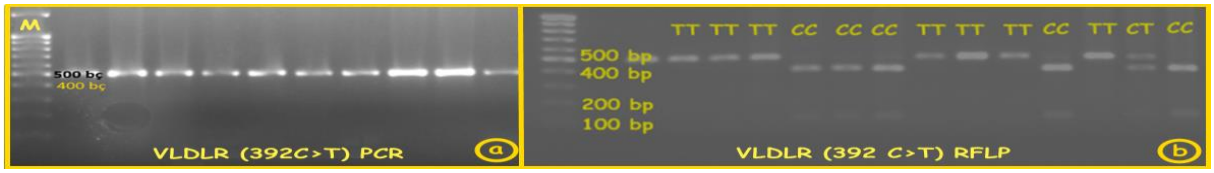
$$Y = \mu + G + e;$$

where Y is the dependent variable (analysed trait), µ is the mean for yields, G is the genotype, and e is the error.

One-way analysis of variance (One-way ANOVA) was performed using the SPSS (version 23) software to determine the relationship between the genotypes obtained in the *VLDLR* (392C>T) gene region and the phenotypic traits. Tukey test for multiple comparisons was performed between groups which were observed to be statistically different.

RESULTS

Initially, records of phenotypic data for a total of 250 animals were collected, but some animals were excluded from the statistical analyses due to various management (survival and laying problems) and laboratory practices (PCR amplification and RFLP digestion problems). Thus, the analyses were conducted based on 191 samples that were recovered during the experiment. It is noteworthy that even the decreased sample size (191 individuals) turned out to be statistically enough for the experiment because the required sample size in power analysis was estimated at 107 individuals. The 493 bp length products obtained by the PCR process were digested with *NlaIII* restriction enzyme, revealing two alleles (C and T) and three genotypes (CC, CT, and TT) (Figure 1). Since the genotypic bands were clearly distinguishable in agarose gel electrophoresis and consistent with the fragment sizes reported by Wu et al. (2015), genotype confirmation via sequencing or other methods was deemed unnecessary.



M: Marker (Thermo 100 bp; Cat.No: SM0241). a) PCR prducts (1 % agarose gel) (493 bp), b) Digestion of VLDLR PCR products by NlaIII (3% agarose gel) (TT genotype: 493 bp; CT genotype: 493 bp, 392 bp, and 101 bp; CC genotype: 392 bp and 101 bp.)

Figure 1 Images of agarose gels from PCR and RFLP processes for *VLDLR* gene intron 11 (392C > T)
Şekil 1. *VLDLR* geni intron 11 (392C > T) için PCR ve RFLP işlemlerinden elde edilen agaroz jellerin görüntüleri

The *VLDLR* (392C>T) gene was found to be polymorphic since studied quail population conserves all possible genotypes. The C and T allele frequencies were 0.22 and 0.78, respectively, while genotype frequencies ranged from 0.14 (CC) to 0.70 (TT) across the population (Table 1).

Table 1. Some descriptive statistics of genotypic and phenotypic data in the studied quail population.
Tablo 1. Çalışılan bıldırcın popülasyonundaki genotipik ve fenotipik verilerin bazı tanımlayıcı istatistikleri.

		Gene Frequencies		Genotype Frequencies			χ2
Gene	**n**	**C**	**T**	**CC**	**CT**	**TT**	
				0.14 (27)	0.16 (31)	0.70 (133)	
	191	0.22	0.78	**Genotypes**			
				CC	**CT**	**TT**	
***VLDLR* (intron 11, 392C>T)**	Egg number of 90 days (Mean±SD)			73.94±4.62[b]	76.07±6.03[ab]	78.31±6.32[a]	53.84**
	Egg weight of 90 days (g) (Mean±SD)			915.80±76.60	933.50±80.20	939.19±81.29	

Different superscript letters (a and b) differ significantly (P < 0.05) in genotypes. χ2 0.01;1:6.63; χ2 0.05;1:3.84;
****: Significant deviation from H-W equilibrium (P ≤ 0.01)**

Significant deviation from Hardy Weinberg equilibrium (P<0.01) was detected in quail population for *VLDLR* gene. The allele and genotype frequencies, as well as the mean number of egg and egg weight per genotype, were summarised in Table 1. The 90-day egg yields were 73.94, 76.07, and 78.31, while 90-day egg weight were 915.80, 933.50, and 939.19 regarding CC, CT, and TT genotypes, respectively. The 90-day egg production of animals with the TT genotype was higher than those with the TC and CC genotypes, according to a one-way analysis of variance across the groups (P<0.05). However, there was no significant difference between the groups in terms of 90-day egg weights.

DISCUSSION and CONCLUSIONS

Various studies have shown that *VLDLR* gene is polymorphic and that variations in this gene were associated with phenotypic traits particularly egg yield and egg weight in several avian species (Wang et al. 2011; Cao et al. 2012; Wu et al. 2015; Zhao et al. 2015; Abdulwahid et al. 2019, Zhou et al. 2020). For example, Wu et al. (2015) focused on two polymorphisms (363T>C and 392C>T) in yellow-feather quail and chestnut feather quail populations and reported that variations in this gene were associated with several phenotypic traits such as first egg, the age of first egg, and egg number of 20-week-old birds. Abdulwahid et al. (2019) showed that variations in the *VLDLR* gene were also associated with egg production and egg weight in Iraqi local Brown Chickens. Moreover, Zhao et al. (2015) detected five SNPs and three InDel variations between exon 14 and exon 16 of *VLDLR* gene in Gaoyou duck breed and reported that of the 11 haplotypes, 4 haplotypes were reported to be associated with body weight at 10 weeks (P<0.05) and abdominal fat percentage (P<0.01).

The results of this study, combined with findings from the literature, support the idea that *VLDLR* polymorphisms could be used in MAS to improve performance traits with higher genetic gain in avian species, including quails. The study showed that animals with TT genotypes are advantageous in terms of the egg number of 90 days trait (p<0.05). While no statistically significant evidence was observed, animals with the TT genotype were also of the highest value in terms of egg weight at 90 days. Similarly, Wu et al. (2015) reported that animals with the TT genotype were of the highest value for egg number of 20-week in chestnut feather quails. However, in this study, the highest allele frequency was observed in TT (0.70), while the CC genotype was of the highest frequency in yellow-feather (0.62) and chestnut-feather quail (0.60) populations (Wu et al., 2015). Differences between genotype distribution between quail populations raised in Turkiye and China could be due to different raising practices. While selection occurs in quail reared in China, no systematic selection process was conducted in the quail population we studied. Besides, quail populations sampled for this study have been closely raised for several generations. This kind of close breeding may cause significant variations in allele and genotype distribution, resulting in deviation from HWE in poultry populations (Karsli et al. 2019, Karsli and Fidan, 2019).

VLDLR gene, directly associated with egg yield in avian species, functions especially in the formation process of yolk. Variations in this gene may also interact with other genes affecting egg yield. For example, the *VLDLR* gene has been reported to affect egg yield via interaction with several genes playing a key role in yolk

protein synthesis (vitellogenin, *VTG*), fat metabolism in ovary (lipoprotein lipase, *LPL)*, hormonal signaling pathway during laying period (estrogen receptor, *ESR*), and folicul development (follicle-stimulating hormone receptor*, FSHR*) (Li et al. 2003; Huang et al. 2016; Ma et al. 2020; Liu et al. 2021). Therefore, the combination of genetic variations in *VLDLR* and related genes in MAS studies could be efficient to improve egg yield in Japanese quails.

Wu et al. (2015) reported that the change in intron 11 of the *VLDLR* gene may be linked to another change in *the VLDLR* gene leading to an amino acid change. Although introns do not encode proteins, they play an important role in gene regulation through mechanisms such as splicing mechanisms, affecting regulatory elements, creating new sites where miRNAs can bind or destroying existing binding sites (Le Hir et al. 2003; Lin et al. 2006; Riethoven 2010).

In conclusion, the effect of a point mutation on the *VLDLR* gene intron 11 (392C>T) on 90-day egg production and egg weight was studied in Japanese quails. A significant association was observed between the *VLDLR* gene (392C>T) variation and 90-day egg production. Quails with the TT genotype for the *VLDLR* gene (392C>T) have better egg production than those with the CC and TT genotypes. Since quail is a model organism for avian species, this study confirms that *VLDLR* gene intron 11 (392C>T) polymorphisms could be utilised for other poultry species in MAS to improve phenotypic traits (egg production). In addition, supporting MAS with a higher number the candidate genes will directly increase the success rate and genetic gain due to the nature of polygenic inheritance.

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Author contributions: All authors contributed equally to the preparation of the article.

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Relationships Between Mixed Feed Production and The Amount of Broiler Meat Consumed Per Person

ABSTRACT

Objective: The objective of this article is to examine data related to the broiler chicken sector in Turkey between 1996 and 2022 and to evaluate the relationship between per capita chicken meat consumption, compound feed production, feed costs, and the number of animals slaughtered. The study aims to reveal the effect of increased broiler chicken production on per capita consumption by analyzing the relationships between these parameters.

Materials and Methods: The study is based on data from 1996 to 2022 obtained from the Ministry of Agriculture and Forestry of the Republic of Turkey and the Turkish Statistical Institute (TÜİK). The data include compound feed production (tons), feed costs (TL per ton), number of animals slaughtered, and per capita chicken meat consumption (kg). The data were modeled and analyzed using quadratic regression analysis in the SPSS 22.0 software package. The statistical model used is: $Y = \mu + 5.753E\text{-}6X_1 - 4.372E\text{-}13X_2^2$. This model explains the relationship between compound feed production and other parameters.

Results: The analyses revealed a significant relationship between compound feed production and per capita chicken meat consumption ($p < 0.005$, $R^2 = 0.96$). As compound feed production increases, per capita chicken meat consumption also increases; however, when production peaks at around 6 million tons, the increase in consumption slows relatively (20-25 kg/person in 2021-2022). In 1995, compound feed production was 1,046,602 tons, while in 2022 it reached 6,131,807 tons (a 5.8-fold increase); feed costs rose from 38 TL/ton to 14,695 TL/ton (a 386-fold increase); the number of animals slaughtered increased from 99,087. 900 to 261,250,314 (a 2.6-fold increase); per capita consumption rose from 8.64 kg to 22.875 kg (a 2.6-fold increase). The increase in exports in recent years (268,000 tons in 2011, 628,000 tons in 2021) is the main reason for the slowdown in domestic consumption. Other factors affecting consumption include income distribution, household size, gender, education level, and price sensitivity.

Conclusion: The study shows that the increase in compound feed production directly increases chicken meat production and indirectly increases per capita consumption. However, chicken meat consumption in Turkey, which was 22.875 kg per capita in 2022, is well below the World Health Organization's recommended animal protein intake target (approximately 54 kg per year). Increased exports are limiting domestic consumption. To increase animal protein intake, compound feed production and slaughtering capacities should be increased, and hatchery and breeding facilities should be developed. This will both support healthy nutrition and contribute to employment and the national economy.

Keywords: Broiler, broiler meat consumption per capita, feed, number of slaughtered animals, feed price

Karma Yem Üretimi ile Kisi Bası Tüketilen Tavuk Eti Arasındaki İliskiler

ÖZ

Amaç: Makalenin amacı, 1996-2022 yılları arasında Türkiye'deki etlik piliç sektörüne ilişkin verileri inceleyerek kişi başına piliç eti tüketiminin, karma yem üretimi, yem maliyetleri ve kesilen hayvan sayıları ile ilişkisini değerlendirmektir. Çalışma, bu parametreler arasındaki ilişkileri analiz ederek, etlik piliç üretiminin artmasının kişi başına tüketim üzerindeki etkisini ortaya koymayı hedeflemektedir.

Materyal ve Method: Çalışma, T.C. Tarım ve Orman Bakanlığı ile Türkiye İstatistik Kurumu'ndan (TÜİK) elde edilen 1996-2022 yıllarına ait verilere dayanmaktadır. Veriler; karma yem üretimi (ton), yem maliyetleri (ton başına TL), kesilen hayvan sayısı ve kişi başına piliç eti tüketimi (kg) içermektedir. Veriler, SPSS 22.0 paket programında kuadratik regresyon analizi ile modellenmiş ve analiz edilmiştir. Kullanılan istatistiksel model: $Y = \mu + 5{,}753E\text{-}6X_1 - 4{,}372E\text{-}13X_2^2$. Bu model, karma yem üretimi ile diğer parametreler arasındaki ilişkiyi açıklamaktadır.

Bulgular: Analizler, karma yem üretimi ile kişi başına piliç eti tüketimi arasında anlamlı bir ilişki olduğunu göstermiştir ($p < 0.005$, $R^2 = 0.96$). Karma yem üretimi arttıkça kişi başına piliç eti tüketimi de artmaktadır; ancak, üretim 6 milyon ton civarında zirve yaptığında, tüketim artışı relatif olarak yavaşlamaktadır (2021-2022'de 20-25 kg/kişi). 1995'te karma yem üretimi 1.046.602 ton iken, 2022'de 6.131.807 tona ulaşmış (5,8 kat artış); yem maliyetleri 38 TL/tondan 14.695 TL/tona (386 kat artış); kesilen hayvan sayısı 99.087.900'den 261.250.314'e (2,6 kat artış); kişi başına tüketim ise 8,64 kg'dan 22,875 kg'a (2,6 kat artış) yükselmiştir. Son yıllarda ihracattaki artış (2011'de 268 bin ton, 2021'de 628 bin ton), iç tüketimdeki yavaşlamanın ana nedenidir. Tüketimi etkileyen diğer faktörler arasında gelir dağılımı, hane büyüklüğü, cinsiyet, eğitim seviyesi ve fiyat hassasiyeti yer almaktadır.

Sonuç: Çalışma, karma yem üretimindeki artışın doğrudan piliç eti üretimini ve dolaylı olarak kişi başına tüketimi artırdığını ortaya koymaktadır. Ancak, Türkiye'de 2022'de kişi başına 22,875 kg olan piliç eti tüketimi, Dünya Sağlık Örgütü'nün önerdiği hayvansal protein alım hedefinin (yıllık ~54 kg) oldukça altındadır. İhracatın artması, iç tüketimi sınırlamaktadır. Kesilen hayvan sayısını artırmak için karma yem üretimi ve kesim kapasiteleri artırılmalı, kuluçka ve yetiştirme tesisleri geliştirilmiştir. Bu, hem sağlıklı beslenmeyi destekler hem de istihdam ve ulusal ekonomiye katkı sağlar.

Anahtar Kelime: Etlik piliç, kişi başı tavuk eti tüketimi, karma yem, kesilen hayvan sayısı, karma yem fiyatı

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INTRODUCTION

The poultry meat sector has serious positive effects on the national economy by providing people with cheap animal protein sources, large employment capacity, import and marketing of feed additives, hatchery and slaughterhouse activities and utilization of slaughterhouse residues. Although the poultry meat sector in our country is dependent on foreign countries for animal material, domestic hybrid studies are being carried out in ministries and institutes. These studies are expected to yield positive results in a short period of time and may be a good domestic alternative as animal material in the future. The history of the poultry meat sector in our country until the 1960s was always obtained by raising domestic chickens or chicks of imported combined fertile animals in the hands of citizens and slaughtering them by slaughterers. The first modern hybrid broiler breeding animals were imported to Turkey in 1968 through private sector companies and the Turkish people were introduced to modern broiler chickens after that time. The first company to import broiler chicks to Turkey in the 1970s was YU-PI and its owner Hanri Benazus. YU-PI company, which initially supplied local chicks named ERBRO from Erbeyli Research Institute, later imported foreign origin breeder chicks used in broiler production in the world and introduced the first broiler chick to Turkey. Although many other poultry companies established later on initially started to buy chicks from YU-PI, later almost all of them imported their own breeder chicks and started to develop their integrated activities (Şengör 2024). In the aforementioned 1970s, chicken meat consumption in the world was 3.9 kg/person/year, while in Turkey it was 2.9 kg/person/year (FAO, 2018). Another important organization in the history of poultry in Turkey is the Development Foundation of Turkey (TKV). TKV is the most important building block for poultry farming in Turkey to become what it is today. It was established on January 13, 1969 in order to increase the income of small producers with limited opportunities in rural areas through agriculture, animal husbandry, forestry and agriculture-based business practices in their own environment, to contribute to the rural and agricultural development of our country by developing models suitable for the realities of the country. Within this framework, TKV has been active in sectors such as poultry, cattle breeding, beekeeping, handicrafts, etc. Among these, it was the application in poultry farming that became industrialized. TKV established KÖY-TÜR Holding A.Ş. in 1985 with the idea that small farmers should also take part in the management of these industrialized activities. Thus, the efficient and effective working style of small producers specific to rural areas was brought together with the known management and technological possibilities of large organizations. A brand new “production, business and marketing” model emerged. This “unique economy” model, which was created by integrating labor and capital into a single sector instead of separate sectors, was born in 1969 and its birthplace was Turkey. Under KÖY-TÜR, broiler production was carried out in an integrated system. The product was produced and offered to the market under healthy conditions at every stage from the beginning of production until it was purchased by the consumer. In other words, the production in those years was identical to the production in today's conditions. The chicks required for KÖY-TÜR main breeding enterprises were supplied every year from large main breeding enterprises (pure line) located abroad. It was one of the dreams of KÖY-TÜR to have one of the large main breeding enterprises, which are as few as the fingers of one hand in the world. KÖY-TÜR realized this dream by purchasing the Danish Scanbrid large mother breeding enterprise in 1992 (Bütün Dünya-January.1999-Başkent University Publications). The continuity of genetic breeding studies in large parent breeding enterprises is essential. These studies were also being carried out in KÖY-TÜR. However, unfortunately, this breed could not maintain its competitiveness against other competitors later on and disappeared. In the second half of the 1980s, one out of every three chickens produced in Turkey carried the KÖY-TÜR brand, which was produced as a TKV model. Unfortunately, KÖY-TÜR, which suffered due to the economic crises that emerged later, entered a downsizing process as of 2001 by disposing of its assets one by one and eventually had to withdraw from the sector completely by not being able to cope with the crises. After KÖY-TÜR, developments in the sector continued at a very high speed. The companies that invested in the sector continued the modernization process by investing all their earnings back into this sector. Today's success lies largely in this working model. Today, the poultry sector in Turkey uses the world's most modern technologies to supply healthy chicken meat to the Turkish people and fulfills its duty to provide healthy nutrition to new generations in the cheapest way (Şengör 2024).

The compound feed sector in Turkey has undergone a significant transformation in parallel with the modernization of animal husbandry and the development of agricultural policies (Tümer, 2018). The development in Turkey can be examined in five different periods. The early Republican period (1923-1950) was a period when the Turkish economy was largely based on agriculture and animal husbandry was carried out using

traditional methods. In the 1920s and 1930s, the state took steps to modernize agriculture and livestock farming; the Industrial Promotion Law was enacted in 1927, and various institutions were established to support agricultural production (Kocagöz, 2005). The sector's first steps (1950-1970) were a period when the livestock sector began to grow with the mechanization of agriculture and the spread of modern agricultural techniques. During this period, the first initiatives for compound feed production emerged, and with the private sector's involvement in feed production in 1964, the sector began to gain momentum (Feed Industry Association, 2015). The first feed factories were generally established through public-private partnerships. The enactment of Feed Law No. 1734 in 1973 was an important step in establishing industry standards and ensuring quality control (Resmi Gazete, 1973). During the period of sector growth and privatization (1970-1990), the state encouraged the private sector to increase compound feed production; new factories were established, and production capacity expanded (Gökalp, 1990). During the period of technological developments and globalization (1990-2000), automation systems became widespread in feed production, and efforts to comply with the European Feed Manufacturers' Federation (FEFAC) brought the Turkish feed sector closer to international standards (FEFAC, 2000). During the modernization and sustainability period (2000-present), the compound feed sector became more competitive through technological investments and R&D studies (Türker and Kocaman, 2020). University-industry collaboration projects played an important role in increasing the technological level of the sector. In 2004, feed definitions and standards were updated in line with the European Union acquis; the Regulation on the Marketing and Use of Feeds regulated the use of feed additives and premixes (Ministry of Agriculture and Forestry, 2004). In terms of production capacity, according to 2023 data, compound feed production in Turkey has reached approximately 27 million tons, and more than 500 feed companies are in operation (Turkish Feed Manufacturers Association, 2023).

Chicken meat is one of the most widely consumed protein sources worldwide and in Turkey, standing out for its affordability, accessibility, and versatility. Its affordability is one of its most important distinguishing features. Chicken meat has lower production costs compared to other protein sources such as beef, lamb, or pork. The main reasons for this include the short rearing periods of poultry (typically 5-6 weeks), high feed efficiency, and the productivity provided by modern poultry farming systems. Thanks to this economic advantage, chicken meat offers an affordable option for both individual consumers and the restaurant and ready-to-eat food sectors. It is particularly preferred as a budget-friendly protein source in low- and middle-income households. In Turkey, the poultry sector benefits from economies of scale and government incentives, keeping chicken meat prices at competitive levels. Additionally, the fact that different cuts of chicken meat (breast, wing, thigh) are offered at various price ranges makes it accessible to all income groups (Goddard E et al. 2017, FAO 2020, Özertan 2008). Another advantage of chicken consumption is that it is easily available everywhere, from supermarkets to local butchers, hypermarkets to small grocery stores, thanks to an extensive distribution network. The development of cold chain logistics has ensured that chicken can be transported over long distances without spoiling and is available throughout the year. In Turkey, the poultry sector is spread across the entire country with regional production facilities, ensuring equal access in both rural and urban areas. Additionally, the availability of chicken meat in frozen or fresh form offers consumers convenience. On a global scale, countries that export chicken meat (such as Brazil, the US, and Turkey) support the continuous availability of this product in international markets. Turkey's geographical location provides easy access to European, Middle Eastern, and Asian markets, creating an advantage in exports (USDA 2023, Keskin and Demirbaş 2016). Chicken meat processing is fast and easy. Compared to other types of meat, chicken meat requires less time and expertise in the cutting, cleaning, cutting, and cooking processes. Integrated production processes, ranging from modern poultry farms to automated slaughterhouses, enable chicken meat to be processed quickly. For example, the slaughtering and packaging process for a chicken is completed much more quickly than for beef. Additionally, the versatile nature of chicken meat makes it suitable for various cooking methods, such as grilling, baking, boiling, or frying. In the food industry, chicken meat is widely used in products like nuggets, döner, ready-to-eat meals, and fast food items. In Turkey, with the growth of the ready-to-eat food sector, processed forms of chicken meat (e.g., salami, sausage) have also become popular. This rapid processing feature saves time and costs in both home kitchens and industrial production (Smith 2014, Barbut 2015, Tuncer 2019). Chicken meat is also important in terms of nutritional value and health. Chicken meat is a rich source of high-quality protein, low fat content, and essential nutrients such as vitamin B12, niacin, and selenium. Especially chicken breast, with its low-calorie and fat-free composition, is an ideal choice for dieters, athletes, and health-conscious consumers. Chicken meat supports muscle development, strengthens the immune system, and contributes to energy metabolism. When

consumed without the skin, it has a low risk of negatively affecting cholesterol levels. In Turkey, with increasing health awareness, chicken meat consumption is on the rise, especially as an alternative to red meat. Additionally, chicken meat is easy to digest and appeals to a wide age group, from children to the elderly (Marangoni et al. 2015, WHO 2018, Çınar and Çelik 2020). Chicken meat production is advantageous in terms of environmental and sustainability aspects. Compared to red meat production, chicken meat production requires fewer environmental resources (water, feed, land) and produces lower greenhouse gas emissions. The feed conversion ratio (FCR) of poultry is high; that is, less feed is used to produce one kilogram of meat. For example, while 6-10 kg of feed is required for beef production, this ratio is around 1.5-2 kg for chicken meat. Additionally, chicken farming requires less land use and has a lower water footprint. In Turkey, efforts to increase feed crop production and integrated production models are enhancing the sustainability of chicken meat production. Globally, chicken meat stands out as a more environmentally friendly protein source in the fight against climate change (FAO 2013, Steinfeld et al. 2006, anonymous 2022).

Chicken meat consumption is also a culturally and socially accepted food in our country. Chicken meat is widely accepted by society due to its compliance with religious and cultural restrictions. Its compliance with halal and kosher standards in Muslim and Jewish communities makes chicken meat a popular choice in these societies. In Turkey, chicken meat has a wide range of uses, from traditional dishes to modern recipes (e.g., chicken döner, grilled chicken, served with rice). Additionally, chicken meat can be easily adapted to different culinary cultures; it can be prepared with various spices and cooking techniques from Asia to Europe. This flexibility enables chicken meat to be widely used in both global and local cuisines. In Turkey, increasing urbanization and changing eating habits have further increased the popularity of chicken meat (Mintel 2021, Aksoy 2017). For a healthy life, foods should be consumed in the required amount and regularly (Şengül; Zeybek, 2020). It is very important to consume animal food sources in a balanced diet (Bircan; Eleroğlu, 2019). It is recommended that 1 in 3 of the amount of protein that should be taken daily for a balanced diet should be of animal origin. This protein need should be met from red meat (large and small head) and poultry meat (poultry and fish meat). Chicken meat has low fat content and high protein content (Stamatopoulou and Tzimitra-Kalogianni, 2022). Chicken meat has an important place in human life due to its easy availability.

In Turkey, children, pregnant and lactating women, and the elderly are the leading groups affected by malnutrition. In adults, chronic diseases such as obesity, hypertension, cardiovascular diseases, diabetes and cancer are the majority. In order to have an adequate and balanced diet, it is necessary to maintain body weight appropriate for height, to consume less saturated fat, to reduce daily cholesterol intake, to reduce sugar and salt consumption, to consume vegetables, fruits, legumes, whole grain products, milk and dairy products and poultry meat products more frequently (Baysal, 2007). When chicken meat, which has an important place in healthy nutrition recommendations, is evaluated in terms of nutrients, it provides lower energy, is a good quality protein source and contains less fat and saturated fat (Table 1) (Cance Widdowson 1998). Energy and Nutrient Content of Raw Chicken Meat / 100 g There are many factors affecting the consumption of chicken meat, which has an important place in our nutrition in our country (Table 1). Regional development differences, consumer income level, socioeconomic and demographic characteristics, individual tastes and habits, product price and food safety factors affect consumer preferences (Aral et al. 2011).

Table 1. Nutritional value of poultry meat (Cance Widdowson 1998).
Tablo 1. Kanatlı etinin besin değeri (Cance Widdowson 1998).

	Chicken Breast Meat	Chicken Thigh Meat	Meat With Skin
Energy (kcal)	116	126	230
Protein (g)	21,8	19,1	17,6
Fat (g)	3,2	5,5	17,7
Sodium (mg)	72	89	70
Potassium (mg)	330	300	260
Calcium (mg)	10	11	10
Iron (mg)	27	22	20
Copper (mg)	0,5	0,9	0,7
Copper (mg)	0,14	0,25	0,16
Zinc (mg)	0,7	1,6	1,0
Vitamin B6 (mg)	0,53	0,30	0,30
Folic acid	8	12	7
Biotin (mcg)	2	3	2
Pantoneic acid (mg)	1,2	1,3	0,9
Thiamine (mg)	1,10	0,11	0,08
Riboflavin (mg)	0,10	0,22	0,14

In Turkey, the broiler meat sector employs 600,000 people, including raw material producer farmers, tradesmen related to the sector, feed, medicine-vaccine, sub-industry, transportation and marketing, and provides the livelihood of approximately 3 million people with their families. The sector has achieved an increase in production, consumption and especially exports with a turnover of 5.5 billion dollars as of 2024 (Besd-Bir 2024). The purpose of this article is to reveal the effect of per capita broiler consumption by evaluating data related to the broiler industry from 1996 to the present. To this end, the relationships between feed production, feed costs, the number of animals slaughtered, and per capita chicken consumption data have been examined.

MATERIAL and METHODS

This study was conducted by evaluating the data of the Ministry of Agriculture and Rural Affairs and TÜİK. The data includes the total amount of compound feed produced, compound feed cost, number of animals slaughtered and broiler meat consumed per capita for broiler chickens from 1996 to 2024.

Table 2. Poultry meat data (Ministry of Agriculture and Forestry and TUIK)

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Years	Broiler Chicken Feed (Ton)	Feed Prices (Ton TL)	Number Of Animals Slaughtered	Broiler Meat Consumption Per Person Kg
1996	1046602	38	99 073 900	8,64
1997	1093620	73	104 870 702	9,47
1998	1150694	102	167 275 380	9,37
1999	1532056	143	167 862 730	9,77
2000	1912270	173	193 459 280	11,05
2001	1503561	305	161 899 442	9,6
2002	1645791	413	188 637 066	9,98
2003	1845421	486	217 133 076	12,12
2004	2131884	548	238 101 895	12,16
2005	2127088	489	257 221 440	15,03
2006	2026069	495	286 121 360	14,42
2007	2471075	590	205 082 159	15,23
2008	2886165	708	180 915 558	16,94
2009	2967431	740	163 468 942	17,4
2010	3593576	770	163 984 725	17,5
2011	4031302	920	158 916 608	18,03
2012	4224111	970	169 034 283	18,79
2013	4083687	1160	177 432 745	18,94
2014	3979945	1260	199 976 150	19,54
2015	4779916	1210	213 658 294	20,29
2016	4566237	1190	220 322 081	20,01
2017	4753989	1380	221 245 322	21,73
2018	5306118	1790	229 506 689	20,89
2019	5363209	2080	221 841 860	20,47
2020	5397526	2720	258 046 340	20,5
2021	5542974	4540	270 393 122	20,68
2022	6022932	10070	251 289 799	21,95
2023	5829005	12730	254 147 577	22,21
2024	6131807	16960	261 183 314	22,85

In this study, data were analyzed with SPSS 2024 package program. The relationships between broiler compound feed (tons), compound feed price (dollar), number of slaughtered animals (number of animals) and poultry meat consumption (per capita /kg) in Turkey over the years (1996-2024) were examined by quadratic regression analysis. As a statistical model

Y: μ+5,753E-6X1--4,372E-13X22 used.

in order to explains the relationship between feed production (tons) and chicken meat consumption (kg), chicken meat consumption (Y) was taken as the dependent variable and feed production (tons) (X) as the independent variable.

RESULTS and DISCUSSION

In this study, the available data were statistically evaluated and a significant relationship was found between compound feed production and poultry meat consumption per capita ($p<0.005$). The established model explains the effect of poultry meat consumption on compound feed production well (R2: 0.96). As compound feed production increases, the amount of poultry meat consumed per capita also increases. However, this increase tends to decrease relatively when compound feed production, which peaked at 6 million tons in Turkey, is considered together with per capita poultry meat consumption (20-25 per capita consumption/kg) (Table 3). This decreasing trend approximately coincides with the 2020s. While compound feed production and per capita consumption of chicken meat increased linearly over the years (1996-2020), this increase has developed negatively in recent years. The most important reason for this is the increase in broiler meat exports in recent years. In this way, the meat of the broilers fed with compound feed produced can be explained by the fact that the remaining part of the exported part is consumed domestically. In other words, it is due to the decrease in the amount of broiler meat consumed domestically. Looking at the export figures, while it was 299 thousand tons in 2012, this figure increased approximately 2.3 times to 697 thousand tons (including broiler meet) in 2022 (Tuik, Besd-bir 2025).

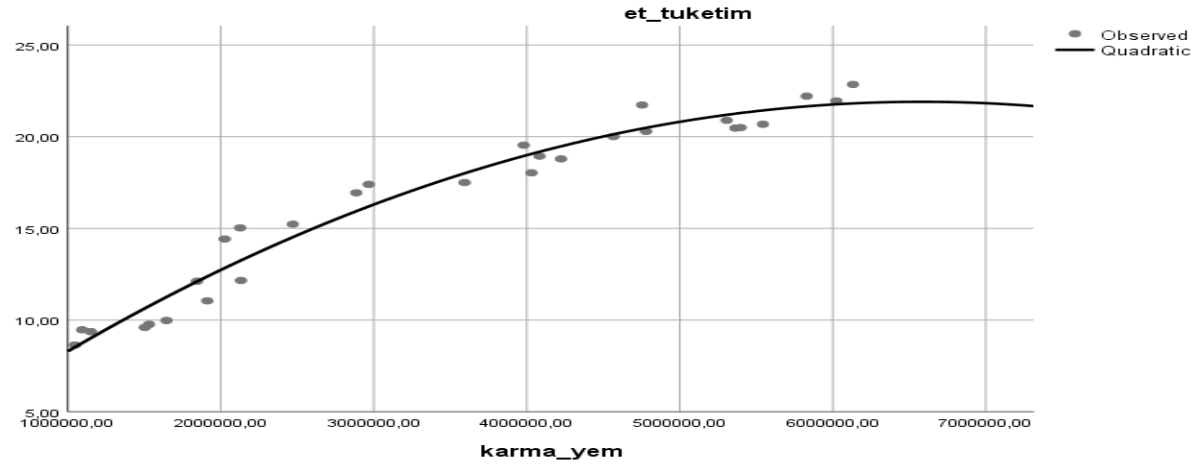

Figure 1. The relationship between compound feed production and per capita poultry meat consumption

Şekil 1. Bileşik yem üretimi ile kişi başına düşen kümes hayvanı eti tüketimi arasındaki ilişki

There are approaches that explain that there may be many reasons for the increase in poultry meat consumption. Karacan (2017) found that while red meat prices increased in Turkey for 2005-2015, demand increased in a similar manner. On the other hand, poultry meat consumption increased more in the same period. In this way, it is reported that if the amount of consumption increases while the price of a good increases, and if the consumption of substitute goods increases at the same time, this situation may be related to income distribution. Uzundumlu et al. (2011) reported that as the income of households increases, both red and poultry meat consumption increases, females are more effective in both red and poultry meat consumption than males, households owning a house increases red meat consumption, while households renting increases poultry meat consumption. As age increases, households' red meat consumption decreases while poultry meat consumption increases. The higher the education level of households, the lower the consumption of poultry and red meat. As the number of individuals increases, red meat consumption decreases while poultry meat consumption increases. When the occupation of the head of the family is a worker, red meat consumption of the household decreases, and when the occupation of the head of the family is a businessman, poultry meat consumption increases. These two studies show that although there are many factors affecting poultry meat consumption per capita, it should not be ignored that the increase in production has a very important share in the production. Akın et al. 2019 with university students, it was determined that 51.7% of the students had a monthly individual food expenditure of less than 250 TL. Again, the proportion of animal food expenditure amounts of 100 TL or less among the individual food expenditures of these students was found to be 59.1%. When the monthly poultry meat consumption rates of the students were analyzed, it was found that 5.8% did not consume poultry meat at all, 31% consumed less than 500 g, and 37.2% consumed between 500-999 g. The students who participated in

the survey reported that they consumed an average of 1.1 kg of poultry meat per month. Cevger ve ark. (2008) (2008) reported that 67.1% of the students spent less than 200 TL for individual monthly food consumption, the rate of those who never consumed red meat types was 4.1% for beef, 42.6% for mutton and 92.6% for goat meat, and the rate of those who consumed 1 kg or more of poultry meat per month was 60.6% for chicken meat, 10.7% for fish meat, and 2.4% for turkey and other poultry meat. In a study conducted by Demir and Aydın (2018) on students, it was determined that 5.8% of the students did not consume chicken meat, 4.7% did not consume eggs, and 18.9% did not consume fish meat at all. On the other hand, the annual per capita chicken meat consumption of the students was 19.6 kg/year, and it was emphasized that the students preferred breast, thigh and wing as the first three in terms of consumption preferences. As a similar result, the average of Turkey in 2018 is 20.89 kg/year per capita. Topçu (2014) divided consumers into 3 groups in his study conducted in Erzurum province. He emphasized that in the group with high poultry meat consumption frequency, there are willing and sensitive consumer segments based on both product image and price sensitivity, while those who consume poultry meat with medium frequency focus on the basic benefit of poultry meat considering sensory and real quality features and the concept that forms their diet under the risk of disease, while those in the group who consume less try to maximize their total benefits through generic branded products. Karakaya and İnci (2014), in their study conducted in Bingöl province, stated that 70% of the surveyed individuals prefer to consume chicken meat, while 30% of them prefer to consume red meat. The individuals who participated in the survey reported that 89% of them bought chicken meat from well-known brands and packaged, while 11% bought unbranded and unpackaged chicken meat. Sayılı (2006) found that avian influenza affected the amount of chicken meat consumption of consumers (42.86%), whereas some producers (57.14%) were not affected by avian influenza. While the price of the product, the name/brand of the company producing the product, the packaging status of the product, weight, appearance, being a village product and color were important, the freshness of the product and being able to trust the product in terms of health were found to be very important. More than half of the families surveyed thought that the disease would not affect people. Therefore, they did not change their consumption even during the illness period. On the other hand, 42.86% of the consumers reported that they reduced their consumption due to the disease. Tümer et al. (2016) found that the ratio of chicken meat expenditure in total food expenditures was 6.01% and the ratio in total meat expenditures was 25.70%. In the study area, the average monthly household consumption of chicken meat was 3.19 kg, red meat 2.12 kg and fish meat 1.35 kg. They emphasized that the most important factors affecting chicken meat consumption are household income, number of individuals in the family and gender of the consumer.

When the data in the study are examined, all values increase steadily over the years (Table 2). For example, while the amount of compound feed produced in 1996 was 1046602 tons, it was 6131807 tons in 2024, exactly 5.8 times more. Considering the costs, while the cost of 1 ton of feed was 38 TL in 1996, it increased to 16960 TL in 2024, 447.7 times more. When the number of animals slaughtered is analyzed, while 99 073 900 animals were slaughtered in 1996, this figure was 261 183 314 in 2024 and exactly 2.6 times more animals were slaughtered. In fact, the amount of poultry meat consumed per capita (kg), which is the most important issue concerning the consumer, increased from 8.64 in 1996 to 22.85 in 2024 and a positive increase of 2.6 times was achieved. When these data are analyzed, the increases in the number of animals slaughtered and the amount of poultry meat consumed per capita are similar. This should be taken into consideration when evaluating agricultural policies on a national basis. These values mean that the effect of the increase in the number of animals slaughtered on the increase in the amount of poultry meat consumed per capita can be achieved by increasing the number of animals slaughtered in the first place when aiming to increase per capita consumption. Poultry meat consumption is extremely important for human nutrition and these values should be targeted upwards. According to World Health Organization (WHO) data, a healthy person should consume 1 gram of protein per kilogram of body weight per day and 42% of this should be of animal origin (Gürer, 2021). The average weight of men and women in the 35-44 age range in Turkey is reported to be 75.7 kg (TUIK 2025). With an average calculation, a daily intake of 31.7 g of animal protein is recommended for a person weighing 75.7 kg. According to this calculation, considering that 31.7 g of protein can be met with poultry meat, it corresponds to 144 g of poultry meat (protein ratio in poultry meat is 22-24%). When we translate these results into annual poultry meat consumption, we get 52.5 kg. As a result, we see that the amount of poultry meat per capita in 2024 is 22.85 kg, well below the target. Even if we consider that even half of the target is met with poultry meat, it comes out as 26.2 kg. When the price and health effects of poultry meat and other meats are evaluated, the target is expected to be much higher than 50%. In this case, it is extremely important to increase per capita consumption of poultry

meat. When we take this as a target, it is understood that the per capita consumption is directly proportional to the number of animals slaughtered and that the target can be reached more easily by increasing the number of animals slaughtered. In addition, when we think of animal protein sources for a healthy diet, we should not only think of broiler chicken meat or red meat, but also of animal products such as eggs, cheese and yogurt. However, when considering these sources, it should be kept in mind that the amount of protein they contain is lower than meat. Eggs, cheese and yogurt contain 12%, 16-20% and 3-4% protein, respectively. In order to increase the number of slaughtered animals, it is necessary to increase hatchery capacities and broiler houses at the same rate. As a result of these increases, it both provides employment and contributes positively to the national economy.

When the total meat consumption in the world is analyzed, it is seen that we are below both the world and European average in 2022. In the year in question, the world average is 64 kg, while the European average is 99 kg. While our country's total meat consumption in 2022 is 51 kg, poultry meat accounts for 41.1% with 21 kg (Our Word in data 2025). When the world poultry meat consumption is analyzed, it is understood that we are still behind the European averages. While the European average accounts for 26.2% of total meat consumption, per capita consumption is 26 kg. While world poultry meat consumption is 17 kg per capita, it accounts for 26.5% of total meat consumption. When comparing Europe and the world with our country in terms of the total amount of meat consumed, it is useful to consider the share of pork consumed. The world average pork consumption is 15 kg and accounts for 23.4% of the world's total pork consumption, while the European average is 35 kg and accounts for 35.3%. Portugal has the highest meat consumption. Total meat consumption per capita is 151 kg and the amount of poultry meat is 32 kg with 21.1%.

The top ten countries in world feed production in 2022 are China (261.424 million tons), USA (231,538), Brazil (80,094), India (44,059), Mexico (38,857), Spain (35,580), Russia (33,000), Turkey (25,300), Japan (24,797) and Germany (24,506). In total, these countries accounted for 65% of world feed production. Moreover, when combined, their feed production grew by 4.4%, outpacing the overall global growth of 2.3%. The poultry sector experienced a slight decline in laying feed tonnage (-1.4%), while broiler feed production increased (+2.3%). The broiler sector also benefited from increased demand for easy-to-cook proteins due to the closure of restaurants during the pandemic and as an affordable option in the face of rising prices of other meat proteins. The most significant increases in Asia-Pacific were in China and India (Alltech Outlook 2025).

CONCLUSIONS

Export and Domestic Consumption Balance: Although the increase in poultry meat exports is a positive economic development, it should be considered to redirect part of the production intended for export to meet domestic demand in order to increase consumption in the domestic market. This could particularly increase protein access for low-income groups.

Increasing Production Capacity: Considering that the increase in the number of animals slaughtered directly affects per capita consumption, increasing the capacity of hatcheries and broiler production facilities should be a strategic priority. This will support both employment and economic growth.

Consumer Education and Health Perception: The health benefits of poultry meat (high protein, low fat) and its price advantage can be more effectively highlighted through consumer education campaigns. Promoting reliable brand and packaging standards can increase consumer confidence.

Income Distribution and Access: Limited consumption among low-income groups can be supported through social assistance programs or subsidies to increase poultry meat consumption. Access to affordable protein sources should be facilitated, particularly targeting students and the young population.

Global Comparisons and Competition: Although Turkey ranks 8th in the world in compound feed production, it lags behind European and global averages in poultry meat consumption. To close this gap, policies that support demand growth in countries such as China and India (e.g., affordable protein demand) can be used as examples.

In conclusion, increasing poultry meat consumption in Turkey depends not only on production increases but also on a comprehensive approach that takes into account socioeconomic factors and consumer habits. Increases in compound feed production, growth in the number of animals slaughtered, and export-oriented

policies have supported the increase in consumption. However, to achieve the protein consumption targets recommended by the WHO and approach global averages, strategies that encourage domestic consumption should be prioritized. This will both improve public health and increase the economic contribution of the poultry meat sector.

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Effects of Multivitamin, Vitamin E/Selenium and Prostaglandin Injections on Reproductive Performance in Kıvırcık Ewes

ABSTRACT

Objective: This study investigated the effectiveness of the single-dose treatment of three different commercial products on reproductive performance in Kıvırcık ewes during the transition to the breeding season.

Material and Methods: Ninety-eight Kıvırcık ewes aged 3-4 years and weighed from 50 to 55 kg have been used in this study. The ewes used in the study were randomly divided into four different (PGF2α, Vit.E/Se, multivitamin and control) groups. All these injections were applied to all groups one week before the ram-mating process.

Results: The difference between the control group and the treatment groups was statistically significant ($P < 0.01$); however, the difference between PGF2α and Vit.E/Se, and multivitamin treatments was insignificant ($P > 0.05$) in terms of multiple-birth rate. The highest value in terms of litter size was found in the PGF2α treatment group.

Conclusion: In the study, all three treatments were more successful than the control group in inducing estrus and pregnancy in Kıvırcık ewes during the transition from anoestrus period to the breeding season and all ewes healthily gave birth.

Keywords: Sheep, reproduction, PGF2α, vitamin E/Selenium, vitamin A, vitamin D3, vitamin B

Multivitamin, Vitamin E/Selenyum ve Prostaglandin Enjeksiyonlarının Kıvırcık Koyunlarının Üreme Performansı Üzerine Etkileri

ÖZ

Amaç: Bu çalışmada üreme mevsimine geçişte Kıvırcık ırkı koyunlarda döl verim performansı üzerine üç farklı ticari ürünün tek doz uygulanma etkinliği araştırılmıştır.

Materyal ve Metot: Bu çalışmada 3-4 yaşlarında ve 50 ila 55 kg ağırlığında 98 Kıvırcık koyunu kullanılmıştır. Çalışmada kullanılan koyunlar rastgele dört farklı (PGF2α, Vit.E/Se, multivitamin ve kontrol) gruba ayrılmıştır. Tüm bu enjeksiyonlar koç katım sürecinden bir hafta önce tüm gruplara uygulanmıştır.

Bulgular: Kontrol grubu ile uygulama grupları arasındaki fark istatistiksel olarak anlamlıydı ($P < 0.01$); ancak PGF2α ile Vit.E/Se ve multivitamin uygulamaları arasındaki fark çoğuz doğum oranı açısından önemsizdi ($P > 0.05$). Yavru büyüklüğü açısından en yüksek değer PGF2α grubunda bulunmuştur.

Sonuç: Çalışmada, anöstrus döneminden üreme mevsimine geçişte Kıvırcık koyunlarında östrus ve gebeliği teşvik etmede her üç uygulama da kontrol grubundan daha başarılı olmuş ve tüm koyunlar sağlıklı bir şekilde doğum yapmıştır.

Anahtar Kelime: Koyun, üreme, PGF2α, vitamin E/Selenyum, vitamin A, vitamin D3, vitamin B

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INTRODUCTION

Various synchronization protocols are implemented in the off-breeding season, aiming to generate the estrus response, ensure pregnancy, and improve fertility since ewes develop estrus seasonally (Abecia et al. 2012). Applying progesterone-containing vaginal equipment in conjunction with an pregnant mare serum gonadotropin injection is a typical synchronization method among ewes (Zohara et al. 2014; Swelum et al. 2015). However, some public concerns such as animal health and welfare, food safety, and environmental impact have recently brought forward reconsidering the use of the progestogen-based protocols of vaginal apparatus used for this purpose (Gonzalez-Bulnes et al. 2020; Ozis Altincekic et al. 2021). At this point, prostaglandin (PGF2α) use becomes more popular due to its cheaper and an environmental pollutant than progestogen intravaginal apparatuses (Fierro et al. 2013). Additionally, since prostaglandins are metabolized rapidly in the lung, they leave no residue in the body (Davis et al. 1980). Therefore, using PGF2α or its analogs among sheep is an alternative synchronization method.

Vitamins are natural compounds with unique properties allowing animals to function optimally. It is well established that vitamin treatments alleviate the harmful impacts of the existing conditions in animals and improve animal productivity (Marai et al. 2008). Animals cannot produce fat-soluble vitamins within their bodies; thus, A, D3, and E vitamins should be outsourced regularly to meet their physiological requirements and sustain high-performance standards (Hafez 2012). In addition to their impacts on growth and physiological processes, vitamins and minerals play a critical role in the reproductive performances of animals (Gabryszuk and Klewiec 2002). According to literature, vitamins A and E have specific functions on ovarian and uterine activities; concurrently, they are indispensable for the body's antioxidant mechanism defending animals against diverse stressors such as the mating season and high ambient temperature (Chauhan et al. 2014). Vitamin D3 plays a critical role in follicle development, oxidative stress, and the generation of steroid hormones, also responsible for the hemostasis of calcium (Ca) and phosphorus (P), which plays a role in fetal development (Yao et al. 2017). B group vitamins in ewes are essential for sustaining animal welfare, ensuring healthy lambing, and functioning efficient reproduction and immune system (Vijayalakshmy et al. 2018). Vitamin C supplement in ewes improves fertility and contributes to lambs gaining live weight (Haliloğlu and Serpek 2000). Selenium (Se) and vitamin E supplements play an essential role in ewes' reproduction and affect the performances of the ewes and lambs equally (Rooke et al. 2008). Injection of Se supplement two weeks before ram-mating with insufficient Se initial status raised their serum Se levels (Ramírez-Bribiesca et al. 2004). The Se supplement is also a component of selenoprotein involved in metabolism, immunity, and other bodily processes (Hefnawy and Tórtora-Pérez 2010). As a result, studies suggested that vitamin E or Se supplements lowered the oxidative stress generated during estrus synchronization practices; consequently, the fertility rate improved conceivably (Sönmez et al. 2009; Kuru et al. 2016). Recent studies also defined the Se supplement as the most critical factor in enhancing animal health and welfare (Surai 2006). However, vitamin treatment in previous studies was applied to ewes with synchronized estrus during the removal of the sponge (Köse et al. 2013), right before the insertion of the sponge into the vagina (Kuru et al. 2017), or while inserting, removing and after the removal of the sponge (Awawdeh et al. 2019).

The objective of this study is to evaluate the effects of PGF2α, Vit.E/Se, or multivitamin injections on fertility and lamb performance in Kıvırcık sheep. For this purpose, pregnancy rate, lambing rate, multiple rate, fecundity, litter size, survival rate, pregnancy prolificacy and total prolificacy were determined as reproductive parameters.

MATERIAL and METHODS

Location

This study was performed at the Application and Research Farm of the Agricultural Faculty, Uludag University in Bursa, a Province in the northwestern region of Turkey. The farm was located at the humid lowland tropics at an altitude of 100 m above sea level, and at longitude 29 °E and latitude 40 °N.

Animals and management

Ninety-eight Kıvırcık ewes aged 3-4 years and weighed from 50 to 55 kg have been used in this study. Before mating, all the ewes grazed the rangeland during the day and were kept in a shelter at night. The ewes were grazed on a pasture consisting of a mixture of common vetch (Vicia sativa L), Hungarian vetch (Vicia

pannonica L), alfalfa (Medicago sativa) and sainfoin (Onobrychis sativa). Minerals salt lick and clean drinking water provided ad libitum. To meet their increased nutrient needs in late pregnancy, the ewes were given 300-400 g from concentrate feed prepared on the farm per ewe that was in addition to ad libitum access to pasture according to the seasonal conditions (Table 1). The metabolizable energy content of the supplement was calculated according to NRC (2007). In Bursa-raised Kıvırcık eves, the estrus season begins in September and lasts through the end of January. As a result, injections and the ram-mating processes were executed in 25th of August; accordingly, this period was designated as the beginning of the breeding season.

The birth process ended in the February-March season. In addition to grazing, approximately 300 to 400 g of concentrate feed was provided per ewes to meet their growing nutritional needs during the early stages of pregnancy. After measuring their birth weights, the lambs received colostrum and had their ear identity numbers immediately in the first postnatal hour. The ewes and their lambs were kept together for about two weeks postnatal, and the lambs received no supplementary feeding. After two weeks, while lambs stayed in the sheep pen when ewes were grazing during the daylight, they were allowed to circle in their mothers in the evening and switched to a feeding system called creep-feeding. When the lambs were three months old, they were weaned and separated from their mothers.

Table 1. Ingredients and chemical composition of diet for Kıvırcık ewes
Tablo 1. Kıvırcık koyunları için diyetin içeriği ve kimyasal bileşimi

Ingredients	Content, %	Chemical Composition	Content, %
Barley	46,0	Dry matter, %	91,30
Corn	25,6	Organic matter, %	84,83
Sunflower meal	26,0	Crude protein, %	15,11
Limestone	1,2	Crude fat, %	3,14
Salt	1,0	Crude ash, %	6,47
Mineral-vitamin premix*	0,2	Metabolic energy, kcal/kg DM	2712

***150 mg ZnSO47H2O, 80 mg, MnSO4H O, 200 mg MgO, 5 mg CoSO47H2O, 1 mg KIO3, 4000, IU vitamin A, 1000 IU vitamin D ve 20 IU vitamin E**

Study design

All ewes were clinically normal with a healthy appearance. The ewes used in the study were randomly divided into four different groups. The first group (n=26) was treated by 12.5 mg/ewe PGF2α injection (Dinolytic, Zoetis). Doses of 12.5 mg of dinoprost (PGF2α)/animal (equivalent to 1ml of medicament). In the second group (n=26), however, ewes were injected with 5 ml/ewe of Vitamin E/Selenium solution (Vit.E/Se) (Yeldif, Ceva) intramuscularly. The third group (n=26) had an injection of a 5 ml/ewe multivitamin solution (Vitaflash, Aksu) intramuscularly as stated in the prospectus. All these injections were applied to all groups one week before the ram-mating process. The fourth group (n=20) contained the ewes for the control group (Table 2). After 24 hours of the treatment, six rams joined the herd as part of the free ram-mating, and they remained there for 34 days (two ram-mating cycles). Rams that were 4 years old and had a condition score of 3 were used in mating.

Table 2. Treatment groups
Tablo 2. Uygulama grupları

Groups	Treatments	Number of ewes (head)
I*	PGF2α (1 ml/ewe)	26
II**	Vit.E/Se (5 ml/ewe)	26
III**	Multivitamin (5 ml/ewe)	26
IV	Control	20

***: 12.5 mg dinoproston tromethamine in 1 ml**
****: 1 mg sodium selenate, 60 mg Vitamin E, 40 mg Vitamin B1 in 1 ml**
*****: 50.000IU vit-A (palmitate), 25.000IU vit-D3, 4mg vit-E (acetate), 2.5mg vit-B1, 2mg vit-B2, 12.5 mg vit-B3, 1.25mg vit-B6, 30mcg vit-B12, 2mg vit-C, and 3mg D-panthenol in 1 ml**

Data collection

Ram-mating and lamb birth dates, as well as lamb birth and postpartum records, were routinely documented. Descriptive values of reproductive parameters were calculated as follows (Kaymakçı 2006).

•*Pregnancy rate (%): Number of pregnant ewes/number of ewes in the group × 100*

•*Lambing rate (%): Number of ewes giving birth/number of pregnant ewes × 100*

•*Multiple rate (%): Number of ewes giving birth to multiple/ number of pregnant ewes × 100*

•*Fecundity (head): Number of lambs born/total ewes mated*

•*Litter size (head): Total number of lambs/number of ewes giving birth*

•*Survival rate (%): Number of living lambs/number of lambs born × 100*

•*Pregnancy prolificacy, kg: Total weight of lambs live-born from every 100 ram-mated ewes.*

•*Total prolificacy, kg: Total weight of lambs obtained from every 100 ram-mated ewes at weaning time.*

Data Analyses

A Chi-square test was used to determine the effects of application methods on reproductive parameters (pregnancy rate, multiple birth rate and survival rate) in ewes (Minitab 19.0).

RESULTS and DISCUSSION

The use of prostaglandins for estrus synchronization in ewes has become more popular with its low cost, ease of application, and ability to minimize vaginal deformations during insertion and removal of vaginal apparatus (Ozis Altincekic and Koyuncu 2017; Adnane et al. 2018). Application of the PGF2α during the anoestrus period aims to induce estrus, initiating a new follicular phase with the disappearance of the corpus luteum and, therefore, releasing progesterone (Kaymakçı 2006). Some literature, such as Doğan and Nur (2006), Ataman et al. (2006), and Yadi et al. (2011), achieved a pregnancy rate of 57.1%, 84.6%, and 70% with the PGF2α injection in anoestrus ewes, respectively. Demiral et al. (2014) reported a 72.1% lambing rate with the PGF2α injection in ewes, indicating that the PGF2α treatment eventuated a more effective response, specifically in the anoestrus periods of ewes previously given birth. Yadi et al. (2011) discovered a 42% multiple-birth rate via PGF2α treatment, stating that the PGF2α was more effective than other synchronization approaches, primarily during the anoestrus season. Ozis Altincekic and Koyuncu (2017) reported comparable and reasonable results with the PGF2α injection in anoestrus ewes regarding fertility criteria with traditional synchronization methods. Similarly, PGF2α treatment in this study led to a 42% pregnancy rate in ewes within the initial few days of the ram-mating; furthermore, 33% more of the ewes also became pregnant during the first ram-mating cycle. Accordingly, PGF2α injection may successfully stimulate the estrus during the transition from anoestrus to the breeding season. Titi et al. (2010), Mirzaei et al. (2017), and Dursun (2019) reported that the PGF2α-based protocol increased estrus rate, lambing rate, and litter size during anoestrus season, boosting the profitability of sheep herd ($P<0.01$). On the other hand, Ayaseh et al. (2021) reported that PGF2α treatment during the anoestrus season appeared to raise the lambing rate in the groups; however, the difference was insignificant in terms of multiple-birth rates, litter size, and BW when compared to the control group ($P>0.05$). This study, however, found that while PGF2α injection performed similarly to vitamin treatments for pregnancy rate and lambing rate, it had significantly higher performance than the control group, outperforming both the other application groups and the control group in terms of multiple rates, fecundity and litter size ($P<0.01$). PGF2α may induce ovulation through a mechanism independent of luteolysis, i.e., by increasing the sensitivity of the pituitary to GnRH, thus increasing LH pulsatility (Randel et al. 1996). Therefore, the possibility that the administered Dinoprost may have acted as a stimulus for cyclicity induction should not be excluded.

Figure 1. The effects of different practices on pregnancy rate, twinning rate and survival rate of lambs in the period before the ram-mating process in ewes
Şekil1. Koyunlarda koç katım öncesi dönemde farklı uygulamaların gebelik oranı, ikizlik oranı ve kuzuların yaşama gücü oranına etkileri

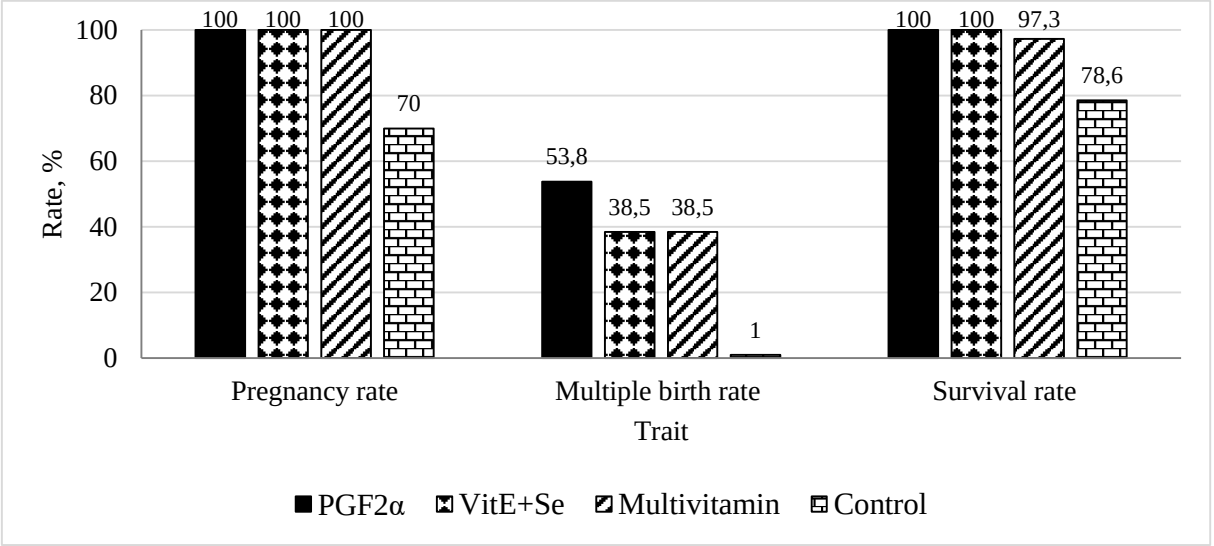

Table 3 illustrates the findings acquired on fertility parameters among the experimental groups in the study. Accordingly, while no multiple births were observed in the control group, multiple births were observed in the PGF2α, Vit.E/Se and multivitamin treatment groups. The difference between the control group and the treatment groups was statistically significant (P<0.01); however, the difference between PGF2α and Vit.E/Se and multivitamin treatments was insignificant (P>0.05) in terms of multiple-birth rate. The highest value in terms of litter size was found in the PGF2α treatment group with 1.54, followed by the multivitamin (1.42), Vit.E/Se (1.38), and control (1.00) groups. The viability (survivability) rate of the lambs until the weaning time was considerably lower (78.6%) in the control group compared to the other groups, where it was 97.3% in the multivitamin group and 100% in the PGF2α and Vit.E/Se groups. While the vitamin E/Se groups had the highest pregnancy prolificacy, the multivitamin group had the highest total prolificacy.

Table 3. Fertility parameters in Kıvırcık ewes
Tablo 3. Kıvırcık koyunlarında döl verim parametreleri

	Treatments					
Parameters	***PGF2α (1ml/ewe)***	***Vit.E/Se (5 ml/ewe)***	***Multivitamin (5 ml/ewe)***	***Control***	***P-value***	***χ^2***
Pregnancy rate (%)	*100.0 [a]*	*100.0 [a]*	*100.0 [a]*	*70.0 [b]*	*0.00*	*24.9*
Lambing rate (%)	*100.0*	*100.0*	*100.0*	*100.0*		
Multiple birth rate (%)	*53.8 [a]*	*38.5 [a]*	*38.5 [a]*	*0 [b]*	*0.01*	*11.4*
Fecundity (head)	*1.54*	*1.38*	*1.42*	*0.70*		
Litter size (head)	*1.54*	*1.38*	*1.42*	*1.00*		
Survival rate (%)	*100.0 [a]*	*100.0 [a]*	*97.3 [a]*	*78.6 [b]*	*0.00*	*17.8*
Pregnancy prolificacy,	*442.5*	*464.7*	*428.2*	*391.33*		
kg: Total prolificacy, kg:	*2338.3*	*2379.5*	*2452.7*	*2.010*		

Studies indicate that Vit.E/Se application facilitates the steroid formation process by acting as an antioxidant before mating and triggers folliculogenesis in the ovaries by stimulating the secretion of gonadotropin from the anterior pituitary gland (Ullah et al. 2020). Thus, Vit.E/Se promotes neutrophil function and enhances uterine health by stimulating ovarian activity. Vit.E/Se also augments fertility by diminishing mortality in the early embryonic period (Amin et al. 2016). Hemingway (2003) reported that Vit.E/Se supplement before mating improved the pregnancy rate among cattle and sheep by reducing early embryonic mortality. Consequently, this study found that Vit.E/Se treatment resulted in pregnancy among 34% of the ewes in the first days of ram-mating, 54% during the first ram-mating cycle, and 12% in the second cycle. Therefore, it is safe to state that Vit.E/Se treatment is a successful stimulus in estrus and that it is synchronized to a large extent in ewes in the transition period. Koyuncu and Yerlikaya (2007) reported that Vit.E/Se injection boosted the fecundity in

Karacabey Merino sheep. Similarly, Munoz et al. (2008) emphasized that the Se element is critical for successful fertilization, implantation, and fetal development. Musa et al. (2018) also noted that injection of Vit.E/Se in Yankasa ewes significantly enhanced reproductive performance by increasing pregnancy, lambing, and fertility rates. The findings of this study, such as all ram-mated ewes got pregnant, gave birth successfully, had the highest pregnancy prolificacy rate in the group receiving Vit.E/Se injection, and all newborn lambs reached the age of weaning healthy, supported the results of the studies described above. Although the multiple-birth rate in the Vit.E/Se and multivitamin groups were similar, it was substantially higher than the control group ($P<0.01$). It could be due to the potential effect of selenium on antral follicles, granulosa cells, and oocytes by regulating the activity of glutathione peroxidases and the ability of Vitamin E to avert oxidative base damage in sheep ovarian epithelium and enhance the uterine environment by preserving the functional integrity of the membranes (Murdoch and Martinchict 2004).Awawdeh et al. (2019) also indicated that while Vit.E/Se injection in Awassi sheep reduced pregnancy losses from 44.8% to 24.3% ($P = 0.08$), it yielded no substantial effect on multiple-birth rate ($P>0.20$). Gabryszuk and Klewiec (2002) reported that Vit.E/Se treatment before ram mating had no impact on estrus rate, fertility and fecundity in Polish Merino ewes compared to the control group ($P>0.05$). Such differences between study results can be caused by several variables such as number of dosages, methods of administration (feed or injection), duration of administration (before and/or after ram mating) and frequency of administration.

Hashem et al. (2016) reported that vitamin A and C supplements before ram-mating increased the number of ovulatory follicles and estradiol (E2) synthesis. Similarly, Abdelrahman and Al-Karablieh (2002) discovered that vitamin AD3E injected in two dosages, 2-3 weeks before and two months after the ram-mating process, increased the fertility rate (82.1%) and lambing rate (86.7%) of Awassi sheep in comparison to the control group (74.0% and 77.9%, respectively). Additionally, these scientists found no statistically significant difference between the treatment and control groups for lambs' birth weights and weaning weights ($P>0.05$). In their analysis of the effects of AD3E vitamins on fertility in Pırlak sheep with synchronized estrus, Birdane and Avdatek (2020) found that the vitamin-treated group had a pregnancy rate of 87.5% and a litter size of 1.54%, compared to the control group's 75% and 1.37%, respectively; however, these increments in rates were statistically insignificant ($P>0.05$). In contrast to the rates observed in both studies, the multivitamin group yielded higher pregnancy and lambing rates in the current study. Besides, multivitamin treatment resulted in pregnancy among 88% of the ewes in the initial days of the ram-mating process. In this context, it is conceivable to argue that the multivitamin is more effective in stimulating and synchronizing estrus in the transitional period than the PGF2α and Vit.E/Se. It is also worth mentioning that despite being comparable to the Vit.E/Se treatment group for pregnancy, lambing, and multiple birth rates, the multivitamin-treated group had significantly higher rates than the control group ($P<0.01$). Vitamins A, C, and E act as antioxidant agents; improving follicle quality or raising the number of healthy follicles may have contributed to such an outcome (Kamiloğlu et al. 2006; Nayyar and Jindal 2010). It may also be related to the fact that vitamin AD3E injection improves animal performance by accelerating sex hormone levels, simply evoking sexual behavior (Al-Asadi et al. 2020). As another issue, the multivitamin group yielded better litter size and fecundity success than both Vit.E/Se and control groups. Whaley et al. (1997) reported that the vitamin A treatment did not reproduce the number of CLs; instead, the larger litter size may be attributable to vitamin A's advancing effect on the number of oocytes and other mechanisms such as improved embryonic survival. Jindal et al. (1996) discovered that vitamin A treatment before mating elevated serum progesterone concentrations and improved early embryonic survival; consequently, it yielded an increment in litter size. Hashem et al. (2016) reported that vitamin A-driven progesterone production during early pregnancy increased fecundity and lambing rate, boosted embryo survival and/or quality, supported the maturation and function of oviducts, uterus, and placenta, and facilitated blastocyst development. Fisher and MacPherson (1991) reported that vitamin B12 supplements played a critical role in embryonic development in ewes, and lambs born from these ewes were highly active, began milking their mothers earlier, and had lower morbidity and mortality rates. Vitamin B2 deficiency, on the other hand, led to a slowing of adolescent growth, and anomalies such as premature birth, stillbirth, and neonatal losses in pregnant animals (Frank et al. 1984). Therefore, higher results for litter size, fecundity, and total prolificacy in the multivitamin group than in the Vit.E/Se group is attributable to the effects of vitamins A and B.

CONCLUSIONS

In conclusion, all ewes in the treatment groups-PGF2α, Vit.E/Se, and multivitamins-got pregnant and delivered healthily. All the injections positively affected ewes' reproductive performance at the beginning of the breeding season. Since the cycles of the sheep were unknown at the time of these injections, it can be assumed that the 34-day relationship of the ewes with the rams and thus the ram effect may have contributed to the occurrence of estrus and the high pregnancy rates. Therefore, such injections can be part of an effective program to improve reproductive performance in sheep breeding by replacing the traditional use of progestagen-containing apparatus + eCG as low-cost, safe and practical applications that provide benefits in terms of animal health and welfare. In addition, these findings will help further explore the frequency, timing, and amount of vitamin injection that may improve the reproductive performance of the ewe. In future studies evaluating the effects of various vitamin levels on reproductive performance, reproductive hormone levels should also be taken into account.

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Juniper Tar Liquid Extract in Whole Milk: Effect on Oxidative Stress, Immune Response, Gut Flora and Renal Health in Holstein Calves

ABSTRACT

Objective: Calves frequently suffer from digestive and respiratory diseases during the suckling period, leading to developmental issues, mortality, and economic losses. This study aimed to evaluate the effects of Juniper liquid extract (JLE) supplementation in whole milk on the health and growth performance of suckling Holstein calves.

Material and Methods: Sixteen newborn Holstein calves were randomly assigned to 4 treatment groups (n=4 per group): Control (G1): Whole milk (WM)+ Calf starter (CS); G2: 1.25% JLE+WM+CS; G3: 2.5%JLE+WM+CS; G4: 5% JLE+WM+CS. Calves were monitored for digestive and respiratory diseases, gut microbiota, renal health, oxidative stress and immune response parameters. The experiment was terminated by weaning the calves when they consumed 800 g/d of calf starter for 3 consecutive days.

Results: Juniper tar liquid extract supplementation significantly reduced digestive and respiratory disease incidence. It suppressed the growth of gut pathogenic bacteria at weaning without affecting lactic acid bacteria. No adverse effects of JLE supplementation were observed on renal functions. JLE decreased oxidative stress levels, while antioxidant defence enzyme activity showed a non-significant increase. Immunoglobulin A, G, and M levels significantly increased, with the best results observed in the 1.25% JLE group.

Conclusion: JLE, a by-product, can be safely used in calf nutrition to improve health by reducing disease incidence, modulating gut flora, and enhancing immune response. The 1.25% JLE supplementation provided the most effective results.

Keywords: Holstein calves, juniper, liquid extract, oxidative stress, immune response, gut flora

Katran Ardıcı Sıvı Ekstraktı Ilavesinin Holstein Buzagılarda Oksidatif Stres, Bagısıklık Tepkisi, Bagırsak Florası ve Böbrek Saglıgı Üzerine Etkisi

ÖZ

Amaç: Buzağılar, emme dönemi boyunca sindirim ve solunum sistemi hastalıklarına sıkça maruz kalmaktadırlar, bu durum gelişim sorunlarına, ölümlere ve ekonomik kayıplara neden olmaktadır. Bu çalışma, tam yağlı süte ilave edilen katran ardıcı sıvı ekstraktının (JLE) emme dönemindeki Holstein buzağıların sağlığı ve büyüme performansı üzerine etkilerini değerlendirmeyi amaçlamıştır.

Materyal ve Metot: Yeni doğan 16 Holstein buzağı rastgele dört deneme grubuna ayrılmıştır (n=4/grup): Kontrol (G1): Tam yağlı süt (TYS)+Buzağı başlangıç yemi (CS); G2: %1.25 JLE+TYS+CS; G3: %2.5 JLE+TYS+CS; G4: %5 JLE+TYS+CS. Buzağılar sindirim ve solunum sistemi hastalıkları, bağırsak mikrobiyotası, böbrek sağlığı, oksidatif stres ve bağışıklık sistemi parametreleri açısından takip edilmiştir. Buzağılar ardışık 3 gün 800 g/gün buzağı başlangıç yemi tükettiklerinde sütten kesilerek deneme sonlandırılmıştır.

Bulgular: JLE takviyesi, sindirim ve solunum hastalıklarının görülme sıklığını önemli ölçüde azaltmıştır (P<0.05). Sütten kesim döneminde bağırsaktaki patojenik bakteri gelişimi baskılanırken laktik asit bakterilerinin gelişimini ise etkilememiştir. Böbrek fonksiyonları üzerine JLE ilavesinin olumsuz etkisi gözlemlenmemiştir. Oksidatif stres seviyeleri azalırken, antioksidan savunma enzim aktivitesinde önemli olmayan bir artış gözlemlenmiştir. IgA, IgG ve IgM seviyeleri önemli ölçüde artış göstermiştir. En iyi sonuçlar %1.25 JLE grubunda elde edilmiştir.

Sonuç: Bir yan ürün olan JLE, hastalık görülme sıklığını azaltarak, bağırsak florasını düzenleyerek ve bağışıklık sistemini güçlendirerek sağlığı iyileştirmek için buzağı beslenmesinde güvenle kullanılabilir. En etkili sonuçlar %1.25 JLE takviyesinde gözlemlenmiştir.

Anahtar Kelime: Holstein buzağı, ardıç, sıvı ekstrakt, oksidatif stres, bağışıklık sistemi, bağırsak florası

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INTRODUCTION

The health and management of calves, which are the future of the dairy herd, are important components of overall herd profitability. The productivity of the dairy herd may be adversely affected by the negative growth of calves, the decreased future milk yield of animals with chronic diseases during the milk suckling period, the spread of infectious diseases from calves to adult cows, increased veterinary costs and limited genetic selection due to high mortality of replacement animals (Lorenz et al. 2011). Of all animals on a dairy farm, the highest morbidity and mortality rates usually occur in calves before they are weaned. Because the placental transfer of immunoglobulins (Ig) is minimal in ruminants and calves born hypogammaglobulinemic, their disease resistance is low (Senturk 2013). Calf deaths' 7.8% occur during the pre-weaning periods and 1.8% occur post-weaning period. Diarrhoea and digestive problems account for 56.5% of pre-weaning deaths, followed by respiratory problems (22.5%) and other or unknown causes (21.0%). Respiratory problems account for 46.5% of post-weaning deaths, followed by diarrheal problems (12.6%) and other or unknown problems (40.9%) (NAHMS 2007). Antibiotics used in the treatment of these diseases reduce non-pathogenic microorganism counts as well as pathogenic microorganisms. Thus, it causes to stop or regress of growth in the early stages of ruminants (Postema et al. 1987; Soltan 2009). The World Health Organization has suggested that, with the misuse of antibiotics, the microorganism gains immunity to specific antibiotics over time and they cannot be effective in protecting human and animal health. For this reason, the use of antibiotics as feed additives in animal production is prohibited and its use in treatment is restricted in EU countries (Anadon 2006). As a result, researchers began to research growth factors that could be alternatives to antibiotics. For this purpose, extensive research on medicinal and aromatic plants and essential oils obtained from them has been carried out and is still being undertaken. Essential oils have been shown to have no health hazards when consumed by humans and animals, and have been classified as safe additives (FDA 2003).

Juniperus oxycedrus L. type junipers naturally found in Türkiye (Ansin and Ozkan 1993) are rich in essential oils, tannins, flavonoids, resins, lignin and triterpenes (Hegnauer 1986). Extracts of *J. oxycedrus L.* are used in traditional medicine in Europe and many countries of the world (Loizzo et al. 2007). Aromatic oil prepared from these plants is used as alternative medicine due to its anti-inflammatory and anti-cancer properties as well as in soap, cream and lotion production due to its dermatological properties (Loizzo et al. 2007; Skalli et al. 2013; Zhang et al. 2015). In addition, medicines prepared with roots, fruits and leaves of *J. oxycedrus L.* have been used as antiseptics in diseases such as pain, cough, and rheumatism, tuberculosis (Tumen and Hafizoglu 2003). It is also known to be good for diabetes, digestive tract diseases, and respiratory tract diseases such as bronchitis and asthma, renal tract diseases, jaundice, sciatica, sinusitis, liver disorders, metabolism disorders and is used against these disorders (Koc 2002; Gurkan 2003).

It was stated that essential oil had higher antiradical activity and iron-reducing properties than liquid extract in a study comparing essential oil and liquid extract obtained from Juniper fruit and leaves, and the liquid extract showed selective antibacterial properties whereas essential oil completely stopped the growth of both pathogenic and non-pathogenic bacteria at increasing concentrations (Isik et al. 2020). In the study conducted with JLE, it was reported that JLE improves growth performance, increases feed consumption, reduces the incidences of diarrhoea and diseases, and allows healthy calves to be raised (Isik and Ozkaya 2021).

In our previous studies, we investigated the effects of JLE on the growth and general health parameters of calves, as well as the total phenolic content, antioxidant, antibacterial and iron-reducing properties (Isik et al. 2020; Isik and Ozkaya 2021). No studies have been found on the effects of JLE, which is a by-product that is released when extracting essential oil, on the performance, immunity, oxidative stress and antioxidative defence mechanism, intestinal flora, and renal system of suckling Holstein's calves. Therefore, in this study, it was aimed to test the hypothesis of whether or not JLE improved the health of calves as a by-product without a negative impact on these parameters.

MATERIAL and METHODS

The study was conducted at the dairy cattle farm owned by Muzaffer Yılmaz, registred in Yassigume Village, Burdur province. Sixteen Holstein calves were included in the experiment. The number of calves was determined by power analysis. In the power analysis, it was determined that the highest value of the enterobacter count in the intestinal flora in the literature reviews was 6.3, the lowest value average was 4.9 and

the standard deviation was 0.92, and it was determined that 4 animals should be present in each group for 95% power.

A commercially available calf starter was used in the study. The calves were given starter feed ad libitum. The calves were given a total of 4L of milk (2L in the morning and 2L in the evening) in two meals. Calves were weaned and the experiment was terminated when they consumed 800 g/d of calf starter for 3 consecutive days.

Calves born on the farm were fed with colostrum for the first 3 days. Then, 4-day-old calves were randomly divided into 4 groups by taking their live weight and body measurements and housed in individual boxes. Juniper tar liquid extract was obtained according to the European Pharmacopoeia (1975) as reported by Isik et al. (2020). The doses of Juniper tar were determined as a result of the minimum inhibition concentration (MIC) analysis and presented as a percentage of the amount of milk fed to the calves by mixing in the milk (Isik et al. 2020).

Experimental groups were designed as follows; G1: Whole milk (WM) and Calf starter (CS) (Control group), G2: fed with 1.25% JLE supplemented WM and CS, G3: fed with 2.5% JLE supplemented WM and CS, G4: fed with 5% JLE supplemented WM and CS (Isik and Özkaya, 2021).

The total phenolic substance amount of JLE used in the study is 1.85±0.04 mg GAE/g. Juniper liquid extract content includes 55.43% α-cedrol, 20.20% Verbenone, 14.72% Verbenol, 6.07% Borneol and 3.59% Trans-pinocarveol (Isik et al., 2020).

Measurements and Sample Collection.

Crude protein, fat (AOAC, 2006), fibre, moisture and ash (AOAC, 2005) content of CS used in the study were determined (Table 1). Fat, protein, lactose, and dry matter of milk were performed with a HasVet Milk analyser and somatic cell count was performed using SOMATOS Mini (Has Vet Medical, Antalya, Turkiye).

Table 1. Chemical composition of calf starter and whole milk
Tablo 1. Buzağı başlangıç yemi ve tam yağlı sütün kimyasal kompozisyonu

	CS	**Milk**			
		G1	G2	G3	G4
DM, %	90.05	12.00	11.90	11.90	11.90
CP, %	18.17	3.40	3.30	3.30	3.30
Crude Fat, %	2.79	3.50	3.60	3.60	3.60
CF, %	9.41				
Moisture, %	9.95				
CA, %	7.52				
Starch, %	28.25				
ME, kcal/kg	2797.59				
Intensity, %		31.90	31.50	31.10	30.90
Lactose, %		5.10	5.00	5.00	5.00
pH		7.00	7.10	7.10	7.10
SSC, cell/ml		364.10	223.50	247.50	226.50

DM: Dry matter, CP: Crude protein, CF: Crude fibre, CA: Crude ash, ME: Metabolic energy, SSC: Somatic cell count

Faeces samples were collected from all animals at 28-day-old and weaning age. After washing the rectums of the calves with betadine solution, faeces samples were taken into sterile faeces containers (3-5g) before the morning meal. Coliform, E.coli, Enterobacteriaceae, and lactic acid bacteria counts in faeces were performed using ready-made media (3M Health Care, St. Paul, MN, USA) whose results were internationally accepted.

Urine samples were collected from all animals at 28-day-old and weaning age. Urine samples were taken into sterile urine containers by massaging the vagina and penis of the calves. The test stick was completely immersed in the mixed urine sample. Excess urine on the stick removed from the container was cleaned. The strip was allowed to stand for 2 minutes for the reaction to occur. The resulting colours were compared with the chromatic scale provided by the manufacturer. Blood bilirubin, urobilinogen, ketone bodies, glucose, protein, nitrite, leukocytes, pH and specific gravity were measured in the urine. Urine analyses were performed using urinary sticks (Acon Laboratories, Inc. San Diego, CA, USA), of which the results are internationally recognized.

Blood samples from calves in each group were taken from the vena jugular of calves at the beginning of the experiment, at weaning age, and on the 5th day of the weaning program. The blood was collected in gel tubes and centrifuged at 3000 rpm for 10 min. The obtained blood serum was analyzed using the Mindray BS-

300 (Mindray, Shenzhen, P.R. China) biochemical blood analyzer. Creatinine (CREA), Urea, Total antioxidant status (TAS), paraoxonase-1 (PON-1), total thiol (TTL), native thiol (NTL), thiol/disulfide homeostasis (TDH), catalase (CAT), superoxide dismutase (SOD) which are markers of antioxidative defence mechanisms and Malondialdehyde (MDA), total oxidant status (TOS) and oxidative stress index (OSI) which are markers of oxidative stress were examined. Immunoglobulins (IgA, IgE, IgG) were also examined. TDH (µmol/L), TAS (mmol/L), TOS (µmol/L), and PON-1 (U/L) tests were measured using commercially available kits (Rel Assay Diagnostics, nufacturer Mega Tip, Gaziantep, Türkiye). OSI value was calculated according to the following formula: OSI (arbitrary unit)=TOS (µmol H2O2 equivalent/L)/TAS (µmol Trolox equivalent/L (Yumru et al. 2009). MDA (nmol/L) level was determined by a method based on the reaction with thiobarbituric acid. SOD (U/ml) activity is measured by the inhibition of xanthine and xanthine oxidase reaction. CAT (U/L) is measured at 405 nm wavelength. IgA is measured at the wavelength of 600 nm. IgG is measured at the wavelength of 600 nm. Antioxidative defence mechanisms, oxidative stress markers and immune system were determined by the spectrophotometric method (Otto Scientific Medical, Ankara, Turkiye).

Digestive system and respiratory tract diseases of calves are recorded daily. When the faeces score (Larson et al. 1977) was ≥3 for 2 consecutive days, it was recorded as a digestive system disease in calves. The calves were checked by the veterinarian when the respiratory score (Heinrichs et al. 2003) was ≥3. Those diagnosed with respiratory tract disease were recorded.

Statistical Analysis

Data were analyzed using ANOVA analysis of variance technique. Starting feed consumption age, which did not show normal distribution, was analyzed with the Kruskal Wallis test, and the differences between the groups' means were examined with the Turkey test (MINITAB v20, Minitab LLC, State College, Pennsylvania, USA). The significance level was taken as $P<0.05$.

RESULTS

No significant statistical differences were observed among the groups in terms of the age at which they started ruminating and consuming CS (Table 2). However, calves in G2 began consuming CS earlier than those in the other groups and, consequently, started ruminating earlier.

Table 2. Effect of juniper liquid extract supplementation on health parameters and gut flora of calves

Tablo 2. Ardıç sıvı ekstraktı ilavesinin buzağıların sağlık parametreleri ve bağırsak florası üzerine ekileri

	G1	G2	G3	G4	P
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	
DD, day	8.25±2.39^{a}	0.25±0.25^{b}	2.75±2.43^{ab}	4.50±1.44^{ab}	0.02
ID, day	2.00±1.15^{a}	0.00±0.00^{b}	0.75±0.75^{b}	0.00±0.00^{b}	0.03
SRA, day	16.25±0.95	14.00±1.47	14.50±2.10	16.00±3.57	0.78
SFCA, day	4.78±0.85	4.00±1.00	5.75±2.43	8.50±1.94	0.30
					28-day-old
Coliforms	6.82±0.13	6.37±0.15	6.42±0.09	6.55±0.22	0.22
E. coli	6.82±0.13	6.46±0.17	6.36±0.15	6.52±0.18	0.24
Enterobacter	6.85±0.18	6.46±0.17	6.43±0.16	6.42±0.14	0.23
Lactic acid	6.85±0.03	6.96±0.05	6.92±0.05	6.81±0.08	0.24
					Weaning age
Coliforms	7.13±0.10^{a}	6.58±0.14^{b}	6.36±0.15^{b}	6.39±0.05^{b}	0.00
E. coli	7.10±0.06^{a}	6.59±0.08^{b}	6.54±0.16^{b}	6.23±0.13^{b}	0.00
Enterobacter	7.09±0.11^{a}	6.63±0.12^{ab}	6.13±0.12^{c}	6.33±0.11^{b}	0.00
Lactic acid	6.89±0.05	6.96±0.05	6.93±0.03	6.94±0.05	0.21

DD: Diarrhoea day, ID: Illness day, SR: Starting rumination age, SFCA: Starting feed consumption age

Table 3. The effect of supplementation of juniper tar liquid extract on the kidney-urinary system of calves

Tablo 3. Ardıç sıvı ekstraktı ilavesinin buzağıların böbrek-üriner sistem üzerine etkisi

	G1	G2	G3	G4	P value
	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	
Crea, mg/dL 1	1.26±0.11	1.29±0.05	1.30±0.07	1.41±0.16	0.80
2	1.20±0.04	1.37±0.06[b]	1.37±0.09	1.17±0.06	0.06
3	1.33±0.06	1.23±0.05	1.27±0.07	1.46±0.02	0.31
Urea, mg/dL 1	8.86±2.14	7.51±0.72	7.65±0.86	11.31±1.62	0.28
2	14.80±4.71	13.27±2.88	13.4±4.11	11.18±2.12	0.88
3	17.12±8.93	10.84±1.08	15.70±8.56	9.25±2.76	0.90
Color					
28-day-old	Light yellow	Light yellow	Light yellow	Light yellow	
Weaning age	Yellow	Light yellow	Light yellow	Light yellow	
Blood, Ery/µL					
28-day-old	18.75±6.25	6.25±6.25	12.50±7.22	15.00±6.12	0.14
Weaning age	125.00±72.20	8.75±5.91	0.00±0.00	127.50±7.08	
Bilirubin					
28-day-old	NO	NO	NO	NO	
Weaning age	NO	NO	NO	NO	
Urobilinogen, mg/dL					
28-day-old	0.15±0.05	0.20±0.00	0.20±0.00	0.20±0.00	0.41
Weaning age	0.20±0.00	0.20±0.00	0.20±0.00	0.20±0.00	
Ketone bodies					
28-day-old	NO	NO	NO	NO	
Weaning age	NO	NO	NO	NO	
Glucose					
28-day-old	NO	NO	NO	NO	
Weaning age	NO	NO	NO	NO	
Protein, g/L					
28-day-old	22.50±7.50	15.00±8.66	7.50±7.50	15.00±8.66	0.51
Weaning age	40.00±2.12	30.00±0.00	30.00±0.00	22.50±7.50	
Nitrite					
28-day-old	NO	NO	NO	NO	
Weaning age	NO	NO	NO	NO	
Leukocyte, µL					
28-day-old	0.25±0.25	0.00±0.00	0.00±0.00	0.00±0.00	0.41
Weaning age	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
pH					
28-day-old	7.00±0.58[b]	8.50±0.29[a]	8.25±0.48[ab]	8.13±0.32[ab]	0.03
Weaning age	8.00±0.41[b]	9.00±0.00[a]	8.00±0.41[b]	8.00±0.41[b]	
Specific gravity					
28-day-old	1.02±0.00	1.01±0.00	1.01±0.00	1.01±0.00	0.62
Weaning age	1.02±0.01	1.02±0.01	1.02±0.00	1.01±0.00	

[ab] difference between the means in the same row, NO: Not observed

Supplementation with JLE significantly (P<0.05) reduced the incidence of diarrhoea. The highest incidence of diarrhoea was observed in G1, and the lowest in G2 (Table 2). The average number of days with respiratory diseases was 2 days in G1 and 0.75 days in G3. However, respiratory diseases were not observed in G2 and G4 (Table 2). Supplementation with JLE did not have a significant effect on the pathogen bacteria count, such as coliform, E.coli, Enterobacteriaceae or non-pathogenic bacteria like lactic acid bacteria in the gut of the 28-day-old calves (Table 2). However, while JLE significantly (P<0.05) suppressed the growth of pathogenic bacteria at weaning age, it did not affect the growth of non-pathogenic bacteria.

No significant differences were observed between serum Urea and CREA concentrations. JLE did not affect the urinary and renal systems of calves (Table 3). The colour of the urine samples taken from the calves was almost the same. Bilirubin, ketones, glucose, and nitrites were not observed in the urine samples of any of the groups. Additionally, no difference was found in blood, urobilinogen, protein, leukocyte, pH and Specific gravity values.

Antioxidative defence mechanism markers and immune responses of the groups were not significantly different at the beginning of the experiment (Table 4). However, while MDA, an oxidative stress marker, was significantly different (P<0.05), TOS and OSI were not. The MDA was lowest in G3.

Antioxidative defence mechanisms, except for the TAS value, and oxidative stress markers were not significantly different (Table 4). The effect of JLE on immune responses was not significant. On the 5th day of the weaning program, JLE had no significant effect on the antioxidative defence mechanism or oxidative stress markers. However, supplementation with JLE increased the concentration of IgA, IgG, and IgM, while resulting in a non-significant increase in IgE (Table 4).

Table 4. The effect of juniper tar liquid extract on oxidative stress, antioxidative defence mechanism and immune response of calves

Table 4. Ardıç sıvı ekstraktının buzağıların oksidatif stress, antioksidan savunma mekanizması ve bağışıklık sistemi üzerine etkisi

		G1	G2	G3	G4	P value
		Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	
TAS, mmol/L	1	0.78±0.06	0.96±0.08	1.01±0.08	0.78±0.07	0.09
	2	0.87±0.03^{bc}	0.97±0.03^{ab}	1.00±0.06^{a}	0.85±0.03^{c}	0.03
	3	0.99±0.04	1.06±0.04	1.00±0.07	0.93±0.03	0.30
TOS, µmol/L	1	3.72±0.90	5.82±1.65	10.83±8.14	4.81±1.12	0.68
	2	3.57±0.43	2.46±0.73	3.08±0.17	2.90±0.72	0.66
	3	6.31±1.17	3.61±3.17	12.65±9.61	4.17±0.84	0.60
OSI, arbitary	1	0.46±0.07	0.64±0.22	0.97±0.68	0.63±0.16	0.81
unit	2	0.41±0.04	0.25±0.07	0.31±0.00	0.34±0.09	0.34
	3	0.63±0.12	0.34±0.28	1.27±0.78	0.44±0.10	0.63
PON-1, U/L	1	89.80±55.30	22.00±5.69	64.50±23.90	40.00±10.90	0.54
	2	369.50±59.90	558.00±44.20	447.00±59.50	487.00±123.00	0.43
	3	462.30±82.10	671.30±37.60	568.80±95.90	577.00±195.00	0.49
TTL, µmol/L	1	483.90±17.70	612.00±109.00	827.00±262.00	634.70±24.40	0.44
	2	452.20±51.20	558.30±71.10	409.00±54.40	428.50±74.80	0.41
	3	502.20±59.40	584.30±94.80	904.00±414.00	453.30±51.70	0.48
NTL, µmol/L	1	375.00±21.80	364.00±76.70	488.80±96.30	451.80±52.60	0.52
	2	334.00±53.60	469.20±47.40	304.70±47.20	344.60±72.90	0.25
	3	373.40±76.40	482.20±52.60	574.00±157.00	362.60±56.90	0.44

		G1	G2	G3	G4	P value
		Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	
TDH, %	1	54.50±17.40	124.20±16.20	169.30±93.90	91.40±21.10	0.47
	2	44.60±14.30	59.10±11.20	52.20±12.00	41.90±10.80	0.74
	3	51.10±22.50	64.39±9.02	40.90±9.05	45.30±25.60	0.63
CAT, kU/L	1	89.80±20.70	37.00±14.00	111.50±59.70	72.80±30.90	0.62
	2	57.00±15.50	104.80±16.40	59.30±26.70	85.00±17.60	0.26
	3	94.00±6.36	227.00±145.00	83.50±17.80	112.00±11.80	0.52
SOD, U/ml	1	706.0±176.0	813.0±283.0	1698±1049	855.00±344.00	0.64
	2	781.0±351.0	1290.0±393.0	541.70±35.20	511.00±208.00	0.29
	3	769.0±166.0	1328±945.00	992.0±448.00	387.00±126.00	0.64
MDA, µmol/ml	1	21.42±4.18^{b}	22.48±5.99^{b}	38.97±5.99^{a}	10.12±1.84^{c}	0.01
	2	15.90±9.11	5.91±0.85	11.31±4.24	9.69±3.96	0.64
	3	8.20±2.99	6.36±0.60	16.00±6.47	18.40±6.90	0.14
IgE, IU/ml	1	56.50±28.00	37.27±4.29	59.20±36.70	31.93±8.06	0.83
	2	25.75±5.98	29.23±2.93	22.33±5.77	21.20±6.06	0.71
	3	23.82±5.49	24.63±3.75	24.63±3.02	24.90±3.12	0.97
IgA, mg/dL	1	10.48±4.57	5.60±2.11	8.32±3.25	9.17±5.08	0.88
	2	1.35±0.68	4.80±1.84	1.38±0.58	1.38±0.81	0.09
	3	1.58±0.54^{c}	7.23±2.65^{a}	1.95±0.75^{c}	3.67±3.67^{b}	0.03
IgG, mg/dL	1	4.83±1.05	6.70±1.46	5.50±1.33	6.08±0.94	0.74
	2	3.95±1.07	5.15±0.69	3.25±0.92	3.52±1.07	0.23
	3	3.53±0.88^{b}	7.08±3.14^{a}	3.23±0.50^{b}	4.00±1.40^{b}	0.04
IgM, mg/dL	1	3.48±0.67	3.63±0.91	3.60±3.47	4.63±4.39	0.78
	2	1.35±0.94	3.20±3.20	2.80±2.80	2.08±2.08	0.20
	3	3.23±1.26^{b}	7.00±4.04^{a}	3.02±3.02^{b}	2.00±2.00^{b}	0.44

TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, PON-1: Paraoxonase-1, TTL: Total thiol level, NTL: Native thiol level, TDH: Thiol/Disulfide Homeostasis, CAT: Catalase, SOD: Super oxide dismutase, MDA: Malondialdehyde, Initial, 2: Weaning age, 3: On the 5th day of the weaning program, ^{abc} Difference between averages in the same row.

DISCUSSION and CONCLUSIONS

Calves begin consuming concentrate feed at approximately 1 week of age and increase feed consumption at 2 weeks of age (Morittu et al. 2021). They typically start ruminating about 14 days after birth (Lopreiato et al. 2018). The development of the calf's rumen and digestive abilities influences the onset of rumination (Lopreiato et al. 2018). Rumination affects feed consumption and feed efficiency, which are associated with calf weight gain, health and welfare (Ambriz-Vilchis et al. 2015). Indeed, all calves in the JLE-supplemented groups began CS intake and rumination at a non-significantly earlier age. Tapki et al. (2020) reported that supplementation of oregano oil led to earlier CS consumption in calves. Similarly, green tea and oregano extract have been shown to promote early CS intake (Heisler et al. 2020).

Milk supplemented with JLE improved the health of calves by reducing the incidence of diarrhoea and respiratory diseases. The antiseptic and antibacterial activities of plant extracts enhance feed efficiency by affecting gastrointestinal system development, rumen microbiological activity, and by reducing digestive and respiratory diseases in newborn calves (Cobellis et al. 2016; Siefzadeh et al. 2017; Campolina et al. 2021). The reduction in digestive and respiratory diseases due to JLE supplementation may be attributed to the positive effects of the active components of the extracts on the secretion of endogenous enzymes, improvement of the intestinal environment, maintenance of a balanced intestinal flora, and enhanced liver functions for better

utilization of fats and proteins (Seifzadeh et al. 2017). In fact, it has been reported that plant oil and extracts reduce the incidence of respiratory (Sastir Koroglu and Kocabagli 2019) and digestive system diseases (Campolina et al. 2021) in calves.

Extracts obtained from plant leaves, flowers, and fruits suppress the growth of pathogenic microbes by reducing their effectiveness (Özkaya et al. 2018; Hassan et al. 2020). Plant extracts reduce the activity of pathogenic microorganisms, primarily due to their bioactive substances such as phenolic compounds, flavonoids, alkaloids, tannins and essential oils. These compounds may exert antimicrobial effects against pathogens through various mechanisms. They disrupt the structural integrity of bacteria by altering cell membrane permeability, inhibit proliferation by interfering with protein synthesis and DNA replication, and disrupt metabolic processes by inhibiting enzyme functions (Hassan et al. 2020). Furthermore, some plant components inhibit bacterial communication by suppressing the quorum-sensing mechanism, thus reducing virulence factors (Çepni and Gürel, 2011). Supplementation of JLE suppressed the growth of pathogens at 28-day-old, though not significantly, but had a significant effect at weaning age. The decrease in pathogen count in faeces can be attributed to the fact that plant extracts possess various antibacterial mechanisms, such as enzyme inhibition, cell membrane disruption, substrate deficiency, and inhibition of bacterial colonization (Hassan et al. 2020; Damjanovic-Vratnica et al. 2011; Bahadar et al. 2016). Numerous studies have reported that plant oils and liquid extracts suppress the growth of pathogenic bacteria (Ünlü and Erkek, 2013; Bi et al. 2017; Özkaya et al. 2018; Hassan et al. 2020).

The presence of high levels of non-pathogenic bacteria in the gut, compared to pathogens, improves the gut health of calves (Bi et al. 2017; Özkaya et al. 2018). Supplementation with JLE did not affect the growth of lactic acid bacteria in the gut. Secondary metabolites such as flavonoids, terpenes and phenolic compounds present in JLE exhibit antimicrobial effects primarily against pathogenic bacteria, which are beneficial components of the gut microbiota. In particular, phenolic compounds of plant origin have been shown to suppress the growth of pathogens by disrupting the cell membrane or inhibiting bacterial enzymatic pathways (Cushnie and Lamb, 2011; Daglia, 2012). However, lactic acid bacteria may be more resistant to such compounds or metabolize them in a way that does not adversely affect their growth. Lactic acid bacteria may have developed natural resistance to antimicrobial compounds present in plant extracts. For example, some lactic acid bacteria strains have “efflux pumps” that pump toxic compounds out of the cell (Makarova et al. 2006). They have also evolved mechanisms to reduce the membrane permeability of antimicrobial agents by altering the lipid composition of cell membranes (Papadimitriou et al., 2016). Such adaptations may minimize the effect of potential antibacterial compounds in JLE on lactic acid bacteria. Lactic acid bacteria adhere to the intestinal epithelial surface and produce organic acids such as lactate and acetate. These acids lower the pH of the intestinal environment and prevent the colonization of many pathogens (Seo et al., 2010). However, since lactic acid bacteria are better adapted to this low-pH environment, this may also protect them from the potential effects of antimicrobial compounds in JLE (Gänzle, 2015). Furthermore, the bacteriocins produced by lactic acid bacteria may further promote their growth by reducing the competitiveness of pathogenic bacteria in the gut (Dobson et al., 2012).

Juniper berries and leaves, or their extracts, have traditionally been used as a diuretic. However, prolonged oral use or high doses may not be safe and may lead to kidney and urinary tract problems (Raina et al. 2019). In this study, however, no adverse effects of JLE supplementation on the kidney-urinary tract functions of calves were observed. This finding is thought to be attributed to the fact that the doses administered were not high. No significant changes or adverse effects on kidney function were detected in the JLE supplementation investigations. Specifically, biochemical parameters indicating kidney function, such as CREA and ürea, remained within normal ranges. These parameters are used to assess kidney damage or dysfunction, as they reflect the filtration capacity of the kidneys and overall health status (Özçelik et al. 2014). Other parameters observed showed that the volume of urine was within normal levels, and there were no abnormal changes in urine pH (Akpolat, 2018). These results support the conclusion that supplementation, when used at low doses, does not have harmful effects on the kidneys and does not have a positive impact on kidney function.

Blood serum TOS and MDA concentrations in G2 decreased at the weaning age and on the 5th day of the weaning program, while levels of antioxidative defence mechanism enzymes increased. Concentrations of TOS and MDA which are lipid peroxidation products, indicate the degree of lipid peroxidation (Davey et al. 2005; Hassan et al. 2020; Özkaya et al. 2023). A decrease in TOS and MDA concentrations reflects a reduction in lipid

peroxidation. The increase in antioxidant defence mechanism enzymes supports the antioxidant properties of 1.25% JLE. The rise in antioxidant defence mechanism enzymes enhances the antioxidant capacity of calves by improving the scavenging of free radicals (Wei et al. 2020). Compounds in herbal extracts disrupt radical reactions by combating reactive host components, thereby halting the propagation of the oxidation chain (Amorati et al. 2013; Hassan et al. 2020; Özkaya et al. 2023). Endogenous antioxidant enzymes contribute to intracellular defence against oxidative stress (Ding et al. 2021).

OSI, an indicator of the degree of oxidative stress, was low in G2, although not significantly. High OSI is a result of high TOS and low TAS values (Ogut et al. 2013; Özkaya et al. 2023). Many researchers have stated that OSI increases in parallel with the increase in TOS value (Marcil et al. 2013; Dagulli et al. 2014; Yucel et al. 2015; Özkaya et al. 2023).

Immunoglobulins (IgA, IgG, IgM, and IgE) provide defense to all tissues reached by the blood and prevent the spread of blood-borne infections, septicemia and microorganisms by neutralizing their entry into the circulatory system (Roomruangwong et al. 2017). In this context, adequate levels of IgA, IgG and IgM are critical for a healthy immune response. These immunoglobulins enable the body to mount a more effective defence against pathogens. Notably, this study found that calves fed 1.25% JLE exhibited a significant increase in IgA, IgG, and IgM concentration compared to the control group, suggesting that JLE has immune response-enhancing effects.

Plant extracts are recognized as valuable sources for immune system enhancement. Secondary metabolites in plants, particularly bioactive components such as flavonoids, alkaloids, and terpenes, have been frequently reported to promote immune function. For instance, Qiao et al. (2013) and Özkaya et al. (2018) investigated the antimicrobial and anti-inflammatory properties of plant extracts and demonstrated their positive effects on the immune system. The beneficial effects of plant-derived compounds on immune function may be attributed to various mechanisms, including the neutralization of microorganisms, activation of immune cells, and protection against free radical damage (Lakhani et al. 2019; Kozyr et al. 2019). Furthermore, Wafa et al. (2021; 2022) reported that plant extracts enhance multiple components of the immune response and strengthen defense mechanisms against microorganisms.

The supplementation of JLE enabled calves to initiate rumination and starter intake at an earlier age while reducing the incidence of digestive and respiratory diseases. JLE suppressed the growth of pathogenic bacteria without affecting the proliferation of lactic acid bacteria. The administration of JLE at a dosage of 1.25% per calf per day enhanced the release of antioxidant defense enzymes and reduced oxidative stress markers concentrations. Additionally, JLE supplementation significantly improved immune response. These findings, considering the adverse effects of synthetic antibiotics and antioxidants on both human and animal health, suggest that JLE can be utilized as a natural preservative. Based on its observed effects on health parameters, pathogenic bacteria, antioxidant defence enzymes and immune response, JLE can be regarded as a feed additive that enhances calf health.

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Egg Poultry in Türkiye and Egg-Feed Relationship#

ABSTRACT

Objective: The aim of this study was to analyze the current situation of the laying hen sector and the egg-feed relationship in Türkiye between 1996 and 2024.

Materials and Methods: In this study, the amount of compound feed produced for laying animals (tons), compound feed price (dollar), number of laying animals (number) and amount of eggs produced (thousand eggs) obtained from the Ministry of Agriculture and Forestry and TURKSTAT data for the period 1996-2024 were evaluated. Quadratic regression model was preferred and Pearson correlation analysis was also used.

Results: The p-values of all models used were significant. The effects of layer feed production (ton) on egg production (R2 0.881), layer feed production (ton) on the number of laying hens (R2 0.921) and the number of laying hens on egg production (R2 0.921) were highly significant and positive. In Türkiye, all the relationships between the variables of egg feed, egg feed price, number of laying hens and quantity of eggs produced were found to be significant and high. The highest correlation was found between the amount of eggs produced and the number of laying hens (0.966). Egg feed price was found to be a determining factor with a decreasing effect relative to the other factors.

Conclusion: Although fluctuations in the price of eggs in Türkiye have a negative effect on feed production and the number of laying animals and eggs produced, this level was not statistically significant. In Türkiye, chicken eggs are highly preferred as a cheap source of protein and their consumption can be expected to increase in the coming years with the increase in population.

Keywords: Laying hens, eggs, compound feed, compound feed price, egg-feed relationship

Türkiye'de Yumurta Tavukçuluğu ve Yumurta-Yem İlişkisi

ÖZ

Amaç: Bu çalışmanın amacı, 1996-2024 yılları arasında Türkiye'de yumurta tavukçuluğu sektörünün mevcut durumunu ve yumurta-yem ilişkisini analiz etmeyi amaçlamaktadır.

Materyal ve Method: Bu çalışmada, 1996-2024 yılları arasında Tarım ve Orman Bakanlığı ile TÜİK verilerinden elde edilen yumurtacı hayvanlar için üretilen karma yem miktarı (ton), karma yem fiyatı (dolar), yumurtacı hayvan sayısı (adet) ve üretilen yumurta miktarı (bin adet) değerlendirilmiştir. Kuadratik regresyon modeli tercih edilmiş ve ayrıca Pearson korelasyon analizlerinden yararlanılmıştır.

Bulgular: Kullanılan tüm modellerin p değeri önemli bulunmuştur. Yem-yumurta paritesinin yumurta üretimine (R2 0,881), yumurta yemi paritesinin yumurta tavuğu sayısına (R2 0,921) ve yumurta tavuğu sayısının yumurta üretimine (R2 0,921) etkileri yüksek düzeyde ve pozitif yönde belirlenmiştir. Türkiye'de yumurta yemi, yumurta yemi fiyatı, yumurta tavuğu sayısı ve üretilen yumurta miktarı değişkenleri arasındaki tüm ilişkiler önemli ve yüksek bulunmuştur. En yüksek korelasyon ise üretilen yumurta miktarı ile yumurta tavuğu sayısı arasında belirlenmiştir (0.966). Yumurta yem fiyatı ise diğer faktörlere nisbi olarak düşürücü bir eki yapmış ve belirleyici bir faktör olarak ortaya çıkmaktadır.

Sonuç: Türkiye'de yumurtanın fiyatında meydana gelen dalgalanmalar her ne kadar yem üretimini ve yumurtalayan hayvan ve üretilen yumurta sayısına olumsuz etki yapsa da bu düzey istatistiki açıdan önemli bulunmamıştır. Türkiye'de tavuk yumurtası, ucuz protein kaynağı olarak çok fazla tercih edilmektedir ve tüketiminin önümüzdeki yıllar itibarıyla da nüfusun artması ile birlikte artış eğiliminde olması beklenebilir.

Anahtar Kelime: Yumurta tavuğu, yumurta, karma yem, karma yem fiyatı, yumurta-yem ilişkisi

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INTRODUCTION

Chicken breeding in Türkiye started in 1930s with the establishment of the Central Poultry Institute in Ankara. In the 1950s, efforts were made to develop village poultry farming. In the 1970s and 1980s, production and research institutions were established for the development of modern poultry production, and the establishment of modern production facilities was encouraged through the Resource Utilization Support Fund and feed support. During this period, an important structural change was achieved with the increase in integrated facilities and the introduction of contract production. With the investments made in the sector in the 1990s, the number and production capacity of modern production facilities increased rapidly and high standard production became widespread. In the 2000s, investments continued and production at European standards became widespread.

Today, the poultry sector in Türkiye has become an important production branch that can make its own production planning and meet a large part of the country's animal protein requirement in a cheap, healthy and high quality way. In Türkiye, 98.6% of the 374 million poultry population in 2023 was composed of chickens with the largest share (Gülaç, 2024a; TÜİK, 2025). It can be said that egg poultry in Türkiye has an integrated production structure with good organization. However, the import of input resources such as raw feed materials and hatching eggs slows down the growth momentum in the sector. The fact that domestic consumption of egg poultry is lower compared to developed countries ranks first among the other factors hindering its growth (Gülaç, 2024a; USDA, 2024). The production of chicken eggs in Türkiye in 2023 was 20.6 billion eggs and 11.4% of the incubated eggs were for laying hen chick production and 83.3% of the 222 thousand tons of shell egg exports in the same year were chicken eggs (TÜİK, 2025).

According to FAO data, world chicken egg production decreased by 0.6% to 82 million tons in 2022 compared to the previous year, while Türkiye saw a 2.7% increase (FAO, 2024). The Netherlands, which meets a significant portion of world chicken egg exports, is the leader with 238 thousand tons of chicken egg exports in 2022. Türkiye ranks ninth in production and third in exports, with a per capita egg consumption of 156 eggs.

The effects of the COVID-19 pandemic and avian influenza, which had a worldwide impact but have diminished in recent years, continue to affect the poultry sector through labor cost increases and feed costs (USDA, 2023a).

This study aims to analyze the current situation of the laying hen sector in Türkiye and the egg-feed relationship in detail. The study aims to assess the impact of feed prices on egg production and consumption, hence the effects of foreign dependency on sectoral growth and the sustainability of egg poultry.

MATERIAL and METHODS

The relationships between egg production, number of laying hens and egg feed costs related to egg poultry in Türkiye were examined in this study. The data reported by the Turkish Statistical Institute (TurkStat, 2025) on egg poultry production between 1996 and 2024 were used (Table 1). For this purpose, quadratic regression model was preferred as it provided the best fit in explaining the relationships between egg production, number of laying hens and egg feed costs and Pearson correlation analysis was also used. IBM SPSS v25 program was used in statistical analyses.

Quadratic regression models

In order to examine the relationship between layer feed production (tons) and the number ofeggs produced (pieces), the quantity of eggs produced (Y) was taken as the dependent variable, and the amount of layer feed (tons) (X) was taken as the independent variable.

Model 1

$$Y= 7871287{,}292 + 10{,}940\ X - 2{,}497E(-6)\ X2$$

Model 2 was created to analyze the effect of ayer feed production (ton) on the number of laying hens. The number of laying hens (Y) was selected as the dependent variable and layer feed production (ton) (X) was used as the independent variable.

Model 2

$$Y = 28627311{,}086 + 85{,}412\ X - 2{,}076\ E(-5)\ X2$$

To evaluate the effect of the number of laying hens on egg production, the amount of eggs produced (Y) was selected as the dependent variable and the number of laying hens (X) was used as the independent variable.

Model 3

$$Y = -273612{,}450 + 0{,}234\ X - 5{,}471E(-10)\ X2$$

Table 1. Egg poultry production in Turkey between 1996 and 2024 (TUİK, 2025)
Tablo 1. Türkiye'de 1996-2024 yılları arasında yumurta tavukçuluğu (TÜİK, 2025)

Years	Layer Feed (tons)	Layer Feed Cost (tons TL)	Number of Layer Hens	Eggs Produced, 1000 pieces
1996	561498	26	53 883 070	9787220,00
1997	510944	52	61 401 783	12089341,00
1998	603624	71	69 722 271	13887864,00
1999	581701	108	71 885 207	14090023,00
2000	535408	135	64 709 040	13508586,00
2001	484152	232	55 675 750	10575046,00
2002	411610	323	57 139 257	11554910,00
2003	429451	390	60 399 520	12666782,00
2004	411592	431	58 774 172	11055556,73
2005	360771	401	60 275 674	12052455,11
2006	316054	415	58 698 485	11733572,20
2007	419362	495	64 286 383	12724958,56
2008	437838	594	63 364 818	13190696,28
2009	461701	590	66 500 461	13832726,47
2010	502646	640	70 933 660	11840396,04
2011	564910	740	78 956 861	12954685,67
2012	638583	810	84 677 290	14910773,95
2013	805766	910	88 720 709	16496751,18
2014	1340217	1020	93 751 470	17145389,09
2015	1957692	1040	98 597 340	16727509,63
2016	1337224	1070	108 689 236	18097604,95
2017	1719070	1220	121 556 027	19281195,84
2018	1859413	1540	124 054 810	19643711,48
2019	2080774	1720	120 725 299	19898126,08
2020	1688035	2190	121 302 869	19788062,82
2021	1984519	3310	121 000 775	19297591,48
2022	1951311	7340	109 806 327	19808538,82
2023	1849782	9110	114 476 843	20637732,44
2024	1957319	12960	116 516 879	21831896,00

Correlation Analysis

Pearson correlation analysis was applied to evaluate the relationships between variables. The analysis was performed between the variables of layer feed production (ton) (feed_egg), feed price-egg parity (feed_price_egg), number of laying hens (egg_animal_number) and quantity of eggs produced (produced_egg). The significance of the correlation coefficients was tested at 0.01 level (2-tailed).

RESULTS and DISCUSSION

The statistical significance of each model was evaluated with the F-test and the p-value of all models was found to be less than 0.05 (Sig. = 0.000), indicating that the models were significant. The explanatory level of the models was measured by R^2 values. The effect of layer feed production (ton) on egg production in Türkiye is highly and positively explained by Model 1 (R2 0.881). It is observed that as the production of egg feed increases, the number of eggs produced also increases, reaching a peak of approximately 20 million eggs with a feed production capacity of approximately 2 million tons (Figure 1).

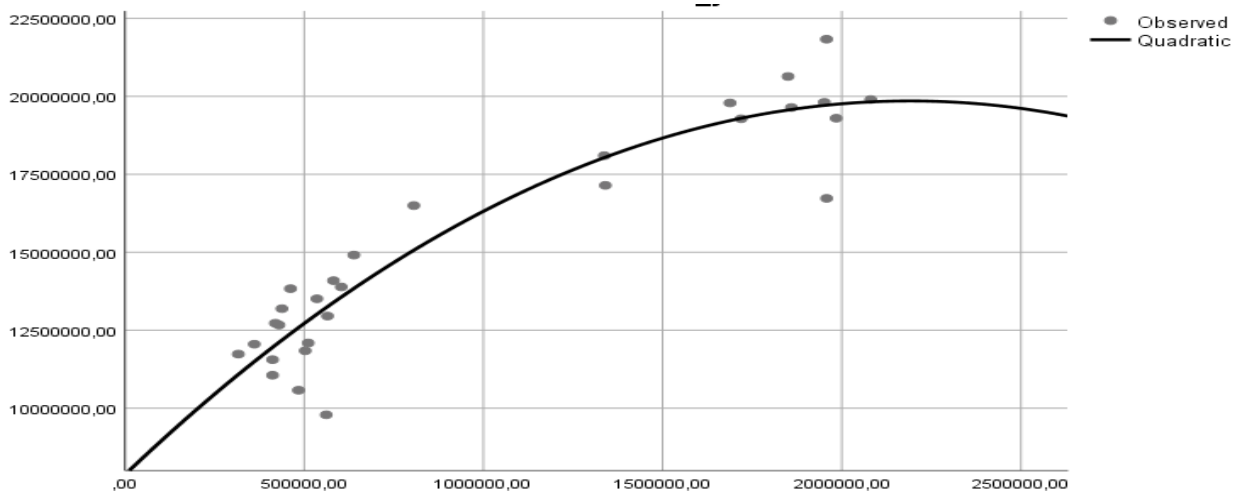

Figure 1. Effect of layer feed production (ton) on egg production
Şekil 1. Yem-yumurta üretiminin yumurta üretimine etkisi

The effect of Türkiye's egg feed parity on the number of laying hens has been determined to be high (R2 0.921) and positive with Model 2.

The effect of Türkiye's egg feed parity on the number of laying hens has been determined to be high (R2 0.921) and positive with Model 2. As the number of laying hens increases, egg feed also increases, reaching approximately 118 million laying hens with an approximate feed production capacity of 2 million tons (Figure 2).

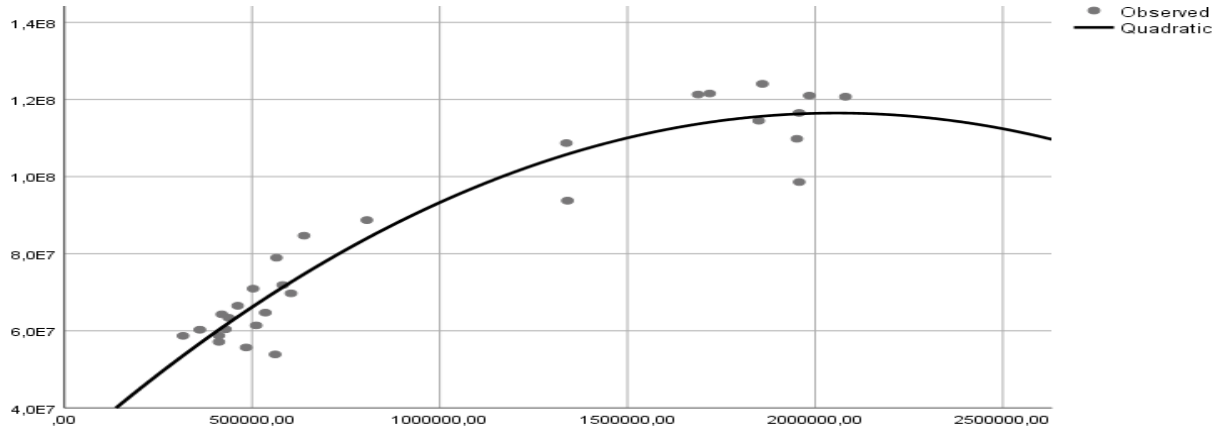

Figure 2. Effect of layer feed production (ton) on the number of laying hens
Şekil 2. Yem-yumurta üretiminin yumurta tavuğu sayısına etkisi

As seen in Figure 1 and Figure 2, there is a relative decrease in the increases that occur when the egg feed production approaches 2.5 million tons. The decrease in the same egg feed capacity after the peak can be explained by the fact that due to the economic crisis and the avian flu in recent years, the growth in the egg poultry industry has stopped and this has affected both the number of laying hens and the number of eggs produced.

When the effect of the number of laying hens on egg production was evaluated, the relationship between them was explained at a high level (R2 0.921) and positively (Model 3). As the number of laying hens increases, egg production also increases (Figure 3). This is only possible when there is maximum utilization of laying hens. Therefore, it can be concluded that managerial tasks such as feeding and health protection are carried out properly in laying hen farms in Türkiye.

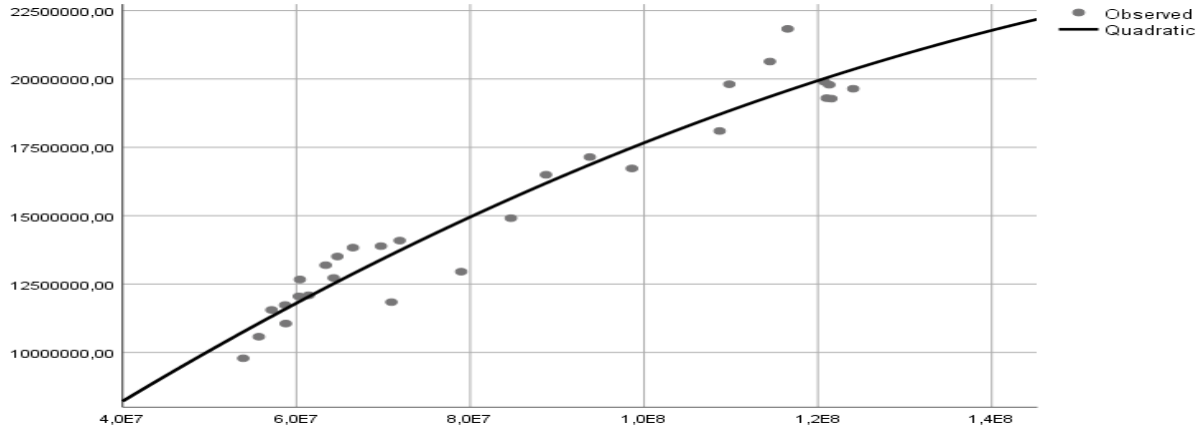

Figure3. Effect of number of laying hens on egg production
Şekil 3. Yumurta tavuğu sayısının yumurta üretimine etkisi

In Türkiye, all the relationships between the variables of egg feed, egg feed price, number of laying hens and quantity of eggs produced were found to be significant and high. The highest relationship was found between the amount of eggs produced and the number of laying hens (0.966). In the next ranking, the relationships between egg feed, number of laying hens and quantity of eggs produced were highly significant. Egg feed price was found to be a determining factor with a decreasing effect relative to the other factors (Table 2).

Table 2. Relationships between the variables of egg feed, egg feed price, number of laying hens and amount of eggs produced
Tablo 2. Yumurta yemi, yumurta yemi fiyatı, yumurta tavuğu sayısı ve üretilen yumurta miktarı değişkenleri arasındaki ilişkiler

		feed_egg	feed_price_egg	egg_animal_count	produced_egg
feed_egg	Pearson Correlation	1	,625**	,947**	,929**
	Sig. (2-tailed)		,000	,000	,000
	N	29	29	29	29
feed_price_egg	Pearson Correlation	,625**	1	,568**	,682**
	Sig. (2-tailed)	,000		,001	,000
	N	29	29	29	29
egg_animal_count	Pearson Correlation	,947**	,568**	1	,966**
	Sig. (2-tailed)	,000	,001		,000
	N	29	29	29	29
produced_egg	Pearson Correlation	,929**	,682**	,966**	1
	Sig. (2-tailed)	,000	,000	,000	
	N	29	29	29	29

****. Correlation is significant at the 0.01 level (2-tailed).**

Feed costs constitute the largest part of the production costs of the poultry sector in Türkiye with 68.0%. Chick expenses account for 15.9% of production costs. Egg feed constitutes 33.1% of the poultry feed produced (TÜRKİYEM-BİR, 2023). Corn and soybean are the main feed raw materials used in poultry farming. The fact that most of these raw materials are imported increases feed costs. In 2023, corn production in Türkiye increased by 25.9% compared to the previous year and reached 8.5 million tons, while corn imports increased by 18.1% compared to the previous year and reached 2.7 million tons. Soybean production in 2023 decreased by 14.8% year-on-year to 155 thousand tons. The amount of soybeans imported was 2.9 million tons, an increase of 15.8% compared to the previous year. In 2023, 24.1% of domestic corn and 94.9% of soybeans were imported (Gülaç, 2024a; TÜİK, 2025). This situation increases the cost of production and reduces the chance of competition in foreign trade. Feed and raw material prices in Türkiye have generally been on an upward trend over the years. Since compound feed prices also increase in line with feed raw material prices, a similar upward trend is also observed here. In 2023, the ton price of egg feed in Türkiye increased by 24.1% to 9,110 TL (TÜRKİYEM-BİR, 2023). Since Türkiye is foreign-dependent in terms of feed raw materials, the increase in exchange rates greatly affected feed prices in 2021 and 2022 and the highest increase was observed in this period. Recently, the increasing demand from Russia and the Middle East has led to a significant increase in imported breeding prices. This leads to increased costs for producers in Türkiye (USDA, 2023b; Gülaç, 2024a).

Although the number of laying hens in Türkiye is decreasing today, it has continued to increase since 2010, reaching its highest level of 121 million in 2020 and 2021. The number of laying hens, which has stagnated for the last three years and constitutes 29.9% of poultry, decreased by 9.3% in 2022 compared to the previous year and reached 110 million. The embargo imposed by Iraq on egg exports is one of the factors for the decrease in the number of laying hens. In 2023, it increased by 4.3% compared to the previous year and reached 114 million (TurkStat, 2025).

In 2023, 59% of the laying hen population was concentrated in three regions of Türkiye. The Aegean Region is the leader in terms of the number of laying hens, with 36.9 million, accounting for nearly one third of Türkiye's total. Western Anatolia ranks second with 17.6 million and Eastern Marmara ranks third with 12.9 million. In 2023, the highest decline is observed in the Northeast Anatolia and Aegean Regions, especially in the Western Black Sea Region (Gülaç, 2024a; TÜİK, 2025).

Almost all of the shell eggs exported in Türkiye are chicken eggs. In 2022, 86.9% of these eggs were table (fresh) eggs, while 17.4% were hatching and breeding eggs. In 2023, fresh egg exports declined to 78% while breeder exports increased to 22% (TurkStat, 2025). The amount of chicken egg exports in Türkiye decreased significantly in 2020-2022. While export losses with Iraq were effective in this decrease, the decrease could not be prevented even though exports to different countries increased. Iraq's ban on egg imports in order to increase domestic production is the most important reason for this decline (Gülaç, 2024a).

The export value of chicken eggs in Türkiye was 403.3 million dollars in 2023 (TÜİK, 2025). Türkiye is a self-sufficient and exporting country in terms of chicken eggs, and while 29.7% of eggs produced in 2018 were exported, this ratio has decreased in the last four years. In 2023, 15.6% of the eggs produced were exported. The amount of egg imports in Türkiye has decreased. Türkiye is dependent on imports in terms of breeder eggs and all of its egg imports consist of hatching/breeder eggs. Hatching eggs are mostly imported from the UK, the US and Canada, while day-old chicks are imported from Germany, the UK and the US. These imports are of great importance for the sustainability of the domestic market (USDA, 2023b). In 2023, the amount of imported hatching/breeding eggs was 2,248 tons. Türkiye produces enough eggs to meet domestic demand and egg consumption is met only from domestic production. Iraq, which has an important place in world egg imports, continues to decrease its egg imports as of 2024. Türkiye's egg production started to increase in 2022 and 2023 after the decrease in 2021 (Gülaç, 2024b).

In some studies, on egg consumption in Türkiye, quality, price, taste, production date, producer company, packaging and advertisement are the most important factors in consumption preference (Parlakay et al., 2017). Durmuş et al. (2007) and Avcılar et al. (2023) reported that the packaging was effective and that the gelatin-coated 30-cell trays were more demanded. However, consumers now prefer ecological chicken products because of their “healthiness and reliability” (Armağan and Özdoğan, 2005; Avcılar et al., 2023). It has been determined that shell color is mostly ignored when purchasing eggs, while egg yolk is mostly preferred as dark yellow (Avcılar et al., 2023).

Egg consumption in Türkiye in 2022 increased by 3.5% compared to the previous year and reached 1 million tons (TÜİK, 2025). Per capita egg consumption increased by 27% compared to the previous year and reached 15.1 kg. In 2020 and 2021, the supply and utilization of eggs tended to decline, but then started to increase in 2022 and onwards. In 2023, the supply and use of eggs in Türkiye was 1.2 million tons (Gülaç, 2024a; TÜİK, 2025).

When the chicken egg/feed parity is analyzed, it is observed that the parity decreases in 2023 and 6.55 kg of egg feed, 4.17 kg of soybean and 10.44 kg of corn can be purchased with 1 parcel (30 eggs) of chicken eggs in 2023. In 2023, chicken egg/egg feed parity increased by 61.9%, chicken egg/soya parity by 46.6% and chicken egg/corn parity by 10.4% compared to the previous year (based on the producer price of 1 carton of chicken eggs) (Gülaç, 2024b; TÜİK, 2025). The increase in costs continues to have an impact on prices.

However, the egg sector continues to be the most affected by the outbreak of highly pathogenic avian influenza (HPAI) in Türkiye as in the world (USDA, 2023a).

In Türkiye, rising feed prices, import dependency, avian influenza, and export restrictions are affecting growth in the egg poultry sector. Sarıözkan (2022) noted that imported raw materials account for more than 70% of feed production and that exchange rate fluctuations increase feed prices, thereby reducing competitiveness.

Erdem and Şahin (2023) reported that highly pathogenic avian influenza (HPAI) caused 5-7% losses in egg production between 2020 and 2023 and, combined with Iraq's import bans, slowed sectoral growth. In terms of consumption habits, there has been an increase in demand for organic eggs; Parlakay and Yılmaz (2024) reported that this demand increased by 15% in the Aegean and Marmara regions and that health and safety concerns shaped consumption. Additionally, Çelik and Koç (2023) emphasized that Iraq's import ban caused a 10-15% decrease in egg exports and that foreign trade policies require regional cooperation for sustainability (Gülaç, 2024a; TÜİK, 2025).

CONCLUSIONS

In Türkiye, chicken eggs are highly preferred as a cheap source of protein and their consumption is expected to increase in the coming years with the increase in population. The increase in avian influenza, which is widespread in Türkiye, in recent years, coupled with the economic crisis, has hindered the progress of the egg poultry sector. This led to an increase in egg prices in the first quarter of 2025, and if exports continue to decline, excess supply is expected to slow the rate of increase in egg prices.

Although fluctuations in the price of eggs in Türkiye had a negative impact on feed production and the number of laying animals and eggs produced, this level was not statistically significant. However, the prices of feed raw materials play a major role in the formation of feed prices in Türkiye. Since most of the feed raw materials are imported, exchange rate increases are also the most important factor in determining feed prices. The realization of the maximum egg production that can be obtained from laying hens raised in laying hen houses is an indication that both the appropriate breeds are used and the managerial conditions are carried out under optimum conditions.

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Investigation of Celtic and Fresian Polledness Variants in Cattle in Eskisehir Region[#]

ABSTRACT

Objective: In this study, the presence of Celtic polled (PolC) and Fresian Pollen (PolF) variants that cause hornlessness in cattle was investigated in the Holstein cattle populations raised in Eskişehir province using PCR and DNA sequence analysis methods.

Material and Methods: DNA obtained from blood samples taken from a total of 100 cattle from some farms in the Eskişehir region were used as animal material for the study. In the study, PolC and PolF variants were detected by PCR and DNA sequence, PCR products were checked on agarose gel and DNA sequence analysis was performed.

Results: While the PolF variant could not be detected in 100 Holstein cattle raised in Eskişehir, a heterozygous cattle with the PolC allele was detected.

Conclusion: While hornless cattle are preferred more in the modern cattle industry, molecular methods will contribute to herd management and planning by guiding the determination of homozygous-heterozygous genotype of polled cattle.

Keywords: Polled, DNA sequence analysis, Holstein Cattle, PolC, PolF

Eskisehir Yöresi Sığırlarında Celtic ve Fresian Boynuzsuzluk Varyantlarının Arastırılması

ÖZ

Amaç: Bu araştırmada; sığırlarda boynuzsuzluğa neden olan Celtic boynuzsuzluk (PolC) ve Fresian boynuzsuzluk (PolF) varyantlarının, Eskişehir ilinde yetiştirilen Siyah Alaca sığır popülasyonundaki varlıkları PCR ve DNA dizi analizi yöntemleri kullanılarak incelenmiştir.

Materyal ve Metot: Araştırmanın hayvan materyali olarak Eskişehir bölgesindeki bazı işletmelerde yetiştirilen toplam 100 baş sığırdan alınan kan örneklerinden izole edilen DNAlar kullanılmıştır. Araştırmada, PolC ve PolF varyantlarının tespiti için PCR reaksiyonu ile çoğaltılmış, PCR ürünleri agaroz jelde kontrol edilerek DNA dizi analizi yapılmıştır.

Bulgular: Eskişehir'de yetiştirilen 100 baş Siyah Alaca sığırla PolF varyantı tespit edilemezken PolC alleline sahip bir heterozigot sığır tespit edilmiştir.

Sonuç: Modern sığır endüstrisinde boynuzsuz sığırlar daha fazla tercih edilirken moleküler yöntemler pol genotipli sığırların homozigot-heterozigot durumunun belirlenmesinde yol göstererek sürü yönetimi ve planlamasına katkıda bulunacaktır.

Anahtar Kelime: Boynuzsuzluk, DNA dizi analizi, Siyah Alaca Sığırı, PolC varyantı, PolF varyantı

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GİRİŞ

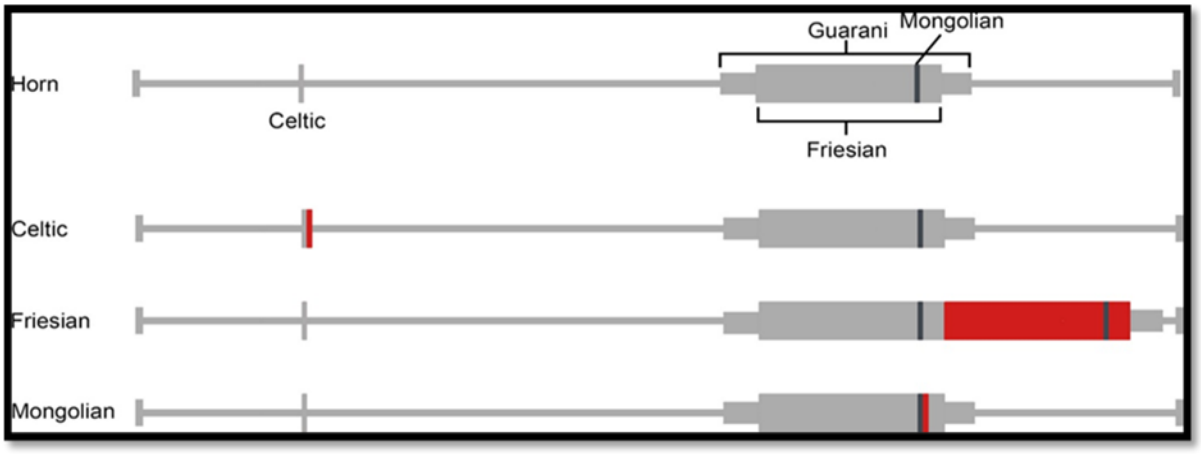
Sığır, keçi ve koyun gibi türlerin kafatası uzantıları olan boynuzlar, yırtıcılara karşı koruma sağlarken, çiftleşme ve kaynaklara erişimde rekabete için rekabete yardımcı olmak için de kullanılırlar (Grobler et al. 2021). Ancak boynuzlu sığırlar diğer sığırlar ve bakıcıları için risk oluşturur ve et işlenirken kesilmesi gereken hasarlı deriler ile çürük dokular nedeniyle ekonomik kayıplara neden olabilir (Kling-Eveillard et al. 2015). Boynuzlarla ilgili sorunları önlemek amacıyla, sığır yetiştiricileri geçmiş yıllarda boynuzları kesmek için sıcak demir ile dağlama (sıcak demirle dağıtma), kepçe boynuz gidericiler kullanılarak tomurcuk amputasyonu (cerrahi) ve boynuz tomurcuklarına kimyasal kostik yakıcı macun (kimyasal dağıtma) gibi çeşitli yöntemler kullanmışlardır. Boynuzlarla ilgili problemleri ortadan kaldırmayı amaçlayan boynuz kesme işlemlerinin hayvanlara verdiği acı ve sıkıntının yanı sıra, yara bölgesinin enfekte olması ve hayvan büyümesini tehlikeye atması gibi nedenlerle hayvan refahı ve etiği konusunda endişelere neden olmaktadır (Spurlock et al. 2014; Caray et al. 2015).

Sığırlarda genel olarak, baş fenotipinde kafatasına sabitlenmiş keratin kaplı sivri çıkıntılara boynuzlu (Horned-Horn), ilkel boynuzlar kafatası yerine cilde gevşek bir şekilde bağlandığında yalancı boynuz (Scurred-Scur) ve boynuzların veya boynuz izlerinin yokluğunda boynuzsuzluk (Polled-Pol) olarak adlandırılmaktadır (Kling-Eveillard et al. 2015; Randhawa et al. 2020). Boynuzsuz sığırların tarih öncesi ve antik çağlarda var olduğu bilinmektedir. Boynuzsuz sığırlara ait izlere Eski Mısır, Roma İmparatorluğu ve İskandinavya gibi bölgelerde rastlanmıştır (Grobler et al. 2021). Chamberlain'in (2017) bildirdiğine göre Bateson ve Saunders (1902), sığırlarda boynuzsuzluk (Pol) fenotipinin boynuzluya baskın olduğunu bildiren ilk kişilerdi. Lloyd Jones and Evvard (1916) da Galloway inekleriyle çiftleştirilen Shorthorn boğalarında boynuzsuzluk genin baskınlığını gösterirken, Williams and Williams'ın (1952) boynuzsuz ve boynuzlu Hereford'ları çaprazlamanın sonuçlarını ortaya koymasıyla desteklemiştir. Polled lokusu ilk olarak 1993 yılında sığır kromozomu 1'e (Bos Taurus Autosome 1- BTA1) haritalanırken (Georges et al. 1993), kromozom 1'in sentromerine çok yakın olan 1q12-14 bölgesinde olduğunu 1995 yılında Schmutz et al. (1995) tahmin etmişlerdir. Pol lokusunun ıslah popülasyonlarında frekansının artırılması ve marker yardımlı seleksiyonda kullanılması için sığır 1. kromozomu üzerindeki TGLA49 ve AGLA17 adlı mikrosatelit markerlarıyla Pol lokusunun ilişkili olduğu bildirilmiştir (Brenneman et al. 1996). Simental sığırlarda boynuzsuzluk lokusu ile BM6438 ve SODIMicro2 markerları arasında tam bağlantı olduğu belirlenmiştir (Brookman et al. 2000). Drögemüller et al. ise 2005 yılında bu bölgeyi 1Mb'lık bir segmente daraltmışlardır. 2009 yılında sığır referans genomunun (Bovine Genome Sequencing and Analysis Consortium, 2009) tamamlanmasının ardından Seichter et al. (2012) farklı sığır ırklarında yürüttüğü GWAS çalışmasıyla bu bölgeyi 381 kb'ye kadar daraltmışlardır.

Moleküler olarak sığır kromozomu 1'in (BTA1) sentromerik bölgesine haritalanmış olan otozomal dominant Polled (boynuzsuzluk) lokusunun (000483-9913) (OMIA, 2024) Bos taurus'ta boynuzsuzluk özelliğini determine ettiği bildirilmiştir. Polled lokusunun BTA1 üzerindeki konumu tanımlanmış ve aday nedensel mutasyonlar belirlenmiştir. Moleküler çalışmalarla sığırlarda boynuzsuzluk fenotipi ile ilişkili dört DNA dizi varyantı tanımlanmıştır; Celtic Polled (PolC) (Medugorac et al. 2012; Allais-Bonnet et al. 2013; Rothammer et al. 2014), Friesian Polled (PolF) (Medugorac et al. 2012; Allais-Bonnet et al. 2013; Rothammer et al. 2014), Mongolian Polled (PolM) (Medugorac et al. 2017) ve Guarani Polled (PolG) (Utsunomiya et al. 2019). Her boynuzsuzluk varyantının kaynaklandığı mutasyon, değişken boyutlu karmaşık bir ekleme-silme (indel) işlemidir (Şekil 1). Bilinen tüm Pol varyantları baskındır ve tek bir pol alleli taşıyan sığırlar, scur lokuslarındaki genotiplerine bağlı olarak boynuzsuz veya scur olacaktır. Farklı popülasyonlarda ve ırklarda başka tanımlanmamış Pol varyantların da bulunması muhtemeldir (örn. Shuxuan ırkı; Chen et al. 2017)

Celtic (PolC) varyantı Kelt coğrafi bölgelerinden kaynaklanan birkaç Avrupa sığır ırkında karmaşık bir ekleme ve silme işlemi olarak tanımlanmıştır. 212 bç'lik bir dizi (1705834–1706045 bç) kopyalanır ve orijinal diziden 6 bç aşağıda olan 10 bç'lik (1706051–1706060 bç) bir dizinin yerini alır (Şekil 1) (Medugorac et al. 2012). Boynuzlu bir Holstein-Friesian boğasından alınan fibroblastların genomuna PolC varyantının 202 bç ekleme-silme mutasyonu CRISPR/Cas12a tekniği ile gen düzenlemesi yapılarak boynuzsuz buzağı üretilerek bu mutasyonun boynuzsuz fenotipine neden olduğu belirlenmiştir (Schuster et al. 2020). Holstein-Friesian sığırlarında tanımlanan Fresien (PolF) varyantı, PolC varyantından yaklaşık 200 kb aşağıdadır ve 1909352 ile 1989480 bç arasındaki dizinin 80128 bç'lik bir kopyasıdır (Şekil 1) (Medugorac et al. 2012; Allais-Bonnet et al. 2013; Rothammer et al. 2014). Kopyalanan segment orijinal dizinin hemen ardında yer alır ve aynı yöndedir. Üçüncü pozisyonda bir T>A transversiyonu ve 45. pozisyonda 2 bç'lik bir delesyon (TG) ile referans diziden farklıdır (Medugorac et al. 2012).

Moğol Yak ve Turano sığırlarında keşfedilen PolM varyantı BTA1'deki 800 kb'lik bir bölgeye yerleşmiştir (Medugorac et al. 2017). PolM varyantına ait iki mutasyondan ilki, orijinal diziden 61 bç aşağı akışta 219 bç'lik bir duplikasyon-eklemedir (P219ID: 1976128 bç'de başlıyor) ve ikincisi, P219ID'den 621 bç yukarı akışta 6 bç'lik bir delesyon ve 7 bç'lik bir eklemedir (Medugorac et al. 2017) (Şekil 1). PolG, Brezilya'dan Nellore sığırlarında (Bos indicus) (Utsunomiya et al. 2019) yaklaşık 110 kb'lik bir çoğaltmanın neden olduğu bir kopya sayısı varyasyonu olarak (1893790–2004553 bç) tanımlanmıştır. Bilinen dört Pol varyatının, herhangi bir kodlama dizisini, intronik bölgeyi veya bilinen herhangi bir düzenleyici bölgeyi etkilemediği bildirilmiştir (Aldersey et al. 2020).



Şekil 1. Celtic, Friesian ve Moğol boynuzsuzluk varyantları. Gri dikdörtgenler, tekrarlanan diziyi temsil eder ve kırmızı dikdörtgenler, kopyaların ekleme bölgesini temsil eder. (Utsunomiya et al. 2019; Aldersey et al. 2020).

Figure 1. Celtic, Friesian, Mongolian and Guarani polledness variants. Grey rectangles represent the duplicated sequence and red rectangles represent the insertion site of the duplicates.

BTA1 (bovine genome assembly ARS-UCD1.2 (GCA_002263795.2): https://www.ncbi.nlm.nih.gov/assembly/GCF_002263795.1/) üzerinde yer alan boynuzsuzlukla ilişkili mutasyonların konumları şöyledir: PolC g.[22429326_ 2429335del;2429109_2429320dupins], PolF g.2629113_2709240dup (80,128 bç), PolM g.[2695261_2695267delinsTCTGAA;2695889_2696047dupins] ve PolG g.2614828_2724315dup (110 kb). BTA1 kromozomunda, Celtic Polled IFNAR2 ve OLIG1 genleri arasında konumlanmıştır. Bilinen herhangi bir kodlama dizisini, bağlantı bölgesini, intronik değişimi veya herhangi bir birleştirmeyi etkilemediği gözlemlenmiştir (Medugorac et al. 2012; Aldersey et al. 2020). Boynuzsuzluk genotiplerinin moleküler yöntemle belirlenmesi için, türe özgü kullanılan birkaç MSAT (mikrosatellit) marker ve SNP (Single Nucleotide Polimorphism) tabanlı testler geliştirilmiştir (Mariasegaram et al. 2012; Grobler et al. 2021).

Bu çalışmanın amacı, farklı ülkelerdeki sığır popülasyonlarında varlığı belirlenen PolC ve PolF varyantlarının, Eskişehir ilinde yetiştirilen Siyah Alaca sığır popülasyonunda varlığını moleküler yöntemlerle araştırmaktır. PolF varyantı Jersey, Holstein ve Witrug ırkı sığırlarda görülürken PolC varyantı Angus, Blonde d'Aquitaine, Dexter, Limousin, Charolais, ve Hereford ırkında görüldüğü bildirilmektedir (Medugorac et al. 2012). Her ne kadar PolC varyantının varlığı Holstein ırkında daha önce bildirilmese de bu çalışmada farklı bir popülasyonla çalışıldığından PolC varyantı da araştırılmıştır. Eskişehir'de yetiştirilen Siyah Alaca sığır işletmelerindeki 100 baş sığırdan alınmış kan örneklerinden DNA saflaştırılarak, PCR yöntemi ile Pol geni varyasyonlarından Celtic (PolC) ve Fresian (PolF) boynuzsuzluk varyantlarının varlığı PCR ve DNA dizi analizi yöntemi kullanılarak incelenmiştir. Yapılan çalışma ile Türkiye'de ilk defa boynuzsuzluk genotipinin varlığı araştırılmıştır. PolC ve PolF varyantların tespiti modern sığır endüstrisinde öne çıkan hayvan refahını iyileştirmek için oluşturulacak boynuzsuz sığır sürülerinin geliştirilmesine katkı sağlanacaktır.

MATERYAL ve YÖNTEM

Hayvan materyali

Araştırmada, Eskişehir Odunpazarı (n:40), Alpu (n:30) ve Beylikova (n:30) ilçelerinde bulunan üç işletmeden toplam 100 baş boynuzsuz Siyah Alaca (Holstein) sağmal inekten alınan kan örnekleri materyal olarak kullanılmıştır. Örnekler DNA saflaştırma çalışmaları yapılana kadar -20 ºC 'de saklanmıştır.

Yöntem

Kandan DNA izolasyonu

Toplam genomik DNA (gDNA) izolasyonu PureLink Genomic DNA Kiti (Invitrogen, Almanya) kullanılarak, üretici firmanın belirttiği DNA izolasyon protokolüne uygun olarak gerçekleştirilmiştir. İzolasyon sonrası gDNA'lar %1'lik agaroz jel kullanılarak kontrol edilmiş Nanodrop yardımıyla miktar ve kalitesi ölçülmüştür. Örnek DNA miktarları 40-60 ng/ul olarak ayarlanmıştır.

PCR çalışmaları

Araştırmada, polled varyantlarından PolC ve PolF varyantlarının tespiti için hedef bölgenin çoğaltılması amacıyla Medugorac et al. (2012) tasarladığı primerlerden yararlanılarak PCR reaksiyon çözeltileri hazırlanmıştır. PCR reaksiyon karışımı 50 µl olacak şekilde (1 U Taq DNA Polimeraz (Thermo Scientific), 10 X PCR tamponu (100 mM Tris-HCl, 500 mM KCl, pH. 8,8), 1,5 mM MgCl2, 200 µM dNTP (Fermentas), 5 pM ve 1 µl DNA (50-100 ng)) hazırlanmıştır. PCR işleminde primer bağlanma sıcaklığı 58 ºC (P202ID) ve 59 ºC (P1909396D2) 60 sn süre ile uygulanırken P202ID 31 ve P1909396D2 için 32 PCR döngüsü yapılmıştır. PCR reaksiyonu tamamlandıktan sonra, mutasyonun meydana geldiği lokusu kontrol etmek için PCR ürünleri %2'lik agaroz jelde koşturularak kontrol edilmiştir.

Genotiplerin belirlenmesi

Eskişehir'de yetiştirilen Holştayn sığırlarında PolC ve PolF varyantlarına ait mutasyonu araştırmak için PCR ve DNA dizi analizi yöntemleri kullanılmıştır (Tablo 1). PolC varyantına sebep olan P202ID insersiyonun değişken bölgesini içeren P1909396D2 primerleri boynuzlu sığırlarda 369 bç ve boynuzsuz sığırlarda 571 bç PCR ürünü verecek şekilde dizayn edilmiştir (Medugorac et al. 2012). PCR ürünlerinin ayırımı ve genotipler %2'lik agaroz jelde elektroforezi yapılarak belirlenmiştir. PolF varyantına sebep olan P80kbID delesyonun değişken bölgesini içeren P1909396D2 primerleri boynuzlu ve boynuzsuz sığırlarda PCR ürünü verecek şekilde dizayn edilmiştir (Medugorac et al. 2012). Elde edilen PCR ürünün büyüklüğü 152 baz çiftidir. Araştırmada, hayvanların çoğaltılan DNA bölgesindeki 2 baz (TG) çifti delesyonunun tespiti için PCR ürünlerinin dizi analizi yapılmıştır. PCR ile çoğaltılan bölgede PolF sığırlarda görülen 2 baz (TG) çifti delesyonu araştırılıp bu delesyona sahip sığırlar PolF haplotipi olarak belirlenmiştir. Dizi analizi sonuçları hizmet alımı yoluyla yapılmış, DNA dizi analizi ham sonuçları laboratuvarımızda incelenip MEGA 12 (Kumar et al. 2024) ve CodonCode Aligner (2021) programları kullanılarak analiz edilmiş ve ilgili genotipler araştırılmıştır.

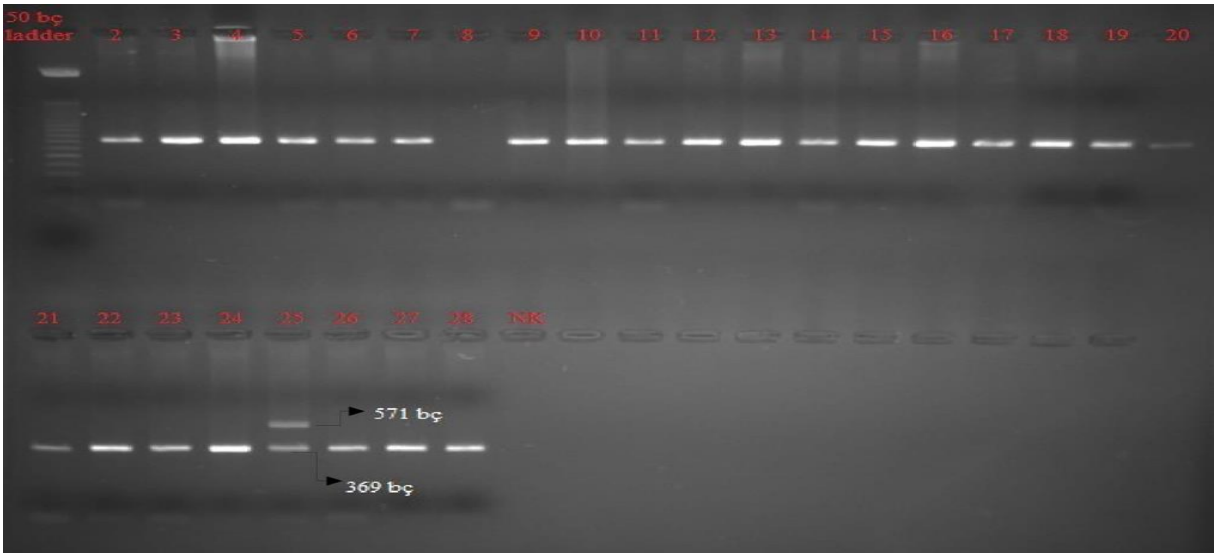
Tablo 1. PolC ve PolF varyant lokuslarına ait primerler
Table 1. Primers for PolC and PolF variant loci

Varyant	Primer	Kaynak
P_{202ID}	Forward: 5'-TCAAGAAGGCGGCACTATCT-3' Reverse: 5'-TGATAAACTGACCCTCTGCCTATA-3'	Medugorac et al. 2012
$P_{1909396D2}$	Forward:5'-GAAGTCGGTGGTCTGAAAGG-3' Reverse:5'-TGTTCTGTGTGGGTTTGAGG-3'	

BULGULAR

PolC PCR reaksiyonu sonuçları

Araştırmada PolC (Celtic varyantı) için yapılan ve P202ID primerleri kullanılarak çoğaltılan PCR ürünleri %2'lik agaroz jele yüklenerek elektroforetik ayrım yapılmıştır. PCR ürünü jel görüntüleri Şekil 2'de verilmiştir. Görüntüleme işleminde PolC varyantı ile ilgili yapılan PCR çalışmalarında, 100 baş sığır içerisinde bir bireyin heterozigot genotipte olduğu belirlenmiştir (Şekil 2).



Şekil 2. PolC için yapılan PCR ürünleri %2'lik agaroz jel görüntüsü (Invitrogen 50 bç DNA Ladder)

Figure 2. The agarose gel image of PCR products for PolC (Invitrogen 50 bç DNA Ladder)

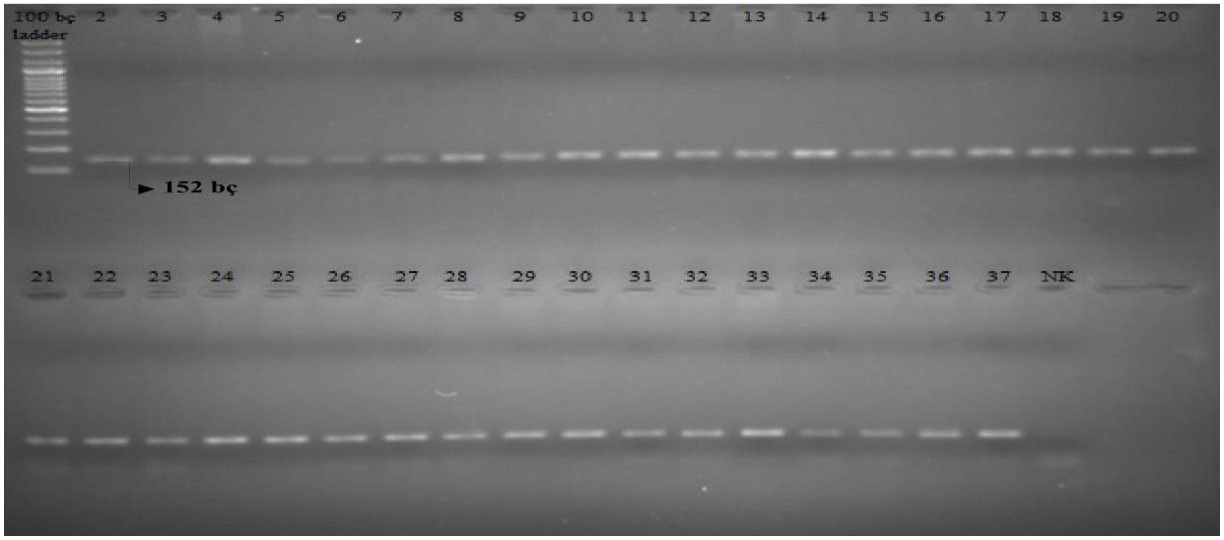
PolF PCR reaksiyonu sonuçları

Boynuzsuzluk (Polled) geninin PolF (Fresien varyantı) için yapılan PCR işlemi sonucu elde edilen ürünler %2'lik agaroz jelde kontrol edilmiştir. Bu PCR ürünlerinin jel görüntüleri Şekil 3'de verilmiştir. PolF varyantını belirlemek amacıyla yapılan PCR sonucunda, 152 bç büyüklüğünde ürünler elde edilmiş ve bu ürünler %2'lik agaroz jelde kontrol edilerek (Şekil 3) DNA dizi analizine gönderilmiştir.

PolF DNA Dizi analizi sonuçları

PolF PCR ürünlerinin hizmet alımı yoluyla gerçekleştirilen DNA dizi analizi ham verileri, MEGA 12 ve CodonCode programları ile analiz edilmiştir. Elde edilen dizi analiz sonuçları, The UCSC Genome Browser Genbankası'nda sığır Polled geni için bilinen mutasyonla karşılaştırılmış ve bu alignment Şekil 4'te gösterilmiştir (UCSC, 2025).

PCR ile çoğaltılan bölgede, PolF varyantına sahip sığırlarda görülen 2 baz (TG) çifti delesyon (P1909396D2) araştırılmış, bu delesyona sahip sığırlar PolF haplotipi olarak belirlenebilirken, çalışmada bu mutasyona sahip herhangi bir örnek tespit edilememiştir (Şekil 4).

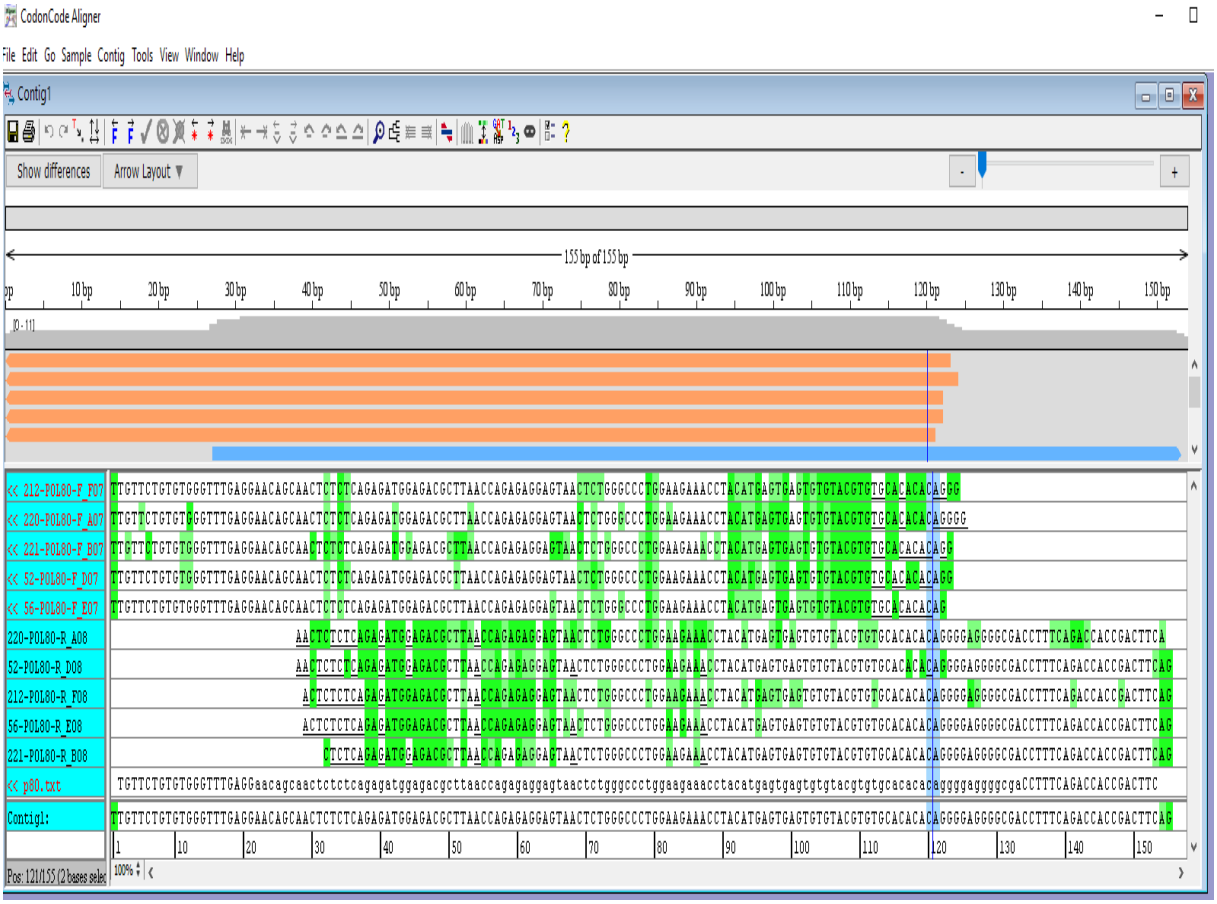


Şekil 3. PolF için yapılan PCR ürünlerinin agaroz jel görüntüsü (Hibrigen 100 bç ladder)

Figure 3. The agarose gel image of PCR products for PolF (Hibrigen 100 bç ladder)

Gen frekanslarının tahmin edilmesi

Çalışmada materyal olarak kullanılan ve Eskişehir ilinde yetiştirilen 100 baş sığır arasında PolF varyantı görülmezken, PolC mutasyonuna sahip bir sığır tespit edilmiştir. Buna göre çalışan popülasyonun PolF bakımından boynuzluluk alleli için monomorfik olduğu belirlenmiştir. PolC (P) mutasyonun frekansı ise PopGene programı kullanılarak 0.005 olarak hesaplanmıştır.



Şekil 4. Codon and Code programında örneklerin PolF varyantı bölgesinin DNA dizi analizi sonuçlarının karşılaştırılması
Figure 4. Alignment of DNA sequence results of PolF variant region of samples with the CodonCode aligner program

TARTIŞMA ve SONUÇ

Türkiye'de ilk defa sığırlarda boynuzsuzluk (polled) geni varyasyonlarından olan PolC ve PolF varyantlarının, Eskişehir ilinde yetiştirilen Siyah Alaca sığırlarında PCR ve DNA dizi analizi yöntemleri kullanılarak araştırıldığı bu çalışmada, Friesian polled varyantı Pol80kbID mutasyonu tespit edilemezken bir sığırın PolC bakımından heterozigot olduğu belirlenmiştir. PolF varyantı Jersey ve Siyah Alaca ırkı, PolC varyantının ise Angus, Limousin, Charolais ve Hereford ırkı sığırlarda (Medugorac et al. 2012) görülürken çalışma materyalini oluşturan Siyah Alaca sığırlarda PolC bakımından heterozigot bir sığır belirlenmesi suni tohumlamada farklı ırklardan boğalarında kullanılmış olabileceği olasılığını akla getirmektedir. Ayrıca çalışma materyali seçilirken sürülerden boynuzsuz olan inekler seçilmiş ancak boynuzların köreltilme durumları belirlenememiştir.

Boynuzlar, sığırların doğal savunma ve rekabet araçları olarak evrimsel süreçte gelişmiştir. Ancak, boynuzlu sığırların taşıdığı potansiyel tehlikeler ve olası zararları nedeniyle, bazı yetiştiriciler ve endüstri uzmanları tarafından boynuzsuz sığırlar daha tercih edilebilir hale gelmiştir. Bu nedenle, genetik mühendislik ve seleksiyon yöntemleriyle boynuzsuz sığırların üretilmesine ve tercih edilmesine yönelik çalışmalar artmıştır. Boynuzsuzluk (Pol) geni taşıyan sığırların yetiştiriciler tarafından daha çok tercih edilmesi, boynuzsuz sığırların daha yaygın ve etkili bir şekilde yetiştirilmesine olanak tanımaktadır.

Götz et al. (2015) bildirdiğine göre Almanya Bavyera'da boynuzsuz Fleckvieh (Simmental) sığırlarının sistemli üretimi, ilk boynuzsuz ineğin 1974 yılında satın alınması ile başlamış 1985'e kadar üç boynuzsuz boğa ve iki baş boynuzsuz inek daha alınarak sürü büyütülmüştür. Embriyo transferi yoluyla 1984'ten itibaren ilk

homozigot boynuzsuz danalar üretilmiştir. Bu araştırma çiftliğinde üretilen boynuzsuz boğa ve inekler kullanılarak 2013 ve 2014 yıllarında sırasıyla yılda yaklaşık 22.000 ve 32.000 boynuzsuz buzağı doğmuştur. Boynuzsuz boğaların suni tohumlamada kullanılmasıyla sığır popülasyonunda boynuzsuzluk frekansının artışının hızlanmasını beklemektedirler. Bu durum yetiştiriciler arasında boynuzsuz sığırların kabul gördüğünü göstermektedir.

Polled genotipleri belirlemek için ilk olarak geliştirilen mikrosatelit markerlara dayalı MSAT testi (Mariasegaram et al. 2012) kullanılmış, sonraları moleküler yöntemlerin gelişmesine bağlı olarak SNP testi kullanılmıştır. Geliştirilen bazı ticari testler (zoetis 2025- Yeni Zellanda-Brahman, Brangus, Limousin, Charolais, Hereford, Simmental vb) bazi ülkelerde yaygın olarak yetiştirilen ırkların bazılarında çalışırken bazı testler (Veterinary Genetics Laboratory 2025) ise belirli varyantları (PolC ve PolF) belirlemeye yönelik geliştirilmiştir. Yeni Zellanda'da geliştirilen PCR-SSCP (Single Strand Conformational Polymorphism) tekniğinin bazı ırklarda güvenilir sonuçlar vermediği görülmüştür (Chamberlain 2017). Yapılan çalışmada PolC ile F varyantlarını belirlemek için kullanılan Medugorac et al. (2012) tespit ettikleri üç SNP noktasının etkili bir şekilde belirleyebileceği başka çalışmalarda (Randhawa et al. 202) da bildirilmiştir. Modern sığır endüstrisinde boynuzlu hayvanların beslenme, taşıma ve çiftlik çalışanlarına karşı potansiyel tehlike oluşturması ve hayvan refahı endişeleri nedeniyle, boynuzsuz sığırlar daha fazla tercih edilirken (Randhawa et al. 2020) moleküler yöntemler pol genotipli sığırların homozigot-heterozigot durumunun belirlenmesine yol göstererek yardımcı olacaktır. Hayvan refahını dikkate alan gelişmiş ülkelerde (ABD- Spurlocket al. 2014; Almanya-Götz et al. 2015) boynuzsuz boğaların suni tohumlamada kullanılmasıyla bu ülke etçi ve sütçü sığır populasyonlarında boynuzsuz sığır sayısı artmaktadır.

Yürütülen çalışmada pedigri kayıtları bilinmeyen az sayıda hayvan kullanılmasına rağmen heterozigot bir ineğin bulunması Türkiye'de de boynuzsuz sığır populasyonun kurulup moleküler yöntemlerle geliştirilebileceği gösterilmiştir. Boynuzsuz fenotipli sığırların pedigri kayıtlarınında incelenerek yapılacak moleküler çalışmalarda Polled genotipli sığırlar belirlenebilecektir.

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A Present Vision for Artificial Insemination of Certain Farm Animals in Egypt

ABSTRACT

It is well known that one of the major limitations in the development of farm animals in Egypt is the extremely low reproductive efficiency. As a result, horizontal expansion would be impossible for dairy animals in Egypt. Therefore, such as in developing countries, artificial insemination (AI) programs are one of the most important options for fast development and genetic improvement in animal breeding studies in Egypt. Meanwhile, the proposed genetic improvement of buffalo milk production is being accelerated using an AI network. According to official statistics, Egypt has only two AI centers for selected buffalo sires, serving four AI units. Based on the current status of AI studies of farm animals in Egypt, this review spots light on recent progresses in the breeding of farm animals such as buffalo, camel, sheep, and goat, based on a comprehensive scientific experience of more than a decade to get a better food security in Egypt.

Keywords: Artificial insemination, farm animals, genetic improvement, Egypt

Mısır'da Bazı Çiftlik Hayvanlarında Suni Tohumlamaya Güncel Bir Bakış

ÖZ

Mısır'da çiftlik hayvanlarının geliştirilmesindeki en büyük sınırlamalardan birinin aşırı düşük üreme verimliliği olduğu iyi bilinmektedir. Bunun bir sonucu olarak Mısır'da süt hayvanı varlığında yatay genişleme imkânsızdır. Bu nedenle, hayvancılığı gelişmiş ülkelerde olduğu gibi, Mısır'da da hayvan yetiştirme çalışmalarında hızlı bir gelişme ve genetik ilerleme için suni tohumlama (ST) programları en önemli seçeneklerden biridir. Halihazırda sütçü mandalar için istenen genetik ilerleme etkili bir ST ağı kullanılarak hızlandırılmaktadır. Resmi istatistiklere göre Mısır, seçilmiş manda boğaları için sadece iki ST merkezine sahip olup bunlar dört ST birimine hizmet vermektedir. Bu derleme, Mısır'da çiftlik hayvanlarında ST çalışmalarının mevcut durumuna dayalı olarak, on yılı aşkın bir süredir manda, deve, koyun ve keçi gibi memeli çiftlik hayvanlarının üreme ve ıslahında kapsamlı bir bilimsel deneyime dayanarak Mısır'da daha iyi bir gıda güvenliği elde etmek için yapılan ve süregelen çalışmalarda kaydedilen ilerleme ile gelecekte yapılacak ya da yapılması gereken ıslah çalışmalarına ışık tutmaktadır.

Anahtar Kelimeler: Suni tohumlama, çiftlik hayvanları, genetik ilerleme, Mısır

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INTRODUCTION

Egypt is one of the top 15 most populous countries in the world with a population of about 100 million (World Population Review, 2018). Egypt is located at the northeastern corner of Africa and the southwest corner of Asia. According to the World Bank (2018a), the gross national income (GNI) per person was $3010, and the GNI index, which measures the wealth distribution within a nation, was 31.8 (World Bank, 2018b). Based on factors including average population education levels and life expectancy at birth, Egypt ranks 115th out of 189 countries in 2017 human development index (United Nations Development Programme, 2018). Economy in Egypt heavily depends on agriculture; it produces over 14.5% of the country's gross domestic product and accounts for 28% of all occupations (United States Agency for International Development, 2018). Despite the importance of agriculture, Egypt struggles with food insecurity. In Upper Egypt food insecurity is more severe, where 38.7% of the population lacks adequate food access (World Food Programme, 2018). As a result, recommendations including improvement in reproductive efficiency of farm animals are required to get better food security in Egypt (Soliman, 2018).

To improve the reproductive efficiency of farm animals in Egypt, artificial insemination (AI) is one of many techniques with significant potential. AI can be used to enhance hereditary characteristics like the ability to produce milk, meat, wool, and racing prowess (Vishwanath, 2003). The success of AI, which in turn depends on the quality of semen produced and its capability for dilution and storage with minimal loss of fertilizing power, is one of the important factors in achievement of the high reproductive activity (Mostafa et al., 2014).

The development of AI technology in Egypt began in the 1940s with experiments carried out in research centers. In 1958, these efforts were transferred to the field at "Bahteim" station in the governorate of Qaliobia. The obtained results had a significant influence on creation of the first AI center for practical field application at a veterinary training center in "Sirs-Lyn" in 1960. This was followed by opening three AI centers, the first in Upper Egypt, the second in Lower Egypt, as well as another one in the eastern Nile Delta in 1962. By the end of the 1960s, there were 161 AI centers spread throughout 18 governorates, but they were reduced to 38 because of budget constraints and a shortage of suitable bulls. Subsequently, a project has been completed in three phases. A sample study was conducted on cattle in the Nile Delta during the first phase, where the high qualified veterinarians were trained in reproductive and AI procedures. The second phase of the project has started with the real implementation of the use of frozen semen and the provision of incoming tools. In January 1977, the final phase concluded with the preparation of frozen semen for both buffalo and Friesian sires (Allam, 2011). In this way, this review focuses on the current progresses in AI of buffalo, camel, sheep, and goat breeding in Egypt.

Artificial Insemination in Buffalo Breeding

According to FAOSTAT (2018), Egypt has 3.37 million head of buffaloes. AI of buffaloes in Egypt was hardly ever used, where only 0.3% of Egyptian buffaloes were a part of AI operations (Borghese, 2010). A good AI program must have high-performance sires with superior semen, dependable and skilled AI technologists, healthy cows, functional communication and transportation networks, and cooperative and competent farmers. The whole AI program fails if even one of these interconnected fundamental components is missing. It should be noted that the natural service, which costs EGP (Egyptian Pound) 75 per service, is still the most popular technique of insemination for dairy buffalo in Egypt. However, it is revealed that on average, 3.5 services are needed for conception, and the specialist may feel the new embryo in the uterus horn by detecting gestation at the third month of pregnancy using a rectal palpation (Soliman, 2008). As a result, AI networks would not only increase genetic improvement in progeny but also enhance the reproductive capabilities of the Egyptian buffalo herds (Soliman and Mashhour, 2019).

Egypt has a competitive advantage in the production of milk from dairy buffalo, but not in the production of red meat (Soliman and Mashhour, 2003). The farm's net income, return on investment, and utilization of the feed unit all benefited dairy production in general and the dairy buffalo in particular. As a result of limited amount of water resources and fertile lands in Egypt, cultivating specialty breeds or expanding the livestock population horizontally is not an option. Therefore, getting the most milk production possible from one additional unit of feed would be a viable work. This showed that the best option for increasing milk production in Egypt is the vertical growth. Internal rate of return (IRR) of 20% or higher has been demonstrated by a recent study resulting from the genetic enhancement of the Egyptian buffalo (Soliman, 2017). An interconnected system of AI centers and AI units is necessary for effective genetic progress however; there aren't enough AI centers in Egypt's

infrastructure. According to Central Agency for Public Mobilization and Statistics (CAPMS) 2000, only two AI centers for raising specific buffalo sires are known to exist, one of them in Cairo and the other in Beni-Suef in Upper Egypt.

Table 1. The required regional spread of Lower Egypt's AI-buffalo network
Tablo 1. Aşağı Mısır'da Mandalarda Suni Tohumlama İçin Gerekli Ağın Bölgesel Dağılımı

Governorate	Buffalo Population (H)	Required Sires (H)	Required AI-centers	Required AI-units
Bahira	466,021	31	3	165
Dakahleya	196,873	13	1	69
Gharbia	227,315	15	2	80
Kafr El-Sheikh	254,628	17	2	89
Monofia	369,684	25	2	132
Qaliobia	161,364	11	1	56
Sharkia	298,048	20	2	104
Total	1,973,933	132	13	695

Source: Central Agency for Public Mobilization and Statistics (CAPMAS), 2016.

A genetic enhancement program utilizing AI would significantly increase milk production in Egyptian buffalo (Soliman and Mashhour, 2019). This is due to the fact that AI would result in about 10 thousand to 20 thousand inseminated females per buffalo sire, but no more than 60 females inseminated per buffalo sire for natural insemination results. Additionally, the buffalo sires raised at AI centers are chosen based on offspring test criteria in addition to phenotypic traits. According to the density of buffalo in each governorate in Lower Egypt, the number of buffalo sires required for the AI network was determined using the rate of buffalo that were inseminated. To guarantee the effectiveness, a management constraint was implemented. Therefore, no more than 100 buffalo sires should be housed in each AI center. As a result, roughly 13 centers for AI were listed (Soliman and Mashhour, 2019). Additionally, according to Table 1, there would be around 695 connected AI-Units in Lower Egypt, as shown geographically in Figure 1. The total number of inseminated buffalos in Egypt was 42,000 with a positive number of 16,000 (Ministry of Agriculture and Land Reclamation, 2015).

Figure 1. Distribution of AI centers concerning the population of buffalo.
Şekil 1. Manda Popülasyonuna Göre Suni Tohumlama Merkezlerinin Dağılımı

Artificial Insemination in Camel Breeding

According to FAOSTAT (2018), Egypt has 85 thousand head of camels (Dromedary Camels). About 75% of the dromedary camel's population is raised in arid and semi-arid regions, making it the strategic stockpile of food security in the Arabian world. Due to its special adaptive traits, it is regarded as an animal that can survive and produce under such difficult environmental conditions. However, a variety of intricate ecological limitations negatively impact the dromedary camel's ability to reproduce. This is demonstrated by the species' low reproductive efficiency. In order to increase the ability of male or female dromedary camels to reproduce, various studies have been done in Egypt since the establishment of a modern, well-equipped laboratory in 1998.

According to El-Hassanein (2003), to collect semen, modified methods often utilized for other domestic species were created as a result of the unique behavior of camel males (position of sitting, and duration of mating). It featured the use of a female camel dummy whose form and position were quite close to those of the female during mating. At the base of the dummy, there was an artificial vagina. An operator was positioned under the dummy in a position that was more comfortable than the natural mating to collect the semen. The typical issues with natural service, such as female injury and male restlessness, are overcome by this position. Additionally, it increases the operator's safety. Rutting, or male camel breeding season, is confined to a few months of the year under the Egyptian environment (from mid-December to late February).

For AI in camel, high viscosity, poor freezing ability and limited post-thawed motility are the major limitations. Pellets and 0.5 mL French straws were two ways that were used to improve semen cryopreservation. After centrifuging the seminal plasma, a tris-lactose extender with 3% glycerol content was utilized to reduce viscosity. Viscosity in camel semen must be removed to create a homogeneous dilution and enhance the evaluation of the material's basic physical qualities. To get around this problem, El-Bahrawy and El-Hassanein (2009) investigated the effects of α-chymotrypsin (0.5%), α-amylase (25%), sodium hydroxide (0.1 N), trypsin (25%), and bromohexine hydrochloride (0.2%) on the viscosity and physical properties of semen, in which α-amylase eliminated semen viscosity and significantly improved sperm motility. However, acrosomal integrity was negatively affected by these mucolytic agents after equilibration.

Contrarily, developing and perfecting a dependable protocol for collection of semen is now crucial for use in AI facilities for camels. El-Bahrawy et al. (2011) investigated the effects of extender, frequency of collection, and temperature of thawing on cryopreserved semen and found that semen extension with tris-lactose extender combined with α-amylase is the best collection program for providing high-quality insemination doses. The physiology of reproduction and AI in such animals have been low focus of research done on the origins of this phenomena, despite the fact that recent studies in the management of camel herds have uncovered the occurrence of unexplained sub-fertility in males. Low libido and short breeding season are thought to be the main causes of low fertility in males, along with their limited breeding opportunities (El-Hassanein, 2003). El-Bhrawy (2005) reported that the intensity and frequency of sexual activity indicators differed across rutting months and between individual bulls. In most species that demonstrate seasonality in reproduction, environmental and physiological factors control when the breeding season begins and how long it lasts.

The term "induced ovulators" refers to female camels, in whom coitus is currently thought to be the main trigger for ovulation. To find the best procedure for synchronizing estrus, inducing ovulation, AI, and early pregnancy detection, numerous studies have been carried out. In line with this development, El- Hassanein et al. (2010) examined three alternative approaches as follow natural mating, intramuscular injection of GnRH, and seminal plasma intrauterine deposition for induction of ovulation in she-camels. El- Hassanein et al. (2010) concluded that synchronization of estrus and induction of ovulation techniques reduced the rate of conception to 83.3% whereas spontaneously mating animals had a 100% pregnancy rate throughout the breeding season. Additionally, no pregnancies were produced following seminal plasma intrauterine deposition for induction of ovulation.

Controlled Intravaginal Drug Releaser (CIDR) and GnRH injection could be used to regulate and induce ovarian activity in she-camels during the anestrus season (Monaco et al., 2012). Ovarian activity is stimulated by this medication during the summer. The mean follicular diameter is reduced with CIDR treatment. According to Monaco et al. (2012), by combining CIDR treatment with PMSG technique before the mating season (September), the synchronization activity is present at day 13 after treatment in both primiparous and multiparous camels. However, in primiparous camels the treatment response was higher. As a result, female camels that have lost a calf can be synchronized for ovulation and artificially inseminated as soon as 37 to 44 days following calving (El-Bahrawy et al., 2011).

Artificial Insemination in Sheep Breeding

Sheep are the most prevalent animal bred for food in Egypt (apart from avian species) (FAOSTAT, 2018). Sheep are a crucial part of agriculture, where its production generates around 30% of all agricultural income in the country. To increase human daily dietary protein intake, sheep breeding is encouraged. Therefore, sheep are a crucial part of the food security strategy in Egypt. About 72,296 tons of red meat was produced by 2.34 million of sheep in 2017, which was about 7.4% of total production of red meat in Egypt (FAOSTAT, 2018). In total, 99,322

tons of sheep milk were produced. Sheep are the most sophisticated livestock in Egypt due to their adaptability to many agricultural circumstances, particularly in reclaimed lands and desert regions. Sheep are very effective in producing meat, milk, and wool from sparse pastures. One of the most urgent issues facing the nation is the lack of protein in the average person's diet. Sheep meat and milk can considerably help with this goal. Therefore, when compared to large ruminants, sheep has the ability to produce both milk and meat without ingesting significant amounts of feed concentrates.

According to FAOSTAT (2018), Egypt has 5.69 million head of sheep, which is more than cattle (5.06 million), buffaloes (3.37 million) and goats (4.35 million). Over the previous two decades, both the number of sheep and cattle has increased, but the number of buffaloes has dropped over the same period. While increasing the number of cattle as they use more feeds to produce milk, the number of sheep has climbed since they can graze more easily and require less concentrate. Figure 2 shows sheep breeds that are commonly raised in Egypt.

Figure 2. Geographic distribution of local Egyptian sheep breeds.
Şekil 2. Mısır'da Yerli Koyun Irklarının Coğrafi Dağılımı

The Egyptian-Finnsheep breeding project was a crossbreeding initiative conducted by Ministry of Agriculture in Egypt with a goal of increasing the production of Rahmani and Ossimi sheep breeds by mating them with the highly productive Finnsheep. According to the project's findings, breeding local breeds of sheep with Finnsheep resulted in a genotype that is 1/4 Finn, 3/4 local (Elshennawy, 1995). However, crossbreeding should not be disregarded as a crucial strategy to increase sheep output in Egypt. To capture breed complementarity and hybrid vigor (heterosis), Egyptian researchers advised commercial flocks to crossbreed the three local fat-tailed breeds (Rahmani, Ossimi, and Barki). Additionally, they recommended crossbreeding with other breeds but only if the exotic variety is suitable for the desert. Egyptian native breeds should be crossed with enhanced breeds that have a moderate level of productive or reproductive efficiency; these exotic breeds should have developed in conditions resembling those of the native breeds (Marai et al., 2009). It is feasible that productive breeds that are not acclimated to desert environments could make a significant contribution in semi-intensive production systems where sheep are fed gathered feeds rather than relying on grazing.

Egypt currently lacks a national, comprehensive sheep breeding program. Although creating a nationwide program for improvement of sheep genetics would be a difficult and impossible task, research institutions and colleges can take the lead in such an endeavor. It may be possible to conduct genetic analyses and subsequently identify genetically superior rams and ewes if sheep farms administered by research institutions and universities adopt a consistent record-keeping system. An essential step in restructuring of sheep sector in Egypt would be the incorporation of AI and embryo transfer (ET) into the programs of sheep breeding at research institutions and universities. Although Egypt already possesses some of the technical expertise to integrate AI and ET into sheep breeding operations, more money will probably be required to fund technical education and equipment acquisitions to increase the process of these technologies which become more widely used.

Position of semen more closely to fertilization site during laparoscopic insemination, is more favorable. For this reason, Laparoscopic Artificial Insemination (LAI) is beneficial in both sheep (Wulster-Radcliffe et al., 2004) and goats (Anakkul et al., 2013) in case of low sperm quantities or low sperm quality. In Egypt, LAI is typically the only way to improve Barki ewe progeny (Elshazly and Youngs, 2019). The technique of standing position artificial insemination (SPAI), which uses advanced research and carefully chosen frozen sperm, can be used to increase the genetic potential of high-quality sheep without putting undue stress on the animals (Elsayad et al., 2014).

Artificial Insemination in Goat Breeding

Goat breeding plays a significant role in Egyptian agriculture. Due to their strong tolerance for heat stress and hard environments, goats play a key role. Goats, unlike other ruminants, can quickly transform scarce crops and forages into milk and meat (Tekin, 2019). Depending on the choice for semen deposition, there are various AI approaches for small ruminants. According to Faigl et al. (2012), fresh semen deposited vaginally (pericervical deposition of semen), cervically (intracervical deposition of semen), or intrauterinally (laparoscopic technique) resulted in satisfactory pregnancy rates (50-70%). The only way to achieve acceptable pregnancy rates when using frozen sperm is through laparoscopic or transcervical intrauterine insemination procedures because, after cervical AI, cryopreservation renders spermatozoa functionally compromised and with disturbed semen transport through the reproductive tract.

During laparoscopic insemination, semen is positioned more closely to the site of fertilization, which is favorable. When sperm quantities are low or sperm quality is poor, deep uterine insemination is beneficial in both sheep (Wulster-Radcliffe et al., 2004) and goats (Anakkul et al., 2013). Insufficient sperm counts have typically resulted in lower pregnancy rates when utilized for AI (Scenna et al., 2005). To achieve the maximum pregnancy rates in goats, it is necessary to determine the ideal number of spermatozoa. Frozen-thawed goat spermatozoa have reported insemination dosages ranging from 5×106 to 200×106 sperm, and these doses mostly rely on the AI procedure used (Anakkul et al., 2014).

Amal Leil (2020) found that the rate of pregnancy following intrauterine insemination (IUI) with 20×106 Zaraiby spermatozoa was the greatest ($P<0.05$) with 71%, while the rate of pregnancy following IUI with 10×106 Boer spermatozoa was the highest ($P<0.05$) with 60%. The lowest ($P<0.05$) pregnancy rate was obtained when 40×106 spermatozoa from Zaraiby or Boer bucks were used (from 0 to 14.29%). When either 10×106 or 20×106 spermatozoa were inseminated laparoscopically by the semen of two breeds, no appreciable changes in the number of kids per goat were found (Amal Leil, 2020). On average, goats inseminated with the spermatozoa of the Boer buck at the aforementioned doses had greater multiple birth rates than goats inseminated with the spermatozoa of the Zaraiby buck (66.67 to 100% vs. 25 to 40%, respectively).

To achieve satisfactory pregnancy results in goats and sheep, Kulaksız and Arı (2016) indicated that cervical or vaginal AI should contain at least 200×106 sperm cells. Ejaculate from bucks or rams may only artificially inseminate 10-15 females due to the large dosage utilized in cervical or vaginal AI. Contrarily, LAI, which uses low doses (10 to 20×106) of frozen-thawed semen, guarantees a greater pregnancy rate (Anakkul et al., 2014; Kulaksız and Arı, 2016). In this case, LAI offers a widespread and more effective mechanism for AI to use semen from a superior buck or ram. Although the semen quality can affect this rate, other factors influencing the pregnancy rate include the body condition of the female, lactation status, the inseminator's skill, the number of prior pregnancies, the time between kidding and AI, seasonality of reproductive, farm and fertility of farm and buck, as well as the technique of semen cryopreservation (Gibbons et al., 2019).

According to Anakkul et al. (2014), using 60×106 or 120×106 spermatozoa in LAI in Saanen goats, there were no significant changes in kidding rates (50.33% vs. 60%, respectively). These variations could be explained by sperm quality elements such as viability, motility, and longevity. According to Bonato et al. (2019), similar kidding rates were observed in various goat breeds. However, some experiments found less impressive findings, occasionally peaking at 85% (Toni et al., 2012).

Finding the ideal quantity of spermatozoa is typical of great importance to increase goat pregnancy rates. LAI is an easy and practical way to increase fertilization rates and promote cross-breeding in goats. It is suggested that the minimal doses of motile spermatozoa for LAI could be 10×106 and 20×106 in Boer and Zaraiby bucks, respectively (Amal Leil, 2020).

CONCLUSION

Artificial insemination has become of great importance in livestock breeding in Egypt. Due to restricted amount of water resources and reclaimed lands in Egypt, spreading the livestock horizontally is not a choice. Therefore, the AI of livestock would be a viable work. Meanwhile, this review represents a current vision for applying the AI of livestock to get a better food security in Egypt.

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Review Article

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CRISPR-Cas9 Technology: in Biotechnology a Breakthrough Innovation

ABSTRACT

CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 is a gene-editing technology based on regularly spaced short palindromic repeats that have revolutionized biotechnology research. This system offers the potential to edit desired changes and genes in the genome in a fast, inexpensive, and simple way. Gene editing has many potential applications, including treating genetic diseases and the enhancement of yield and quality in agricultural products. The CRISPR-Cas9 system has a wide range of applications, from treating genetic diseases in medicine, improving crop yields in agriculture, obtaining disease-resistant animals, studying antibiotic resistance in microbiology, nitrogen fixation, biofuels, biosensors, greenhouse gas emissions, pesticide reduction, water management, etc. For example, this system has been used to successfully regulate mutations in intestinal organoids in the treatment of cystic fibrosis. In plants, successful results have been achieved in creating herbicide resistance in rice, powdery mildew in tomatoes, and reducing high amylopectin content in potatoes. In animals, studies are underway to provide resistance to bacteria that cause mastitis in cows. At the same time, research is underway to reduce milk allergens in goats by silencing the beta-lactoglobulin gene. However, the ethical aspects of CRISPR technology are also an important topic of debate. Given the potential risks and social implications of genetic engineering, ethical debates on this issue should continue. In conclusion, CRISPR-Cas9 is a revolutionary tool in genetic engineering and offers new opportunities for innovative applications in many fields. This article reviews studies on CRISPR-Cas9 technology, its use in medicine, agriculture, animal husbandry, and ethical debates.

Keywords: CRISPR/Cas9, biotechnology; genome editing; agriculture; environment

CRISPR-Cas9 Teknolojisi: Biyoteknolojide Devrim Niteliğinde Bir İnovasyon

ÖZ

CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9, biyoteknoloji araştırmalarını devrim niteliğinde değiştiren düzenli aralıklarla yerleşmiş kısa palindromik tekrarlar temelinde bir gen düzenleme teknolojisidir. Bu sistem, genomda istenilen değişiklikleri ve genleri hızlı, ucuz ve basit bir şekilde düzenleme potansiyeli sunmaktadır. Gen düzenlemenin birçok potansiyel uygulaması vardır; bunlar arasında genetik hastalıkların tedavisi ve tarımsal ürünlerin verimliliği ile kalitesinin artırılması yer alır. CRISPR-Cas9 sistemi, genetik hastalıkların tedavisinden tarımda ürün verimliliğini artırmaya, hastalıklara dirençli hayvanlar elde etmeye, mikrobiyolojide antibiyotik direncini incelemeye, azot fiksasyonu, biyoyakıtlar, biyosensörler, sera gazı emisyonları, pestisit azaltımı, su yönetimi vb. bir dizi farklı alanda uygulamalara sahiptir. Örneğin, bu sistem, kistik fibrozisin tedavisinde bağırsak organoidlerinde mutasyonları başarılı bir şekilde düzenlemek için kullanılmıştır. Bitkilerde ise pirinçte herbisit direnci, domateste unlu mantar hastalığı ve patateslerde yüksek amilopektin içeriğinin azaltılması gibi başarılı sonuçlar elde edilmiştir. Hayvanlarda ise ineklerde mastitise yol açan bakterilere karşı direnç sağlamak için çalışmalar devam etmektedir. Aynı zamanda, keçilerde beta-laktoglobulin geninin susturulmasıyla süt alerjenlerinin azaltılması amacıyla araştırmalar yapılmaktadır. Ancak, CRISPR teknolojisinin etik yönleri de önemli bir tartışma konusudur. Genetik mühendisliğin potansiyel riskleri ve toplumsal etkileri göz önüne alındığında, bu konuda etik tartışmaların devam etmesi gerektiği söylenebilir. Sonuç olarak, CRISPR-Cas9, genetik mühendislikte devrim niteliğinde bir araçtır ve birçok alanda yenilikçi uygulamalar için yeni fırsatlar sunmaktadır. Bu makale, CRISPR-Cas9 teknolojisi, tıptaki, tarımdaki, hayvancılıktaki kullanımı ve etik tartışmalarla ilgili yapılan çalışmaları incelemektedir.

Anahtar Kelimeler: CRISPR-Cas9, biyoteknoloji, genom düzenleme, ziraat, çevre

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INTRODUCTION

The term biotechnology was first defined in 1918 by the Hungarian engineer Karl Ereky. However, the basic applications of biotechnology are actually as old as human history. Following Ereky, biotechnology has been defined in different ways by different scientists. According to one definition, biotechnology combines the principles of engineering and biological sciences to produce new and useful products from raw materials of biological origin (Verma et al., 2011).

Another definition of biotechnology is the use of living organisms or products derived from them to improve human health or the environment. Biotechnology is important not only for its beneficial aspects, but also for its risks, which need to be handled with care (Verma et al. 2011).

There are several sub-fields of biotechnology:

• Medical biotechnology: Includes topics such as drug development, gene therapy and disease diagnosis.

• Agricultural biotechnology: Aims to produce more resilient and productive crops.

• Environmental biotechnology: Provides solutions to reduce pollution and make sustainable use of natural resources.

• Food biotechnology: The use of biotechnology in food production.

• Industrial biotechnology: Covers biotechnology applications in industrial production.

• Marine biotechnology: Focuses on the use of biological materials from the sea.

Traditional biotechnology uses the natural biological processes of living organisms, such as bread, cheese and other foods produced by fermentation. Modern biotechnology uses advanced scientific methods such as genetic engineering, DNA technologies, monoclonal antibody production and biological therapies. These technologies offer significant innovations and opportunities in the fields of health and the environment. As a rapidly developing field, biotechnology has the potential to provide solutions to future problems (Gupta et al., 2017).

Many scientists have made great efforts, sacrifices and revolutionary contributions in the development of molecular biotechnology. One of the most prominent of these developments was realised in 1978 by Genentech, a US-based pharmaceutical company. The company isolated the gene that codes for human insulin and transferred it to Escherichia coli bacteria. These bacteria, working like biological factories, produced human insulin and this molecule was made available to diabetics. Shortly after this important development, in 1980, Genentech's shares soared on the New York Stock Exchange. The shares experienced one of the fastest jumps in stock market history, rising from $35 to $89 in 20 minutes. This surge was driven by high expectations of the potential applications of biotechnology. These include the production of micro-organisms that can replace chemical fertilisers, and the development of genetically engineered pest-resistant and nutrient-enhanced crops. However, this process has also given rise to ethical, social, legal and religious debates (Tarım, 2004).

New techniques for editing plant genomes have been developed that can target specific chromosomal regions. These techniques are implemented using sequence-specific and customisable nucleases. These nucleases create a double-stranded DNA break at the target region. The cell recognises this break as DNA damage and activates the appropriate enzymatic mechanisms to carry out the repair. When this repair is done by homologous recombination (HR), the broken region is repaired using information from a DNA template. This template can be a homologous chromosome, a sister chromatid or user supplied DNA. Custom templates can contain modifications such as nucleotide changes or transgenes and can be incorporated into the target site by HR. Another repair mechanism, non-homologous end joining (NHEJ), is usually used in somatic cells and although correct repair is often achieved, small deletions or rare insertions can occur. In addition, some mutations can result in the removal of a few amino acids in the coding sequences, but this can alter the function of the gene without affecting the reading frame. Similarly, insertions or deletions in promoter regions can affect gene expression by disrupting the structure of regulatory sequences. To generate targeted mutations, SSNs must be able to recognise specific DNA sequences in complex genomes (Songstad, 2017).

A number of basic tools have been developed to edit plant genomes. These include homologous recombination (HR), zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), pentatricopeptide repeat proteins (PPR), the CRISPR/Cas9 system, RNA interference (RNAi), cisgenesis and intragenesis. In addition, sequence-directed editing techniques and oligonucleotide-based mutagenesis methods offer the possibility of modifying the genome at the level of a single nucleotide. In recent years, innovations such as adenine base editors (ABEs) have been developed. ABEs use a combination of Cas9 nickase and a deoxyadenine deaminase (TadA) enzyme with reduced catalytic activity to convert AT base pairs to GC base pairs (Mohanta et al. 2017).

CRISPR-Cas9 Technology

In 1989, Francisco Mojica joined the University of Alicante for his Ph. D and became involved in the study of an archaeal microbe, Haloferax mediterranei, which is extremely salt-tolerant in the marshes of Santa Pola (Doudna and Gersbach, 2015).

Mojica delved deeper into the microbe's DNA, and in his initial research he discovered a palindromic structure of 30-base-long repeating sequences separated by about 36 bases. These sequences were unlike any known microbial repeat group. Fascinated by this unusual structure, Mojica dedicated himself to solving the puzzle. Over time, he found similar structures in other halophilic archaea, as well as in close relatives such as H. volcanii. A search of the literature suggested that such structures might also be present in eubacteria; in particular, a group of researchers had reported a similar structure in Escherichia coli. However, this observation had not attracted much attention. Mojica thought that the structure might have an important biological function because it was repeated in different microorganisms and published a paper describing it. He initially called them 'short regularly spaced repeats' (SRSRs), but later changed the term to 'clustered regularly interspaced palindromic repeats' (CRISPRs) (Lander, 2016).

In 2000, initial observations showed that microorganisms such as Streptococcus thermophilus, Mycobacterium tuberculosis, Clostridium difficile and Yersinia pestis add new spacer sequences to the CRISPR region after viral attack. These DNA sequences were found to be compatible with specific parts of the virus genome, and changes in the spacer sequences can affect the bacteria's resistance to viruses. These findings confirm that CRISPR sequences play an important role in the immune system of bacteria (Vidyasagar, 2018).

To understand how life works, researchers need to alter the genetic make-up of cells. In the past, this process was slow, complex and sometimes impossible. But with CRISPR/Cas9 genetic scissors, the code of life can now be changed in just a few weeks. Emmanuelle Charpentier discovered a previously unknown molecule, tracrRNA, while studying bacteria such as Streptococcus pyogenes. Charpentier's research showed that tracrRNA is part of CRISPR/Cas, the bacteria's ancient immune system, which cuts the DNA of viruses and neutralises them. Charpentier published his findings in 2011. That same year, she began working with biochemist Jennifer Doudna. Together, they recreated the bacterium's genetic scissors in a test tube, making them more accessible. They enabled these scissors to cut DNA at a predetermined point. This discovery opened up the possibility of modifying the genetic code of life (Charpentier and Doudna, 2020).

Mechanism of the CRISPR System

CRISPR-Cas systems are an immune mechanism used by bacteria and archaea and consist of CRISPR arrays and CRISPR-associated (Cas) genes. In the operation of this system, Cas genes, which are usually located near CRISPR arrays, are responsible for the production of proteins involved in the immune response and play an important role in CRISPR activity. CRISPR arrays are formed by inserting short pieces of foreign genetic material, such as the virus that infects the bacteria, between repeated sequences. This mechanism allows certain parts of the viral DNA to be inserted into the CRISPR site along with the repeat sequences (Gök and Tunalı, 2016).

The CRISPR-Cas immune system works in three basic steps: adaptation, expression and targeting. The first step, adaptation, involves the integration of short interval sequences from the invading DNA into the CRISPR site. This process starts with the recognition of protointerval adjacency motifs (PAMs) in the foreign DNA (Barrangou and Marraffini, 2014). PAM motifs prevent the system from targeting its own DNA, while mutations allow the phage to escape immunity. In the second step, sequences at the CRISPR locus are transcribed into precursor CRISPR RNAs (pre-crRNAs), which are converted into small crRNAs by Cas enzymes. In the final step, targeting,

the crRNA pairs with the invading DNA and Cas proteins cut the target DNA, preventing replication of the invading genetic material (Savic and Schwank, 2016; Barrangou and Marraffini, 2014).

Applications of CRISPR Technology

Since the discovery of CRISPR/Cas9 by Charpentier and Doudna in 2012, the use of this technology has expanded rapidly. CRISPR/Cas9 has contributed to many important discoveries in basic science and has enabled the development of crops resistant to pests, mould and drought in agriculture. In medicine, clinical trials of new cancer treatments are underway. CRISPR/Cas9 has revolutionised the life sciences and brought significant benefits to humanity (Charpentier and Doudna, 2020).

The Role of CRISPR in Medicine

Transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) are monogenic disorders that are common worldwide and cause significant health problems. Approximately 60,000 cases of TDT and 300,000 cases of SCD are diagnosed worldwide each year. These diseases are caused by mutations in the haemoglobin β-subunit gene and lead to severe complications such as ineffective erythropoiesis, haemolysis, anaemia, organ damage and painful crises. Current treatments aim to manage symptoms with transfusions and iron chelation, but these approaches do not correct the underlying genetic defect. However, CRISPR-Cas9 gene-editing technology offers a revolutionary approach to treating these diseases. By targeting the enhancer region of the BCL11A gene in erythroid cells, this technology increases γ-globin expression and reactivates fetal haemoglobin production. In clinical trials, patients with TDT and SCD treated with CRISPR-Cas9 have shown positive results, including improved haemoglobin levels, transfusion independence and reduced complications. These developments show that CRISPR-Cas9 is a promising tool for the treatment of genetic diseases (Frangoul et al. 2021).

One study aimed to correct the Q61K mutation in the NRAS gene, which causes malignant melanoma (skin cancer), using CRISPR/Cas9 genome editing. Using the SK-MEL-30 cell line carrying the Q61K mutation, guide RNA (gRNA) sequences were designed and integrated into plasmid vectors. These genetic constructs were transferred into cells by electroporation and analysed using techniques such as fluorescence microscopy and flow cytometry. Using the HDR-assisted repair mechanism, knock-in and knock-out procedures were performed in the targeted regions and the efficiency of these procedures was verified by real-time PCR and end-point analysis methods. The results of the study show that the Q61K mutation can be successfully corrected using CRISPR/Cas9 and that this approach provides an innovative and effective method for treating cancer mutations. The study provides an important foundation for gene editing technologies and directs future research in this field (Duran, 2018).

This study investigates the efficiency and specificity of the CRISPR/Cas9 system for gene editing in human triple-nucleus (3PN) zygotes. The accuracy and off-target effects of gene editing in human embryos are critical to the success of clinical gene therapy and embryo editing studies. The study showed that CRISPR/Cas9 enables genetic editing in 3PN zygotes, and that editing occurs mainly by error-prone NHEJ, with limited linear editing by HDR. In addition, off-target effects and genetic mosaicism were observed in edited embryos. These findings suggest that although CRISPR/Cas9 has potential for genetic therapy, off-target effects should be further investigated in clinical applications (Kose et al. 2020).

Innovative Practices in Agriculture

This paper focuses on the development of herbicide-tolerant carrot genotypes using a combination of CRISPR/Cas9 and cytidine base editing techniques. CRISPR/Cas9 technology offers genetic editing by targeting specific regions on DNA, while cytidine base editing offers the ability to modify specific bases in DNA. These two powerful biotechnology tools were used to make carrot plants resistant to herbicides. Although herbicides used in agriculture are effective in controlling weeds, they can have adverse effects on plants. Therefore, the development of herbicide-tolerant plants is of great importance in terms of increasing agricultural productivity and reducing environmental impact. The aim of this study is to increase the yield of carrot genotypes developed using these biotechnological methods by providing a more resistant and sustainable production against herbicides (İpek et al. 2024).

Researchers at the University of Florida used CRISPR-Cas9 technology to reduce the bending angle of sugar cane leaves while increasing dry biomass, internodes and branch number. This change allowed the plants to access more sunlight, increasing biomass production. The research has demonstrated the potential of genome

editing to increase sugarcane productivity. The study particularly highlights the importance of sugarcane for biofuel production (Brant et al. 2024).

A research team from the Chinese Academy of Agricultural Sciences also used the CRISPR-Cas9 system to create mutations in GmFT2a, an integrator in the photoperiod flowering pathway of soybean. The modified soybean plants showed late flowering, resulting in an increase in vegetative size. The mutation was found to be stably inherited in the next generation (Cai et al. 2017).

A New Era with CRISPR in Industry

To investigate the biological feasibility of decaffeinating coffee plants using CRISPR-Cas9 gene-editing technology and to examine the impact of this technology in a social context. The aim was to stop caffeine production by regulating some genes involved in the caffeine biosynthesis pathway. The study used a methodology in which the CRISPR-Cas9 system was used to create mutations in target genes and this genetic editing was transferred to plants by Agrobacterium tumefaciens-mediated transformation. The results showed that the regulation of these genes is promising for the production of decaffeinated coffee. In particular, regulation of the DXMT gene can lead to the accumulation of theobromine, which can affect the bitterness of coffee, while regulation of XMT can completely stop caffeine production. However, it has been stressed that decaffeinated coffee plants produced using CRISPR-Cas9 technology have a significant impact on commercial success (Leibrock et al. 2022).

CRISPR/Cas9 technology is playing an important role in energy science. This gene-editing tool makes it possible to modify the genomes of microorganisms used in biofuel production more quickly and precisely. In particular, microorganisms such as Yarrowia lipolytica can be modified using CRISPR/Cas9 to make them more efficient at converting sugars into lipids and hydrocarbons. This approach allows biotech products to be produced in a more sustainable and economical way than synthetic methods. In addition, biofuel and chemical production processes are being developed using CRISPR/Cas9 in bacteria such as Clostridium autoethanogenum, which has great potential for renewable energy production (Kaboli and Babazada, 2018).

CRISPR Revolution in Animal Genetics

CRISPR-Cas9 gene editing technology is being used to improve hornlessness in dairy cows. Horned cattle pose a safety risk to both other animals and farm workers. Instead of cattle whose horns are physically removed using traditional methods, cows have been developed that are genetically rendered hornless using CRISPR. This method both prevents animal suffering and makes the production process safer. However, the classification of gene-edited cows as GMOs requires regulatory hurdles and safety testing. However, recent regulatory changes in the US suggest that these barriers may be overcome and that gene-edited cows may become more common (Sandøe et al. 2021).

The aim of this study is to use CRISPR/Cas9 technology to correct the single nucleotide change that causes IARS (Isoleucyl-tRNA synthetase) syndrome in Japanese Black cattle. The CRISPR/Cas9 system is designed to make the correct nucleotide substitution by targeting the mutated region. The genetic modification was transferred into bovine fetal fibroblast cells using donor DNA containing the AcGFP cassette, followed by embryo production by somatic cell nuclear transfer (SCNT). The result showed that the mutation was correctly repaired and no additional DNA was identified. These results suggest that genome editing technology could be an effective tool for improving livestock productivity and restoring genetic diversity (Ikeda et al. 2017).

To create mutant dogs with muscle hypertrophy by editing the myostatin (MSTN) gene in beagle dogs using CRISPR/Cas9 technology. MSTN is a gene that regulates skeletal muscle mass. Mutations in this gene can increase muscle development. First, the MSTN gene was disrupted using Cas9 mRNA and sgRNA, and then embryos were transferred to female dogs by microinjection. By introducing mutations in the MSTN gene, the researchers were able to successfully produce genetically modified dogs with marked changes in muscle structure. These results support the potential of CRISPR/Cas9 technology to generate new canine models for biomedical research (Tian et al. 2023).

Atlantic salmon is an important commercial aquaculture species. In this study, we aimed to improve the salmon genome through gene editing and induced mutations in the pigmentation-related tyr and slc45a2 genes using the CRISPR/Cas9 system. Microinjection experiments showed that low incubation temperatures and

physical characteristics of salmon eggs had limiting effects on mutation efficiency. Analyses showed that mutation rates in target genes were low compared to zebrafish and similar to tilapia. DNA analysis from fin clips was also suggested as a practical method for assessing gene editing efficiency. Phenotypic effects of mutations were observed and associated with loss of pigmentation. This study demonstrates that CRISPR/Cas9 technology can be successfully applied in salmonids and can be used as an important tool for genetic improvement in aquaculture (Edvardsen et al. 2014).

ETHICAL DISCUSSIONS

CRISPR-Cas9 technology has great potential for the treatment of genetic diseases. This technology can be applied in two different categories: somatic and germline gene editing. While somatic editing only affects the individual being treated, germline editing can make permanent changes that can be passed on to subsequent generations. However, technical problems such as off-target effects and mosaicism make it difficult to use this technology safely. Although promising, germline editing raises ethical questions. Reduced genetic diversity and practices such as 'designer babies' can lead to social inequalities. The first editing of a human embryo in 2015 fuelled ethical debates in the field. Globally, the use of CRISPR technology is regulated differently in different countries. While some countries prohibit germline editing, others allow its use for research purposes (Tosun and Kesmen, 2022).

CRISPR technology has the potential to bring huge benefits in areas such as food security, health and biotechnology, but it raises ethical and safety concerns. Applications such as food modification and gene drives could benefit the undernourished, but there is a risk that these benefits could increase inequality through limited access. In addition, gene drives can permanently alter species, which may have uncertain effects on the balance of ecosystems (Brokowski and Adli, 2019).

While the commercialisation of CRISPR technology is accelerating progress in genetic engineering, it raises questions about patent rights and intellectual property. The patenting of these technologies should be shaped not only for commercial interests, but also for the general benefit of society. Patents should be organised in such a way as to ensure the protection of innovation, while at the same time not preventing large sections of society from benefiting from these innovations. Otherwise, powerful technologies such as genetic engineering may deepen inequalities and ignore the public good (Mulvihill et al. 2017).

To improve the safety of the CRISPR-Cas9 system, several innovative approaches have been developed to reduce off-target effects (OTE). At the forefront of these is the design of more specific and targeted sgRNAs. These designs aim to minimise the likelihood of off-target mutations. Modifications to the Cas9 protein are also aiding this process; for example, Cas9 nicase mutants increase the sensitivity of editing by making only single-strand breaks in the target region. In addition, tools such as optogenetics and transposons can be used to make gene editing more controlled and safer. These strategies increase the safety of genetic interventions, allowing treatments to be delivered more effectively and with less risk (Kose et al. 2020).

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7. Araştırma makaleleri Türkçe veya İngilizce dillerinden birisi ile genel olarak; Başlık, Özet, Abstract, İngilizce ve Türkçe Anahtar Sözcükler, Giriş, Materyal ve Yöntem, Araştırma Bulguları, Tartışma, Sonuç, Kaynaklar ana başlıkları altında hazırlanmalıdır. İstenirse Araştırma Bulguları ve Tartışma bölümleri tek başlık altında yazılabilir.
8. "Özet" ve "Abstract" çalışmanın kısa amacı, materyal ve metod, önemli araştırma bulguları ile sonucu içeren yapılandırılmış düzende olmalıdır.
   a. Yurt dışından gelecek makalelerde bulunan "Abstract"ların Türkçe "Özet" çevirisi editör kurulu tarafından yapılacaktır.
   b. "Özet" ve "Abstract" en çok 200 sözcük olmalıdır ve ana metinden ayrı olarak konumlandırılmalıdır.
   c. Kısaltmalar, diyagramlar ve literatürler "Özet" ve "Abstract" da yer almaz.
   d. "Özet" ve "Abstract"dan bir satır boşluk bırakıldıktan sonra 4 - 6 sözcük olmak üzere "Anahtar Kelimeler" ve "Key Words" yer almalı ve başlıkta geçen kelimelerden farklı olmalıdır.
9. Makalede yer alan türlerin bilimsel isimleri italik karakterde olmalı ve ondalık sayılar nokta işareti ile ayrılmalıdır.
10. Grafik, harita, fotoğraf, resim ve benzeri sunuşlar "Şekil", sayısal değerlerin verilişi "Çizelge" olarak isimlendirilmelidir. Şekil ve Çizelgelere ait Türkçe isimlendirmelerin altında İngilizce isimlendirmeler de yer almalıdır. Verilen tüm çizelge ve resimlere metin içerisinde atıf yapılmalı ve şekil ve çizelgeler makale sonunda ayrı ayrı sayfalarda verilmelidir.
11. Hayvansal Üretim' de yayımlanacak araştırma ve derleme makalelerinde derginin daha önceki sayılarında yayımlanan en az bir yayına atıf yapılması önem arz etmektedir.
12. Makale düzeni;
    a. Microsoft Word yazılımıyla (docx format; Word 2007 ve üstü) Times New Roman yazı karakterinde ve tek sütun halinde toplam 20 sayfayı geçmeyecek şekilde, A4 kağıdına kenarlarda 2.5 cm boşluk olacak şekilde çift satır aralıklı yazılmalıdır.
    b. Makalede her sayfaya numara verilmeli ve satırlar sürekli şekilde satır numaraları içermelidir.
    c. Makalenin Türkçe ve İngilizce başlığı koyu, 14 punto, ortalı ve ilk harfleri büyük olacak şekilde küçük harflerle yazılmalıdır.
    d. En fazla 3. düzeyde bölüm başlıkları kullanılmalıdır. Birinci düzey başlıklar sola yaslı, koyu, 12 punto ve her kelimenin ilk harfi büyük olmalıdır. İkinci düzey başlıklar koyu, sola yaslı ve yalnız ilk kelimenin ilk harfi büyük olmalıdır. Üçüncü düzey başlıklar her ne kadar önerilmese de eğer gerekli ise kullanılabilir ve sola yaslı ve sadece ilk kelimenin ilk harfi büyük şekilde yazılmalıdır.
    e. Metnin ana gövdesi çift aralıklı, Times New Roman, 12 punto ve iki yana yaslı yazılmalıdır. Tüm paragraflar sol kenardan başlamalıdır. Metin tümüyle iki yana yaslı hizalanmalıdır. Hiçbir heceleme olmamalıdır. Kalın veya altı çizili yazı kullanımı ile metin vurgulama önerilmez.
    f. Yazar/yazarların isimleri, makale başlığının altında bir satır boşluktan sonra ünvan belirtilmeden koyu 12 punto ile ön ismi açık ve küçük harfle, soyadı büyük harfle ve sekme (tab) ile boşluk bırakılarak yazılmalıdır.
    g. Yazarlarla ilgili akademik ve/veya diğer profesyonel kurumları rakam üst simgesi kullanılarak 10 punto ile belirtilmelidir. Ayrıca sorumlu yazarın elektronik posta adresi ayrı bir satırda yıldız işareti ile gösterilmelidir.
13. Makale içindeki atıflarda özel durumlar dışında "yazar ve tarih" sistemi kullanılmalıdır. Birden çok kaynağa aynı anda atıf yapılacaksa yayınlar noktalı virgül ile ayrılmalı ve kronolojik sıra ile verilmelidir. Örneğin: (SoyadıA, 2002; SoyadıB ve

ark., 2008; SoyadıC, 2008; SoyadıD1 ve SoyadıD2, 2012). İki yazarlı eserlerde yazar isimleri "ve" ile ayrılmalı, çok yazarlı eserlerde "ve ark." (yabancı dildeki kaynaklarda ise "et al.") kullanılmalıdır. Örneğin: Soyadı1 (2007), Soyadı1 ve Soyadı2 (2005), Soyadı1 ve ark. (2003). Birden fazla yazarlı veya tek yazarlı yayınların çoklu kullanışlarında tarihsel sıralanmalı, aynı yılda bir çok yayının kullanılmasında (yazar grupları aynı olmasa bile) ise küçük harf ile ayrılmalıdır. Örneğin: Sönmez, R.,Kandemir, Ç., and Taşkın, T. 1999a; Sönmez, R.,Kandemir, Ç., and Taşkın, T. 1999b; Sönmez, R., Kandemir, Ç., and Taşkın, T 1999c (çünkü metin içinde hepsi " Sönmez ve ark., 1999" olarak geçecektir).

14. Metin içinde anılan bütün literatür, "Kaynaklar Listesi" nde yer almalıdır. Kaynaklar listesi alfabetik sırada ve yazar-tarih sistemine göre verilmelidir. Aynı yazarın iki veya daha fazla yayını kullanılmış ise Kaynaklar Listesinde eski tarihli yayın önce verilmelidir. Kitap ve kitap bölümünün adının her kelimesinin ilk harfi büyük harf olmalıdır. Bir kuruluşun yayınları ise yayın numarasıyla verilmeli, değilse basıldığı matbaa adı ve şehri belirtilmelidir. Literatürün yayımlandığı dergi adı kısaltma yapılmadan açık olarak yazılmalıdır. Kaynakların yazılışında ilk satır sola yaslanmalı, izleyen satırlar 0.5 cm içeri çekilmelidir. Literatür yazım şekli için örnekler aşağıda verilmiştir.

Kaynak makale ise:

Altan Ö, Oğuz İ, Akbaş Y. 1998. Japon bıldırcınlarında (*Coturnix coturnix japonica*) canlı ağırlık yönünde yapılan seleksiyonun ve yaşın yumurta özelliklerine etkileri. Turkish Journal of Veterinary and Animal Sciences 22(6):467-473.

Kaynak kitap ise:

Düzgüneş O, Eliçin A, Akman N. 1991. Hayvan ıslahı. 2. Baskı, Ankara Üniversitesi Ziraat Fakültesi Baskı Ünitesi, Ankara.

Kaynak bir kitaptan bölüm ise:

Karaca O. 1997. Keçilerde yetiştirme işleri. Editör: Kaymakçı M, Aşkın Y. Keçi yetiştirme. Baran Ofset, Ankara, s.102-114.

Kaynak sempozyum veya kongre makalelerinden ise:

Akbulut Ö, Bayram B. 1999. Buzağılarda yaş-ağırlık-yem tüketimi ilişkisinin fonksiyonel analizi. Uluslararası Hayvancılık'99 Kongresi, 21-24 Eylül 1999, Ege Üniversitesi Ziraat Fakültesi, İzmir, s.52-58.

Kaynak Web sitesi ise *(varsa yazarlar, yayının tarihi ve belgenin adı. Tam URL adresi ve Erişim tarihi):*

Rayens B. 2004. Practical nonparametric statistics http://www.ms.uky.edu/~rayens/teaching/sta673/sta673.html (15 Nisan 2004).

Efe E, Bek Y, Şahin M. 2000. SPSS'te çözümleri ile istatistik yöntemler. http://www.ksu.edu.tr/kisisel/eefe/spss.pdf (15 Nisan 2004).

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