

Effects of Environmental Factors on Some Milk and Reproductive Traits of Anatolian Buffaloes Raised in Bitlis Province

Önder Bayram ÇOBAN¹, Gürsel DELLAL², Seyrani KONCAGÜL³

¹Muş Alparslan University, Faculty of Applied Science, Faculty of Animal Production and Technologies, Muş, Türkiye, ^{2,3}Department of Animal Science, Faculty of Agriculture, Ankara University, Ankara, Türkiye

¹<https://orcid.org/0000-0002-5179-8687>, ²<https://orcid.org/0000-0002-8129-982X>, ³<https://orcid.org/0000-0001-7596-0485>

✉: ob.coban@alparslan.edu.tr

ABSTRACT

This study aimed to determine the effects of some environmental factors on milk and fertility traits of Anatolian buffaloes raised in Bitlis province within the scope of the "Anatolian Buffalo Breeding in Public Hands Project" carried out by the Ministry of Agriculture and Forestry (MAF), General Directorate of Agricultural Research and Policy (GDARP). In the study, phenotypic parameters of performance traits, including lactation period, total milk yield during lactation, 305-day milk yield, average daily milk yield, calving interval, and service period, were estimated using 2012-2019 records. The average total values of the corresponding traits were 241.28±0.301 days (lactation duration), 1011.38±3.58 kg (lactation milk yield), 1278.37±3.77 kg (305-day milk yield), 4.19±0.012 kg (average daily milk yield), 438.32±1.110 days (calving interval), and 111.39±0.148 days (service period). The coefficients of variation for these values were 12.27%, 28.35%, 23.66%, 23.66%, 23.69%, 24.86% and 13.12%. The statistical analyses showed that environmental factors such as year and season of calving, order of lactation, and place of rearing had significant effects on these traits ($p<.05$). Eight years of research data indicate that positive phenotypic progress has been recorded in terms of milk and fertility in the Anatolian buffaloes population. The findings demonstrate the effectiveness of selection programmes and reveal that productivity can be sustainably increased by optimising genetic improvement and environmental management strategies.

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ÖZET

Bu çalışma ile Tarım ve Orman Bakanlığı (TOB), Tarım Araştırmaları ve Politikası Genel Müdürlüğü (TAGEM) tarafından yürütülen "Halk Elinde Anadolu Mandası Islahı Projesi" kapsamında Bitlis ilinde yetiştirilen Anadolu mandalarının süt ve döl verim özellikleri üzerine bazı çevresel faktörlerin etkisinin belirlenmesi amaçlanmıştır. Çalışmada, 2012-2019 kayıtlarına dayalı olarak laktasyon süresi, laktasyon set verimi, 305 günlük süt verimi, ortalama günlük süt verimi, malaklama aralığı ve servis periyodu gibi verim özelliklerinin fenotipik parametreleri tahmin edilmiştir. İlgili özelliklerin ortalama toplam değerleri sırasıyla 241,28±0,301 gün (laktasyon süresi), 1011,38±3,58 kg (laktasyon süt verimi), 1278,37±3,77 kg (305 günlük süt verimi), 4,19±0,012 kg (ortalama günlük süt verimi), 438,32±1,110 gün (malaklama aralığı) ve 111,39±0,148 gün (servis periyodu) olarak bulunmuştur. Bu değerlere ait varyasyon katsayıları sırasıyla %12,27, %28,35, %23,66, %23,66, %23,69, %24,86 ve %13,12 olarak bulunmuştur. İstatistiksel analizler, malaklama yılı ve mevsimi, laktasyon sırası ve yetiştirme bölgesi gibi çevresel faktörlerin bu özellikler üzerinde önemli etkileri olduğunu göstermiştir ($p<.05$). Sekiz yıllık araştırma verileri, Anadolu mandası popülasyonunda süt ve döl verimi açısından olumlu bir fenotipik

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ilerleme kaydedildiğini göstermektedir. Bulgular, seleksiyon programlarının etkinliğini kanıtlarken, genetik ıslah ve çevresel yönetim stratejilerinin optimize edilmesiyle verimliliğin sürdürülebilir şekilde artırılabilirliğini ortaya koymaktadır.

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INTRODUCTION

One of Turkey's native farm animal species is the Buffalo (*Bubalus bubalis*), and only the Anatolian buffalo breed is raised as a native breed. This buffalo breed originated from the Mediterranean buffalo, a sub-class of the River buffalo group, and has a chromosome number of 50 (Dellal, 1994).

In Turkey, Anatolian buffalo are raised by farming families in rural areas, mainly for milk and meat production, and are also used for labor. While the number of Anatolian buffalo was 1.140.000 head in 1961, it decreased to 85.000 head by 2007. The reasons for this decline include the development of mechanization in agricultural production, the decrease in wetland and pasture areas suitable for buffalo, low consumer preference for buffalo meat and dairy products, and the lack of support for buffalo breeding. However, due to the State's support for buffaloes since 2007 (84.700 heads) and the positive developments in consumer preferences for buffalo meat and dairy products, the total buffalo population increased by 91%, reaching 162 thousand heads in 2024 (TÜİK, 2025).

There have been three important changes in buffalo breeding in Turkey since the early 2000s. The first is the establishment of intensive farms for the Murrah buffalo breed, driven by rising consumer demand for buffalo dairy products (mozzarella cheese, yogurt, etc.). The second is the implementation of the “National Anatolian Buffalo Breeding Project in Public Hands” developed by MAF/GDARP in 2011. The third is the establishment of the Türkiye Buffalo Breeders Central Union in 2012.

The Anatolian buffalo is strategically important for Turkish livestock farming due to its high adaptability to environmental stress and its status as a native genetic resource. As the effects of climate change on animal production become increasingly evident, the role of such breeds in sustainable production systems is growing more significant. However, most existing studies on the milk and fertility traits of Anatolian buffaloes are based on short-term data or limited analyses that combine different ecological regions. In particular, studies evaluating long-term records from national breeding projects are scarce in regions such as Bitlis province, which has high altitude and harsh climatic conditions. This study analyses eight years of data collected in Bitlis province as part of the Anatolian Buffalo Breeding Project in the Hands of the People, revealing the effects of environmental factors on milk and fertility yields, and thus aims to address an important gap in the literature.

MATERIAL and METHOD

Research material

The material of the study consisted of 3000 Anatolian buffaloes bred within the framework of the “National Breeding Project for Anatolian Buffaloes under Agricultural Conditions” by the General Directorate of Agricultural Research and Policy of the Ministry of Agriculture and Rural Affairs (GDARP) in Bitlis Province, as well as milk and reproduction data of these animals kept between 2012 and 2019.

Research area

Bitlis is located in the Eastern Anatolia region of Turkey, between 41°33'-43°11' east longitude and 37°54'-38°58' north latitude. Its total area is 6,707 km². The province has a transitional climate with Mediterranean and continental climate characteristics. However, the annual climate data can vary greatly. Winters are cold and snowy; springs are rainy; summers are short and hot. According to meteorological data for the period of 1991-2020, the average temperature is 1.0 °C, the lowest temperature is 8.5 °C, the highest temperature is 29.4 °C, the average annual precipitation is 1025.8 mm, and the average annual number of rainy days is 119.6 days (Anonymous, 2025; TSMS, 2025).

Herd management

Similar breeding and animal husbandry practices are used in the buffalo farms where the project is carried out. The buffalo's diet is largely based on roughage, mainly from pastures and forage crops such as alfalfa. A limited

amount of concentrated feed is fed at critical times. Mating normally takes place in the pasture, where the animals can mate freely. Milking is done twice a day (morning and evening) by hand, usually by women in the barn. During milking, the calf is kept next to its mother and is allowed to suckle. The calves are weaned around 6-8 months after birth. Due to the long, harsh winters in the region, the buffalo are kept in enclosed shelters until they can go out to pasture.

Data collection

The defined milk and reproductive traits are: lactation period (LLL), lactation milk yield (LMY), 305-day milk yield (305-DMY), average daily milk yield (ADMY), calving interval (CI), and service period (SP). Milk yield checks were performed monthly, and data on these traits obtained from registered farms within the project scope were collected by technical personnel and recorded in the 'Buffalo Star' recording programme, developed as part of the Anatolian Buffalo Improvement Project. This software supports routine record maintenance, the removal of erroneous and incomplete data, and the standardisation of the dataset, thereby increasing the reliability of the data used in analyses (Tekerli, 2019).

Selection practices

Selection for milk yield focuses on LL and LMY. For this purpose, female and male calves whose mothers have LL >200 days and LMY >1000 kg are selected as stud calves. Generally, at least 10% of female calves are selected as breeding stock per year. However, this rate varies depending on factors such as the buffalo milk market and breeder demand. The number of male calves selected for breeding per year is at least half of the number of available bulls. Male calves stay in the herd for a maximum of two years.

Data analysis

LL is the time from calving to drying off of a buffalo cow. LMY was calculated for each buffalo cow according to the "Fleischmann method" (Barillet et al., 1992). 305-DMY is obtained by multiplying the average daily milk yield (ADMY) by 305 days. ADMY is calculated as lactation milk yield (LMY) divided by lactation length (LL). CI is the number of days between two consecutive calvings. SP was calculated by subtracting 320 days of gestation from the calving date. Data were prepared for analysis using Excel and SAS (2017).

This study is limited to data obtained in the province of Bitlis between 2012 and 2019, and the generalisability of the results is restricted. Environmental impacts were assessed using general climate data, while detailed variables such as microclimate conditions and feed quality could not be measured directly.

Some restrictions were applied to the data before analysis. Individuals with at least four milk control records were included in the analysis to ensure a reliable representation of lactation. Records with a lactation period shorter than 180 days or longer than 410 days, calving intervals shorter than 11 months or longer than 36 months, and service periods shorter than 60 days or longer than 200 days were excluded from the analysis, as they were considered insufficiently representative of the production characteristics of the Anatolian buffalo. Due to their known biological effects on milk production traits, service period and calving interval were included in the model as covariates. PROC GLM (SAS, 2017) was used to estimate the effects of environmental factors on these traits. The LSMEANS/Tukey command was used to calculate least squares means and test the significance of differences between group means. The following model was used to analyze milk and reproductive traits:

$$LL_{ijklm} = \mu + CY_i + CS_j + CV_k + LO_l + \beta_1 SP_{ijklm} + \beta_2 CI_{ijklm} + e_{ijklm}$$

$$LMY_{ijklm} = \mu + CY_i + CS_j + CV_k + LO_l + \beta_1 SP_{ijklm} + \beta_2 CI_{ijklm} + \beta_3 LL_{ijklm} + e_{ijklm}$$

$$ADMY_{ijklmn} = \mu + CY_i + CS_j + CV_k + LO_l + \beta_1 SP_{ijklm} + \beta_2 CI_{ijklm} + \beta_3 LL_{ijklm} + e_{ijklm}$$

$$305 - DMY_{ijklm} = \mu + CY_i + CS_j + CV_k + LO_l + \beta_1 SP_{ijklm} + \beta_2 CI_{ijklm} + e_{ijklm}$$

$$SP_{ijklm} = \mu + CY_i + CS_j + CV_k + LO_l + \beta_1 LL_{ijklm} + e_{ijklm}$$

$$CI_{ijklm} = \mu + CY_i + CS_j + CV_k + LO_l + \beta_1 LL_{ijklm} + e_{ijklm}$$

Here; CY_i : Effect of calving year; CS_j : Effect of the calving season; CV_k : Effect of the calving village; LO_l : Effect of lactation order; LL_{ijklm} : Lactation length (covariate); LMY_{ijklm} : lactation milk yield; $ADMY_{ijklm}$: Lactation average daily milk yield; $305-DMY_{ijklm}$: 305-day lactation milk yield; CI_{ijklm} : Calving interval (covariate); SP_{ijklm} : Service period (covariate); e_{ijklm} : Random error.

RESULTS and DISCUSSION

General descriptive values

Table 1 presents descriptive statistics for milk and reproductive traits in Anatolian buffaloes, including mean values and coefficients of variation. The high coefficient of variation values indicate significant phenotypic

differences within the population for milk and fertility traits, creating potential for genetic selection. The lactation length (LL) is an average of 241.28 days, the lactation milk yield (LMY) is 1011.38 kg, and the 305-day milk yield (305-DMY) is 1278.37 kg. These values show high variability in milk yield characteristics, with coefficients of variation of 28.35%, 23.66%, and 23.69%, respectively, indicating significant differences in milk production among the buffaloes. The average daily milk yield (ADMY) is 4.19 kg, with a calving interval (CI) of 438.32 days. The service period (SP) has an average of 111.39 days and a relatively low coefficient of variation (13.12%). These findings highlight the significant influence of genetic and environmental factors on milk and reproductive performance, indicating opportunities for improvement through genetic selection and management strategies.

Table 1. Descriptive values of milk and reproductive traits in Anatolian buffalos

Çizelge 1. *Anadolu mandalarında süt ve üreme özelliklerinin tanımlayıcı değerleri*

Traits	Number of Records	$\bar{X} \pm S\bar{x}$	Min.	Max.	% CV
LL (Day)	6417	241.28 $\pm$ 0.301	180.00	367.00	12.27
LMY (Kg)	6407	1011.38 $\pm$ 3.58	249.35	1894.77	28.35
305-DMY (Kg)	6407	1278.37 $\pm$ 3.77	400.14	2164.52	23.66
ADMY (Kg)	6407	4.19 $\pm$ 0.012	1.26	7.100	23.69
C (Day)	3708	438.32 $\pm$ 1.110	334.40	881.60	24.86
SP (Day)	1414	111.39 $\pm$ 0.148	75.20	196.80	13.12

*Lactation Length (LL), Lactation Milk Yield (LMY), 305 Days Milk Yield (305-DMY), Average Daily Milk Yield (ADMY), Calving Interval (CI), Service Period (SP), Average ($\bar{X}$), Standard Errors Of Mean ($S\bar{x}$), Coefficients of Variation (CV)

The average lactation period (LL) calculated in this study for Anatolian buffaloes (241.28 $\pm$ 0.301 days) in general, agrees with the LL averages reported by the Turkish Breed Registration Board and various researchers for the same breed of buffalo. For example, Uslu (1970) reported an LL of 244.4 days, and Koçak et al. (2019) reported an LL of 245.43 days. However, these LL averages for Anatolian buffaloes are much lower than the LL values for buffalo breeds genetically improved for milk production, which range from 280 to 351 days (Kay, 1977; Alexiev, 1998; Rasali et al., 1998; Khattab, 2017). This difference in LL may be due to Anatolian buffaloes having traditionally been selected for their adaptability to local environmental conditions rather than for their higher milk yield. These buffaloes are often kept in more extensive systems where factors such as climate, feed quality, and management practices can influence their lactation length and overall productivity.

In contrast, breeds such as Murrah and Nili-Ravi have undergone systematic genetic improvement programs focused on milk yield, supported by controlled management practices. These long-term selection efforts have resulted in significantly higher lactation milk yields compared to Anatolian buffaloes. For example, Murrah buffaloes with an LMY of 2034 kg (Kumar et al., 2014) and Nili-Ravi buffaloes with an LMY of 2430 kg (Cady, 1983) have benefited from decades of breeding programs to improve the efficiency of milk production, which is not yet the case for Anatolian buffaloes.

In this study, overall mean values for lactation milk yield (LMY) and 305-day milk yield (305-DMY) of 1011.38 $\pm$ 3.58 kg and 1278.37 $\pm$ 3.77 kg, respectively, were recorded in Anatolian buffaloes. In previous studies conducted on Anatolian buffaloes in Turkey, LMY values between 710 and 1223 kg were determined (Uslu, 1970; Şekerden & Küçükkebaşı, 2013; Koçak et al., 2019; Berkin et al., 2020; Şahin et al., 2020; Alkoyak & Öz, 2021; Öz et al., 2022; Kaplan & Tekerli, 2024), while the 305-DMY values ranged from 894 to 1230 kg (OGRT, 2004; Tekerli et al., 2001). However, these averages are much lower than the LMY and 305 DMY averages of foreign dairy buffalo breeds. For example, Murrah buffaloes have an LMY of 2034 kg (Kumar et al., 2014), Nili-Ravi buffaloes average 2430 kg (Cady, 1983), Kundi buffaloes 1357 kg (Ghaffar et al., 2007), and Meshana buffaloes average 1708 kg (Galsar et al., 2016). Similarly, the 305 DMY values for Nili Ravi buffaloes average 2191 kg (Hussain et al., 2006), while Murrah buffaloes reach 1893 kg (Kour & Narang, 2021) and Jaffarabadi buffaloes average 1477 kg (Sharma et al., 2017).

This discrepancy in milk yield between Anatolian buffaloes and foreign breeds can be attributed to several factors. First, the lack of a systematic, long-term genetic selection program in Turkey to improve milk production in Anatolian buffaloes until 2011 means they were not selected as intensively for milk yield as their foreign counterparts. In addition, management practices, including feeding, housing, and disease management, may also contribute to these lower averages. Although Anatolian buffaloes have proven their resilience in harsh conditions and are well adapted to local ecosystems, their milk yield has not been the focus of breeding programs.

However, it is important to note that the situation is improving with the recent initiation of systematic breeding programs. The National Anatolian Buffalo Breeding Project, launched in 2011, aims to improve the genetic potential of Anatolian buffaloes, including increasing milk yield. As the breeding program progresses and selection

becomes more targeted, it is expected that the milk yield of Anatolian buffalo will gradually approach that of other high-yielding breeds, and the current productivity gap will be closed.

In this study, the average daily milk yield (ADMY) of Anatolian buffaloes was calculated to be 4.19 ± 0.012 kg. This value is significantly higher than the average values of Şekerden and Avşar (2008) and Kul et al. (2016), who found ADMY values of 2.6 ± 1.42 kg and 2.76 ± 0.051 kg, respectively. However, it agrees well with the values reported by Şahin & Ulutaş (2014), Kaplan & Tekerli (2024), and Öz et al. (2022), who observed ADMY values of 5.21 ± 0.096 kg, 4.44 ± 0.095 kg, and 4.07 ± 0.02 kg, respectively. The higher ADMY averages observed in these studies, including the current study, are likely due to the positive effects of selective breeding practices aimed at improving milk yield in the buffalo herds studied. These practices are part of the “Anatolian Publicly Owned Buffalo Breeding Project” initiated by the General Directorate of Agricultural Research and Policy of the Ministry of Agriculture and Forestry (MAF-GDARP) in 2011. This targeted selection for improved milk yield has probably contributed to the improved milk production observed in recent studies.

However, it is important to note that ADMY averages for Anatolian buffaloes still lag behind those of foreign dairy buffalo breeds that have undergone extensive selection for higher milk yield over many years. For example, the ADMY values for Egyptian buffaloes are 6.52 kg and 6.91 kg (Marai et al., 2009; İbrahim, 2012). Murrah buffaloes in India give an average of 6.80 kg (Sing & Barwall, 2012) and 6.85 kg (Dev et al., 2015), while Kundi buffaloes in Pakistan have 6.19 kg (Ghaffar et al., 2007). These higher values can be attributed to years of intensive genetic selection and management practices specifically tailored to maximize milk production.

As far as calving interval (CI) is concerned, an average of 438.32 ± 1.110 days was recorded for Anatolian buffaloes in this study. A short CI is crucial for maintaining high lifetime milk production in dairy buffaloes, with an optimal CI usually being less than 410 days (Thomas, 2008; Borghese, 2005). With this in mind, the CI of around 438 days observed in Anatolian buffaloes is promising and suggests that CI could be further reduced in the coming years through further improvements in breeding practices. Compared to other buffalo breeds, CI values vary considerably. For example, Murrah buffaloes in India have an average CI of 538.6 days (Lundström et al., 1982), while Brazilian Murrah buffaloes have a CI of around 385 days (Tonhati et al., 2000). In Egypt, native buffalo have a CI of 518.9 days (Aziz et al., 2001), and in Iraq, native buffalo have an average CI of 408 days (Thomas, 2008). These variations are influenced by genetic differences among buffalo breeds and by environmental conditions and management practices across regions. The differences in CI observed in the present study compared to other studies emphasize the importance of both genetic selection and management practices in improving reproductive performance.

In this study, the average service period (SP) was calculated to be 111.390 ± 0.148 days, which is close to the SP time recommended for efficient and economical milk production in dairy buffalo herds, which is normally 100 days (Thomas, 2008). However, the SP average observed in this study differs significantly from the SP averages of other Anatolian buffalo herds in Turkey, which are 97.5 days (İlaslan et al., 1983), 106.1 days (Şekerden & Küçükkebaşı 2013) and 105.3 days (Kaplan & Tekerli, 2024). These differences can be attributed to various factors, including regional differences in farming methods, food systems and environmental conditions.

There are also notable differences in the SP values of Anatolian buffaloes compared with those of foreign buffalo breeds. For example, Hussain et al. (2006) reported an SP of 96.7 days for Nili Ravi buffaloes in Pakistan, while Thiruvankadan et al. (2010) observed an SP of 108.5 days for Murrah buffaloes in India. Other studies, such as those by Gupta et al. (2012) and Ayad et al. (2022), have reported SPs of 110–120 days across different buffalo breeds in India and Egypt. While breed-specific genetic makeup contributes to differences in SP values reported among buffalo breeds, the primary determinants are environmental conditions and management practices, as reproductive traits are known to have low heritability. Therefore, variation in SP is largely due to environmental and farm management factors specific to the region, such as feeding levels, climatic conditions, estrus detection success, milking and breeding management, housing conditions, and health practices.

The differences in SP observed in the studies could also be influenced by factors such as the frequency and timing of artificial insemination, health management, and the overall reproductive management system. It is well known that a shorter SP is desirable for higher reproductive performance and higher lifetime productivity of buffaloes. The current SP value of 111.39 days observed in Anatolian buffaloes suggests room for improvement, particularly through more intensive breeding strategies, better estrus synchronization, and improved health and nutrition management practices.

Since genetic selection for reproductive efficiency has not been applied as systematically in Anatolian buffaloes as in other buffalo breeds, it is reasonable to assume that SP in Anatolian buffaloes could be further reduced by the ongoing National Buffalo Breeding Program, which focuses on improving milk yield and overall herd productivity.

Least squares means

The least square means of the milk yield traits of Anatolian buffalos such as lactation length (LL), lactation milk yield (LMY), 305-day milk yield (305-DMY), average daily milk yield (ADMY) are presented in Table 2; the least square means of the reproductive traits calving interval (CI) and service period (SP) are presented in Table 3. The effect of calving year on these traits was generally significant ($p<.05$). The least squares mean for LL decreased from 263.20 ± 1.490 days in 2012 to 235.93 ± 1.223 days in 2019 ($p<.05$), despite variation across years. This result is in line with other studies investigating the influence of calving year on LL in Anatolian buffalo (Soysal et al., 2018; Koçak et al., 2019; Şahin et al., 2020; Berkin et al. 2020; Alkoyak & Öz 2021; Öz et al. 2022) and other buffalo breeds (Egypt: Aziz et al., 2001; Murrah: Thiruvankadan et al., 2010; Egypt: Ayad et al., 2022; Murrah: Sigdel et al., 2015; Surti: Pawar et al., 2019).

A significant increase in LMY, 305-DMY, and ADMY was observed between 2012 and 2019 ($p<.05$), with fluctuations. These increases are likely due to the positive effects of the selection program initiated in 2011 to improve milk yield, the increasing age of buffalo, and the efficient management of environmental factors. Similar results were found in other buffalo breeds where the effect of calving year on LMY, 305-DMY, and ADMY was also significant (LMY: Anadolu: Öz et al., 2022; Murrah: Patil et al., 2018; Nili-Ravi: Afzal et al., 2007; Egypt: Elmaghraby, 2010; 305-DMY: Rege, 1991; Atil et al., 2001; Amimo et al., 2007; ADMY: Anadolu: Kaplan & Tekerli, 2024; Murrah: Jamuna et al., 2015; Egypt: Kushwaha et al., 2013).

Higher milk yield in winter and lower yield in summer can be attributed to differences in heat stress and feeding conditions, highlighting the importance of appropriate stress management and feeding strategies during the summer. The observation that milk yield peaks in the fourth lactation suggests that animals reach physiological maturity at this stage, indicating that advanced lactation performance should be considered in herd management and breeding selection.

Although fluctuations in the average values of CI and SP were observed between 2012 and 2019, the differences between these years were not significant. However, considering that the SP period should be around 100 days for economic milk production in dairy buffalo (Thomas, 2008; Borghese, 2005), the SP period of 111.39 ± 0.148 days in Anatolian buffalo can be considered sufficient. This could be due to factors such as the relatively short suckling period of calves (approx. 6-8 months) and favorable climatic and grazing conditions, which shorten the postpartum anoestrus period. Many studies support this interpretation (El-Fouly et al., 1976; Jainudeen et al., 1983; Perera, 1987; Usmani et al., 1990; Arya & Madan, 2001; Baruselli et al., 2001; Borghese, 2005; Perera, 2008; Thomas, 2008).

In this study, the effect of calving season on LL, LMY, 305-DMY, and ADMY in Anatolian buffalo was found to be significant ($p<.05$). The means of these traits were highest in the winter, followed by spring, autumn, and summer, with the winter-summer difference being particularly significant ($p<.05$). These results align with findings from other studies (LL: Anatolian: Öz et al., 2022; LMY: Anatolian: Kaplan & Tekerli, 2024; Murrah: Jakhar et al., 2016; Nili-Ravi: Bashir 2015; Native Egyptian: Ayad et al., 2022; 305-DMY: Kour & Narang, 2021; ADMY: Anatolian: Kaplan & Tekerli, 2024; Egypt: Ramadan, 2018; Murrah hybrid: Bhattarai, 2020).

The seasonal differences in milk production in buffaloes are attributed to variations in genetic structure and environmental factors (Ayad et al., 2022). In our study, the significantly higher averages of LL, LMY, 305-DMY, and ADMY in winter compared to summer may be explained by the following reasons: (1) During winter, calves are kept with their mothers and are not exposed to the outside environment, which positively influences milk synthesis through suckling and stimulating maternal instincts. (2) Buffaloes calving in winter and spring benefit from longer daylight hours, which positively impact milk synthesis, as well as access to higher quality pastures and water resources compared to those calving in summer. (3) Increased temperatures negatively affect milk synthesis in buffaloes calving in summer (Jainudeen et al., 1983; Zicarelli, 1997; Naqvi, 2000; Baruselli et al., 2001; Perera, 2008; Thomas, 2008).

In this study, the least square means for CI and SP in Anatolian buffaloes calving in winter, spring, summer and fall were calculated as follows: CI (454, 438, 440, 464 days) and SP (113, 109, 113, 118 days), respectively. The differences between the winter-autumn and spring-summer seasons for CI and between all seasons for SP were significant ($p<.05$). According to these results, CI and SP were longest in the fall and shortest in the spring. These results for CI and SP are consistent with some previous studies, while they are not consistent with others (CI: Soysal et al., 2018; Koçak et al., 2019; Alkoyak & Öz, 2021; Kaplan & Tekerli, 2024; Ahmad et al. 1981; Gill & Ruki 1985; Maymone & Pilla 1961; Barkawi et al. 1998; Campo et al., 2002; Kushwaha et al., 2013; Jakhar et al., 2016;

Table 2. Least squares means ($\pm$ SE) of certain milk yield traits in Anatolian buffaloes according to calving year, calving season, lactation number, and location

Çizelge 2. Anadolu mandalarında buzağılama yılı, buzağılama mevsimi, laktasyon sayısı ve lokasyona göre bazı süt verimi özelliklerinin en küçük kareler ortalamaları ($\pm$ SE)

	LL (day)		LMY (kg)		305-DMY(305) (kg)		ADMY (kg)	
	N	$\bar{X} \pm S\bar{x}$	N	$\bar{X} \pm S\bar{x}$	N	$\bar{X} \pm S\bar{x}$	N	$\bar{X} \pm S\bar{x}$
Calving year								
2012	899	263.20 $\pm$ 1.490 ^a	900	736.66 $\pm$ 10.237 ^a	900	951.95 $\pm$ 12.367 ^a	900	3.07 $\pm$ 0.041 ^a
2013	398	244.16 $\pm$ 1.601 ^b	405	987.51 $\pm$ 12.083 ^b	405	1219.65 $\pm$ 14.882 ^b	405	4.00 $\pm$ 0.048 ^b
2014	423	242.19 $\pm$ 1.522 ^b	424	989.93 $\pm$ 11.409 ^b	424	1238.41 $\pm$ 13.976 ^b	424	4.07 $\pm$ 0.045 ^b
2015	662	249.70 $\pm$ 1.477 ^c	661	1060.68 $\pm$ 9.909 ^c	661	1331.70 $\pm$ 12.124 ^c	661	4.35 $\pm$ 0.039 ^c
2016	576	259.95 $\pm$ 1.546 ^a	565	1158.79 $\pm$ 10.257 ^d	565	1460.67 $\pm$ 12.397 ^d	565	4.75 $\pm$ 0.041 ^d
2017	1339	262.25 $\pm$ 1.306 ^a	1332	1127.70 $\pm$ 8.703 ^e	1332	1410.97 $\pm$ 10.494 ^e	1332	4.59 $\pm$ 0.034 ^e
2018	1289	233.09 $\pm$ 1.285 ^d	1289	1145.02 $\pm$ 8.496 ^{de}	1289	1442.02 $\pm$ 10.394 ^d	1289	4.74 $\pm$ 0.034 ^d
2019	831	235.93 $\pm$ 1.223 ^d	831	1144.29 $\pm$ 8.493 ^{de}	831	1448.83 $\pm$ 10.397 ^d	831	4.76 $\pm$ 0.034 ^d
Calving season								
Winter	1037	258.14 $\pm$ 1.308 ^a	1034	1063.41 $\pm$ 8.718 ^a	1034	1333.42 $\pm$ 10.568 ^a	1034	4.34 $\pm$ 0.035 ^a
Spring	3803	251.03 $\pm$ 1.033 ^b	3798	1034.93 $\pm$ 6.870 ^b	3798	1307.51 $\pm$ 8.380 ^b	3798	4.27 $\pm$ 0.027 ^b
Summer	1379	240.54 $\pm$ 1.167 ^c	1377	1035.13 $\pm$ 7.879 ^b	1377	1298.45 $\pm$ 9.659 ^b	1377	4.26 $\pm$ 0.031 ^{ab}
Autumn	198	245.50 $\pm$ 1.918 ^d	198	1041.81 $\pm$ 14.355 ^{ab}	198	1312.72 $\pm$ 17.604 ^{ab}	198	4.30 $\pm$ 0.057 ^{ab}
Lactation order								
1	2669	236.19 $\pm$ 0.760 ^a	2669	941.41 $\pm$ 5.430 ^a	2669	1188.09 $\pm$ 6.618 ^a	2669	3.90 $\pm$ 0.021 ^a
2	1642	243.72 $\pm$ 0.845 ^b	1641	1026.84 $\pm$ 5.871 ^b	1641	1296.22 $\pm$ 7.193 ^b	1641	4.24 $\pm$ 0.023 ^b
3	1098	247.80 $\pm$ 0.966 ^c	1092	1038.04 $\pm$ 6.782 ^{bc}	1092	1309.57 $\pm$ 8.299 ^{bc}	1092	4.28 $\pm$ 0.027 ^{bc}
4	609	248.39 $\pm$ 1.277 ^c	608	1066.39 $\pm$ 8.478 ^d	608	1338.80 $\pm$ 10.397 ^c	608	4.37 $\pm$ 0.034 ^c
5	274	254.89 $\pm$ 1.805 ^d	272	1063.66 $\pm$ 11.932 ^{cd}	272	1336.40 $\pm$ 14.569 ^{bc}	272	4.36 $\pm$ 0.047 ^{bc}
6	98	255.25 $\pm$ 2.890 ^{cd}	98	1060.28 $\pm$ 19.014 ^{bcd}	98	1331.99 $\pm$ 23.291 ^{bc}	98	4.34 $\pm$ 0.076 ^{bc}
7	27	255.45 $\pm$ 5.406 ^{bcd}	27	1062.12 $\pm$ 35.643 ^{bcd}	27	1391.14 $\pm$ 43.700 ^{bc}	27	4.54 $\pm$ 0.143 ^{bc}
Location								
1	855	241.34 $\pm$ 1.361 ^a	851	1055.30 $\pm$ 9.386 ^{ab}	851	1333.75 $\pm$ 11.511 ^{ab}	851	4.37 $\pm$ 0.037 ^{ab}
2	1354	242.92 $\pm$ 1.245 ^{ac}	1354	1017.68 $\pm$ 8.607 ^{cd}	1354	1279.26 $\pm$ 10.554 ^{cd}	1354	4.19 $\pm$ 0.034 ^{cd}
3	915	255.56 $\pm$ 1.329 ^b	915	1040.15 $\pm$ 9.204 ^{ac}	915	1301.82 $\pm$ 11.207 ^{ac}	915	4.24 $\pm$ 0.036 ^c
4	960	252.32 $\pm$ 1.322 ^{bd}	962	1043.11 $\pm$ 9.052 ^a	962	1310.57 $\pm$ 11.062 ^a	962	4.28 $\pm$ 0.036 ^{ac}
5	966	245.99 $\pm$ 1.349 ^{ce}	966	1006.87 $\pm$ 9.149 ^d	966	1262.36 $\pm$ 11.214 ^d	966	4.13 $\pm$ 0.036 ^d
6	788	248.31 $\pm$ 1.347 ^e	786	1093.02 $\pm$ 9.054 ^e	786	1373.85 $\pm$ 11.073 ^e	786	4.49 $\pm$ 0.036 ^e
7	579	249.39 $\pm$ 1.483 ^{de}	573	1074.18 $\pm$ 10.191 ^{be}	573	1352.70 $\pm$ 12.488 ^{be}	573	4.41 $\pm$ 0.040 ^{be}
Overall	6417	241.28 $\pm$ 0.301	6407	1011.39 $\pm$ 3.582	6407	1278.37 $\pm$ 3.77	6407	4.19 $\pm$ 0.012
SP		0.032 $\pm$ 0.0261		-0.292 $\pm$ 0.1772		-0.173 $\pm$ 0.2175		-0.00063 $\pm$ 0.000071
CI		-0.006 $\pm$ 0.0155		0.252 $\pm$ 0.1061*		0.281 $\pm$ 0.1302*		0.00092 $\pm$ 0.00042*
LL		---		4.255 $\pm$ 0.0713*		-0.173 $\pm$ 0.2175		0.00159 $\pm$ 0.00028
R ²		0.19		0.60		0.46		0.46

LL: Lactation length, LMY: Total lactation milk yield, LMY(305): 305 day lactation milk yield, ADMY: Average Daily milk yield *p<.05; a, b, c, d Means in a column with different superscripts differ significantly (p<.05)

Table 3. Least squares means ($\pm$ SE) of certain reproductive traits in Anatolian buffaloes according to calving year, calving season, lactation number, and location

Çizelge 3. Anadolu mandalarında buzağılama yılı, buzağılama mevsimi, laktasyon sayısı ve lokasyona göre bazı üreme özelliklerinin en küçük kareler ortalamaları ($\pm$ SE)

	CL(day)		SP(day)	
	N	$\bar{X} \pm S\bar{x}$	N	$\bar{X} \pm S\bar{x}$
Calving year				
2012	51	447.90 $\pm$ 5.617 ^{ae}	15	113.28 $\pm$ 0.816 ^{abc}
2013	442	387.93 $\pm$ 5.944 ^b	269	113.94 $\pm$ 0.864 ^{abc}
2014	475	490.39 $\pm$ 5.655 ^c	113	113.82 $\pm$ 0.821 ^{abc}
2015	357	471.11 $\pm$ 5.486 ^d	124	113.95 $\pm$ 0.799 ^b
2016	327	443.59 $\pm$ 5.784 ^{ae}	132	112.94 $\pm$ 0.843 ^{abc}
2017	634	459.83 $\pm$ 4.892 ^{ad}	178	112.99 $\pm$ 0.717 ^{abc}
2018	734	438.51 $\pm$ 4.738 ^e	390	111.58 $\pm$ 0.695 ^c
2019	688	455.89 $\pm$ 4.501 ^{ad}	193	113.01 $\pm$ 0.659 ^{abc}
Calving season				
Winter	444	454.11 $\pm$ 4.867 ^a	84	113.82 $\pm$ 0.714 ^a
Spring	2214	438.35 $\pm$ 3.781 ^b	773	109.15 $\pm$ 0.560 ^b
Summer	899	440.82 $\pm$ 4.306 ^b	497	113.620 $\pm$ 0.629 ^c
Autumn	151	464.31 $\pm$ 7.132 ^a	60	118.19 $\pm$ 1.033 ^d
Lactation order				
1	171	411.49 $\pm$ 2.652 ^a	48	113.96 $\pm$ 0.410 ^a
2	1574	497.02 $\pm$ 2.987 ^b	654	114.25 $\pm$ 0.454 ^a
3	1020	474.90 $\pm$ 3.490 ^c	350	113.38 $\pm$ 0.523 ^{ab}
4	541	475.01 $\pm$ 4.697 ^c	212	115.49 $\pm$ 0.689 ^a
5	274	456.50 $\pm$ 6.745 ^{cd}	99	110.49 $\pm$ 0.978 ^b
6	100	433.28 $\pm$ 10.820 ^{ad}	37	114.30 $\pm$ 1.562 ^{ab}
7	28	397.55 $\pm$ 20.261 ^{ad}	14	110.44 $\pm$ 2.921 ^{ab}
Location				
1	537	453.26 $\pm$ 5.098 ^{ab}	194	112.40 $\pm$ 0.734 ^a
2	594	457.19 $\pm$ 4.659 ^a	218	113.85 $\pm$ 0.671 ^a
3	591	446.33 $\pm$ 5.014 ^{ab}	247	113.32 $\pm$ 0.722 ^a
4	598	454.81 $\pm$ 4.973 ^a	184	112.33 $\pm$ 0.716 ^a
5	576	439.26 $\pm$ 5.053 ^b	254	113.05 $\pm$ 0.728 ^a
6	501	446.84 $\pm$ 5.051 ^{ab}	197	113.95 $\pm$ 0.728 ^a
7	311	448.05 $\pm$ 5.565 ^{ab}	120	112.90 $\pm$ 0.802 ^a
Overall	3708	438.32 $\pm$ 1.110	1414	111.390 $\pm$ 0.148
LL	0.11 $\pm$ 0.0423*		0.00781 $\pm$ 0.00061	
R ²	0.15		0.13	

CI: Calving interval, SP: Service period *p<.05; a, b, c, d Means in a column with different superscripts differ significantly (p<.05)

Shafik et al., 2017; Ramadan, 2018; Ayad et al., 2022; Kumar et al., 2022 and SP: Zicarelli, 1997; Naqvi, 2000; Arya & Madan, 2001; Agrawal, 2003; Nanda & Nakao, 2003; Borghese, 2005; Lemcke 2008; Perera, 2008;).

As various factors influence the relationship between, CI and SP, the interpretation of these relationships is complex. However, when the results of the studies that have investigated these relationships are analyzed together, it can be assumed that the calving season influences CI in three main ways: photoperiod, climatic conditions and nutrition. At least one of these three factors is effective in different countries, regions and buffalo breeds. These pathways primarily influence SP physiology, which in turn affects the length of the CI period. Since buffalo are susceptible to heat stress, postpartum anoestrus and gestation length are shorter when they calve in cooler weather and under good nutritional conditions than when they calve under hot, undernourished conditions (Barkawi et al. 1998; Campo et al., 2002; Thomas, 2008). Although buffaloes are polyestrous animals, their reproductive activity is more positively influenced by shorter photoperiods. Consequently, SP and CI last shorter in buffaloes that calve during shorter days (e.g. in winter), while they last longer in buffaloes that calve during longer days (e.g. between March and June) (Ganguli, 1981; Thomas, 2008). The shorter SP and CI observed in our

study in buffaloes calving in spring could be due to the cooler weather and better grazing and feeding conditions during this period, which probably stimulate estrus activity.

In this study, the effect of lactation order on LL, LMY, 305-DMY, and ADMY in Anatolian buffaloes was generally found to be significant ($p<.05$). As lactation order increased, LL also increased consistently ($p<.05$). On average, LL increased by 19 days from the 1st lactation (236 days) to the 7th lactation (255 days). This increase is likely due to the extension of the milk synthesis period in the udder as lactation order increases. Similarly, the effect of lactation order on LL was determined to be significant in Anatolian buffaloes (Berkin et al., 2020) as well as in other buffalo breeds (Nili-Ravi: Hussain et al., 2006; Murrah: Kour et al., 2021; Jaffarabadi: Marques et al., 1991; Venezuelan native: Nava-Trujillo et al., 2018).

In this study, the least squares means for LMY, 305-DMY, and ADMY in Anatolian buffaloes were lowest in the 1st lactation, peaked at the 4th lactation, and then declined by the 7th lactation. Similar findings have been reported in several studies (LMY: Anatolia: İlaslan et al. 1983; Alkoyak & Öz 2021, Native Italian: Salerno 1974, Native Indian: Kay 1977, Nili-Ravi: Afzal et al 2007, Murrah: Jakhar et al. 2016; 305-DMY: Murrah: Jamuna et al. 2015, Sigdel et al. 2015, Jakhar et al. 2016, Egypt: Shafik et al. 2017, Abo-Gamil et al. 2017; ADMY: Egyptian Buffalo: İbrahim 2012; Ramadan 2018, Murrah: Sigdel et al. 2015). Collectively, these studies indicate that the peak milk production observed during the 4th and 5th lactations is a direct consequence of the buffalo's reaching full anatomical and physiological maturity. This period corresponds to maximal mammary gland functionality, optimal metabolic support for lactation, and the highest efficiency in nutrient utilization. After these parities, age-related declines in epithelial cell renewal, increases in connective tissue, and higher disease susceptibility ultimately lead to reductions in milk yield.

In this study, the average CI in Anatolian buffaloes showed a significant decrease ($p<.05$), especially from the 2nd lactation (497 days) to the 6th lactation (433 days), and the average SP decreased from the 2nd lactation (114 days) to the 5th lactation (433 days). Thus, both traits show a consistent downward trend with increasing lactation order, with a decline after the 4th lactation. These results, especially regarding the relationship between lactation order and SP, are consistent with studies conducted on Italian (Borghese et al., 1993) and Pakistani (Naqvi, 2000) buffaloes. Naqvi (2000) reported a similar trend in Pakistani buffaloes in which SP, which was 287 days in 1st lactation, decreased to 237 days by the 8th lactation. These researchers emphasized that the most important factor influencing SP length in buffaloes, as in other mammals, is the postpartum anoestrus period.

Moreover, in this study, the village factor was found to be a significant ($p<.05$) source of variation for the least squares means of LL, LMY, 305-DMY, and ADMY in Anatolian buffaloes. Since the same selection method for milk yield was applied to the buffalo herds in all seven villages, it can be concluded that the differences observed between villages are due to the genetic responses of buffalo populations to selection and environmental factors that could not be effectively controlled in each village (e.g., pasture and water availability, temperature variation, diseases, etc.). Similar results have been reported in other studies on Anatolian buffalo (Şahin & Ulutaş 2014; Soysal et al., 2018; Berkin et al., 2020; Alkoyak & Öz, 2021; Kaplan & Tekerli, 2024).

In this study, although not statistically significant, the relationship between LL and SP was estimated to be positive (0.032 ± 0.0261), while the relationship between LL and CI in Anatolian buffaloes was estimated to be negative (-0.006 ± 0.0155). The negative relationship between LL and CI, albeit at a very low level, can be attributed to irregular fluctuations in LL from 2012 to 2019 and the influence of calving, especially in fall and winter. Although buffaloes are polyestrus, short photoperiods tend to have a more positive influence on their reproductive activities (Thomas, 2008). Therefore, in this study, the short days in buffalo calving in fall and winter may have stimulated estrus shortly after calving and thus shortened the calving interval.

As for the relationships between lactation traits and milk yield, the regression coefficients of LL, CI, and SP on LMY were calculated as 4.255 ± 0.0713 ($p<.05$), 0.252 ± 0.1061 ($p<.05$), and -0.292 ± 0.1772 , respectively. Positive relationships between LMY, LL, and CI are generally expected, as LL and CI should also increase with increasing LMY. Indeed, in this study, a gradual increase in LMY, LL, and CI was observed between the 1st and 7th weeks. Although an increase in SP with an increase in LMY is generally expected, the insignificant inverse relationship observed in this study may be explained by the small number of records for the 6th and 7th weeks.

In this study, the regression coefficient of CI and SP on 305-DMY was calculated as 0.281 ± 0.1302 ($p<.05$) and -0.173 ± 0.2175 , respectively. The positive and significant relationship between 305-DMY and CI can be attributed to the positive effect of higher milk yield on CI expansion, as observed for LMY. Although higher milk yield is generally expected to increase SP, the negative but non-significant relationship between 305-DMY and SP may be explained by factors similar to those responsible for the negative relationship between LMY and SP.

In addition, the regression coefficients of average daily milk yield (ADMY) on calving interval (CI), lactation length (LL), and service period (SP) were estimated to be 0.00092 ± 0.00042 ($p<.05$), 0.00159 ± 0.00028 ($p<.05$), and -0.000063 ± 0.000071 , respectively. The positive and significant relationships between ADMY and both LL and CI

suggest that increases in ADMY are associated with longer lactation and calving intervals. The negative but non-significant relationship between ADMY and SP may be attributed to the same factors that influenced the inverse associations between SP and both LMY and 305-DMY.

In addition, a positive ($p<.05$) correlation (0.11 ± 0.0423) was estimated between CI and LL. This positive relationship can be interpreted as a result of selection for higher milk yield in Anatolian buffaloes since 2011, which has also affected the increase in LL and CI. Similar to other agricultural mammals, there is generally a positive relationship between LMY and LL in buffaloes, and in this study, a very high (4.255 ± 0.0713 ; $p<.01$) positive relationship was found between these two traits in Anatolian buffaloes. Environmental factors that affect lactation continuity also significantly affect the duration of LL and CI (Sabra et al., 1997). Therefore, the increase in LMY and LL due to greater awareness among farmers participating in the genetic improvement project may also have contributed to the increase in CI.

In this study, the regression of LL on SP in Anatolian buffaloes was estimated at 0.00781 ± 0.00061 . The fact that this relationship is positive, albeit at a very low level, indicates that SP is also prolonged by LL's expansion. However, this situation is undesirable for well-managed dairy buffalo herds. This is because the lengthening of the SP shortens the LL, thereby decreasing the amount of milk obtained. This study argues that this positive but undesirable relationship between SP and LL is mainly due to the suckling of calves by the buffalo mother after birth, which extends milk synthesis and thus LL or SP.

The findings of this study indicate that buffalo calves calving in winter months exhibit higher milk yield and more favorable lactation characteristics. This suggests that shifting the calving season to winter months in regional Anatolian buffalo operations could be considered a management strategy to increase milk production. Furthermore, given the yield increases associated with lactation order, optimizing the age at first calving may improve overall herd productivity. However, some limitations of the study should also be considered. Although strict data control criteria were applied, the exclusion of some records due to missing or inconsistent information was unavoidable and may have led to a partial loss of variation. Furthermore, because the classification of regional climatic conditions was based on general meteorological data, microclimatic differences between villages may not have been fully captured. Finally, it was not possible to fully control for individual variations arising from operational management practices, animal health status, and nutritional differences within the scope of this study. In the future, the inclusion of more detailed environmental measurements and advanced modeling approaches will help reduce these limitations.

CONCLUSION

The results of this study indicate that the selection programme initiated in 2011 in Bitlis Province to increase milk and fertility yields in Anatolian cattle generally had a positive effect on the relevant yield traits. However, these observed improvements are at the phenotypic level, and genetic progress must also be evaluated. Despite the gains achieved, the mean and least squares mean values for the traits examined remain lower than those of foreign dairy breeds. Furthermore, macro-environmental factors such as year and season of birth, dam age, village, and calving interval were found to have significant effects on milk and fertility traits.

These findings highlight the importance of considering environmental factors in the development of selection models, as well as seasonal calving management (especially in winter and spring), the regular and standardised application of lactation monitoring systems, and the integration of regional environmental conditions into breeding programmes. Additionally, raising awareness of management practices through breeder education is a key element in improving productivity. In this context, the results provide guidance for both breeders and policymakers on developing sustainable breeding programmes that account for climate and environmental conditions. In future studies, it is recommended to conduct analyses to predict genetic parameters and trends, demonstrate selection response, and evaluate genomic selection approaches alongside environmental stress indices.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the articles declare that they have no conflict of interest.

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