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CONTENTS

Research Article

Forecasting of Bull Carcasses Prices for the Future with Box-Jenkins Models: The Case of Tokat Province
Mehmet ÇELİK, Mehmet Saltuk ARIKAN **1-10**

Effects of Different Temperature and Humidity Regimes on Reproduction and Development of Lucilia sericata (Meigen,1826) Female Populations
Nevra POLAT, Salih MOLLAHALİLOĞLU, Murat KOÇ **11-25**

Quail Meat Under Threat: Hidden Microplastics Pose Risks to Public Health and Environment
Veysel DOĞAN, Çağatay SALUM, Sedat GÖKMEN, Mehmet Sait ATAR **26-38**

Case Report

Clinical Manifestation of A Cat With Acute Acetaminophen Toxicity
Esma KISMET, Halid RECEP, Fatma AKDAĞ, Erdem GÜLERSOY **39-46**

Surgical Treatment of Cranioschisis and Meningocele in a Newborn Calf
Kerem YENER, Mehmet Salih KARADAĞ, Kübra DİKMEN İLGİNOĞLU, Mehmet Sıdık HURMA **47-53**

Veterinary Journal of Kastamonu University (2024): 3(2) Research Article
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Forecasting of Bull Carcasses Prices for the Future with Box-Jenkins Models: The Case of Tokat Province

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Abstract
Aim to study: The purpose of this study is to make future forecast of bull carcass meat prices in Tokat province using econometric methods. It is aimed to analyze bull carcass prices in Tokat province with time series analysis and estimate these prices for 2025.
Material and methods: The data set of the study consists of monthly bull carcass prices slaughtered between June 2018 and May 2024. The estimates were made with the Box-Jenkins method, one of the econometric analysis methods.
Results: As a result of the time series analysis, the most suitable model for bull carcass prices was determined as Auto Regressive Integrated Moving Average (ARIMA) (0,1,0) (1,1,0). The estimation results from the determined ARIMA models predict that the average bull carcass price in Turkey will reach 550.37 TL/kg (16,98 US$) in May 2025.
Conclusion: In conclusion, time series analysis can be utilized to forecast future bull carcass prices, though these estimates should be regularly updated. This approach can support the future planning efforts of both producers and consumers.
Keywords: Box-Jenkins, bull, carcass, forecast, price, Tokat.

Tosun Karkas Fiyatlarının Box-Jenkins Modeller ile Geleceğe Yönelik Kestirimleri: Tokat İli Örneği

Öz
Çalışmanın amacı: Bu çalışmanın amacı; Tokat ilinde tosun karkas et fiyatlarının ekonometrik yöntemlerle geleceğe yönelik tahminlerinin yapılmasıdır. Tokat ilinde tosun karkas fiyatlarının zaman serisi analizi ile çözümlenerek, bu fiyatların 2025 yılı için tahmin edilmesi amaçlanmıştır.
Materyal ve yöntemler: Çalışmanın veri seti; 2018 Haziran ve 2024 Mayıs ayları arasında kesilen aylık tosun karkas fiyatlarından oluşmuştur. Tahminler ekonometrik analiz yöntemlerinden Box-Jenkins yöntemi ile yapılmıştır.
Bulgular: Yapılan zaman serisi analizi sonucunda tosun karkas fiyatları için en uygun model Otoregresif Bütünleşik Hareketli Ortalama (ARIMA) (0,1,0) (1,1,0) olarak tespit edilmiştir. Model doğrultusunda tosun karkas fiyatlarının kestirim sonuçlarına göre Türkiye'de Mayıs 2025 döneminde tosun karkas fiyatının ortalama 550,37 TL/kg (16,98 US$) ulaşacağı tahmin edilmiştir.
Sonuç: Sonuç olarak, zaman serisi analizi gelecekteki tosun karkas fiyatlarını tahmin etmek için kullanılabileceği, ancak bu tahminlerin düzenli olarak güncellenmesi gerekmektedir. Bu yaklaşım, hem üreticilerin hem de tüketicilerin ileriye yönelik yapacağı planlamalara katkı sağlayabileceği sonucuna varılmıştır.
Anahtar kelimeler: Box-Jenkins, tosun, fiyat, karkas, tahmin, Tokat.

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Introduction

With the increase in the world population and the development of socio-economic conditions of consumers, the need for animal protein, in other words, the amount of meat consumed per person, has increased. Increasing the production of animal products to meet the demands of the increasing world population has become the primary goal of states (Sakarya & Aydın, 2011).

According to the data of the Turkish Statistical Institute (TurkStat), the number of slaughtered cattle increased by 6% in 2023 compared to the previous year and reached 5 million 811 thousand, and cattle meat production increased by 6% and reached 1 million 670 thousand tons (TurkStat, 2024).

In meat production, the choice of cattle breed affects unit costs and sales income, while knowing the fattening performance of cattle breeds improves the ability of producers to make economically sound decisions (Arıkan & Gökhan, 2018). The most suitable animal material for fattening activities in Turkey is generally Brown Swiss, Holstein, and Simmental breeds and their crossbreeds with local animal breeds (Çiçek, 2002). However, today, in order to close the red meat gap, different beef cattle breeds such as Angus, Hereford, Charolais and Limousin are also imported (Arıkan & Gökhan, 2018).

Time series is a method that uses econometric methods to estimate future prices using past and present data sets (Matta et al., 2021).

When producers fatten their butchered animals, they have no prediction about how much the carcass meat they obtain will be traded at the market when the fattening process is over. Time series analyses accept the prices that have occurred in a market before as data and make estimates about the price of that product in that market in the future (Montgomery et al., 2015).

The future course of carcass prices is of great importance for producers and consumers. By knowing the prices, breeders can predict the estimated price range of the carcasses they obtain at the end of the fattening process in the market they are in. On the other hand, industrialists who want to buy carcass meat can have a forecast on which price range the carcass meat will be in which time period.

This study was conducted to make future carcass price estimates by analyzing the time series of bull carcass prices in Tokat province using the Box-Jenkins method.

Material and Methods

Data Set

The data set of the study consisted of the prices (TL/kg) of 2,359 bull carcasses slaughtered in the slaughterhouses located in the center and districts of Tokat province between June 2018 and May 2024 (TOB, 2024). In the study, the average bull carcass price of each month was calculated to be used in the time series analysis and a 72-month data set was obtained. In the analysis of the data, trend analysis was performed in order to obtain and estimate the series value under the influence of only long-term movements by purifying the time series from the effects of irregular fluctuations. Within the scope of this study, the ARIMA model from the Box-Jenkins models was used in the time series analysis. Descriptive statistics of the data were performed and the parameters of the model with high goodness of fit in price estimation were estimated and price estimation was made. SPSS, version 25.0 (IBM Corporation, 2018) program was used for the analysis of the time series.

Time Series Analysis

In the study, ARIMA (0,1,0) (1,1,0) model was used for future predictions of beef carcass prices. When the autocorrelation function graph of the

series was examined, it was determined that the series was not stationary. The first difference was taken in order to make the series stationary. In order to determine the appropriate ARIMA (p,d,q) model for the predictions of beef carcass prices, the autocorrelation and partial autocorrelation functions of the series were examined, p and q levels were determined and the significance of the parameters was checked.

In the models (Wickramarachchi et al., 2017);

p: Autoregressive model degree,

q: Moving average model degree,

d: Non-seasonal difference degree.

The ARIMA (p, d, q) model is expressed as shown in Equation [1].

$$Z_t = \phi_1 Z_{t-1} + \phi_2 Z_{t-2} + \ldots\ldots\ldots\ldots + \phi_P Z_{t-P} + a_t - \theta_1 a_{t-1} - a_t - \theta_2 a_{t-2} - \ldots\ldots\ldots\ldots - a_q - \theta_q a_{t-q}$$

[1] Here:

ϕ_P: Parameter values for the autoregressive operator, a_t: Error term coefficients, θ_q: Parameter values for the moving average operator, Z_t: Time series with d degree difference taken from the original series, That is, $W_t = Y_t - Y_{t-1}$, t = 1,2,…,t [2]

and the first difference series is defined as shown in equation [2] Here:

W_t = First difference series,

Y_t = Set of random variables of the original time series.

If the first difference series is not stationary; stationarity control is performed by taking the difference of the first difference series again. This is also modeled as given in the equation [3].

$Z_t = W_t W_{t-1}$ t = 1,2,…,t [3]

When the differencing degree d=0 (indicating that the original series is stationary), the ARIMA model reduces to an AR, MA, or ARMA model. This characteristic means that ARIMA models encompass all Box-Jenkins models.

Box-Jenkins ARIMA models include four basic steps in the establishment phase. In the first step, it is generally determined which class the model is in.

The autocorrelation and partial autocorrelation function graphs are used in selecting the general model. Considering both functions, the properties of theoretical functions are utilized for ARIMA models (Box et al., 2015).

In the second step, a temporary model that fits the data is established. Autocorrelation and partial autocorrelation functions are utilized in establishing this model. In determining the model, a model such as AR, MA, ARMA, ARIMA or SARIMA is decided (Tekindal et al., 2016).

In the third step, the parameters of the temporary model are estimated using statistical methods and their significance is determined by considering the standard errors of the coefficients. In the last step, the suitability of the decided model for estimation is determined. To evaluate this, the autocorrelation function is analyzed using the graph of autocorrelation coefficients for the residuals of the provisional model. If a specific pattern appears, it suggests that the residuals are not randomly distributed, indicating that the provisional model is unsuitable. In this case, the process returns to the second step, and a new provisional model is tested until an appropriate one is identified. Once a model passes this suitability check, it can be used for predictions or estimations (Yenice & Tekindal, 2015; Tekin & Tekindal, 2019; Kaymaz, 2018).

In evaluating the estimation consistency of the model; Forecast Error, Mean Forecast Error, Mean Absolute Deviation, Mean Absolute Percentage Error and Mean Square Error parameters were used (Arıkan et al., 2018).

Results

Within the scope of the research, a price series was created for bull carcasses with a total of 72 months of data between 2018/10-2024/5. Average beef carcass prices (TL/kg) in Tokat province between 2018-2024 are presented in Table 1.

When the table is examined, the bull carcass price increased between 2018/10-2024/5. The highest increase in the bull carcass price occurred in December 2023. Compared to the previous month, the bull carcass price increased by 23.36% in February 2024. Future estimates of bull carcass prices in Tokat province were made with time series analysis using price data between 2018/10-2024/5. The time series graph indicating the bull carcass prices is presented in Figure 1.

Table 1. Average bull carcass prices in Tokat province between 2018/10-2024/5 (TL/kg)

Years/Months	2018	2019	2020	2021	2022	2023	2024
January	-	28,79	31,50	35,00	65,00	127,71	280,50
February	-	28,55	32,17	35,22	67,56	160,33	296,11
March	-	29,00	33,57	39,08	73,38	186,47	335,31
April	-	29,56	34,33	39,57	85,00	209,69	361,67
May	-	30,85	35,60	40,00	95,00	224,12	361,67
June	-	30,92	37,29	43,63	94,20	227,78	-
July	-	31,00	37,52	44,20	96,50	233,89	-
August	-	31,23	37,77	45,50	96,78	234,50	-
September	-	31,47	37,90	45,00	96,91	240,75	-
October	29,80	32,80	35,67	44,67	100,83	245,29	-
November	29,00	30,27	35,40	51,36	102,92	218,88	-
December	28,33	30,83	35,42	57,33	107,63	270,00	-

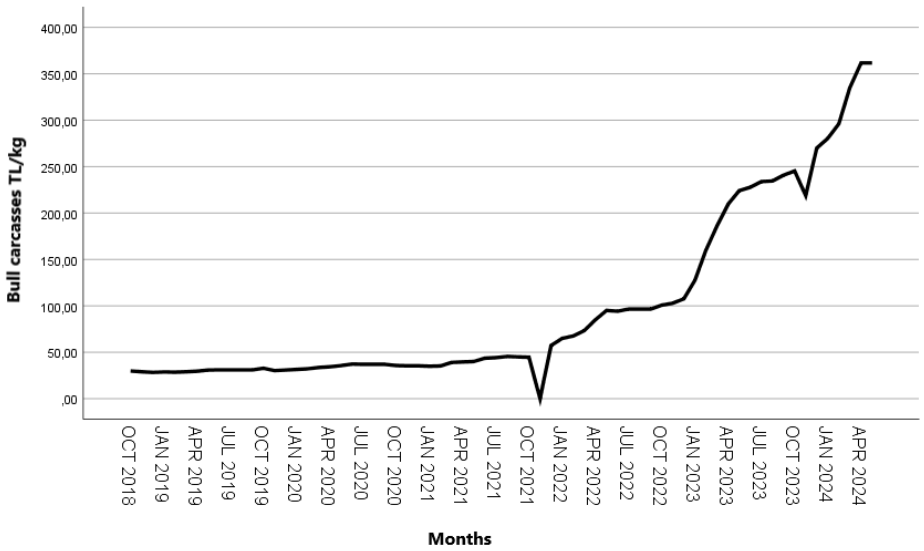

Figure 1. Time series graph of bull carcass prices (TL/month)

When Figure 1 is examined, it can be said that the time series increases with a trend along with fluctuations. The presence of a certain trend in the series shows that the time series of bull carcass prices is not stationary. In order to determine the stationarity of the time series of bull carcass prices, the autocorrelation function (ACF) and partial autocorrelation function (PACF) graphs of the series are presented in Figure 2.

When Figure 2 is examined, it can be stated that the series is not stationary because multiple lags in the time series are outside the confidence limits. In order to partially stationarize the series, the logarithm of the series was first taken and thus the differences between the values were reduced. However, since the series was still not stationary, the difference was taken until the series became stationary. Once the difference was taken, it was seen that Figure 3 the trend of the time series became stationary.

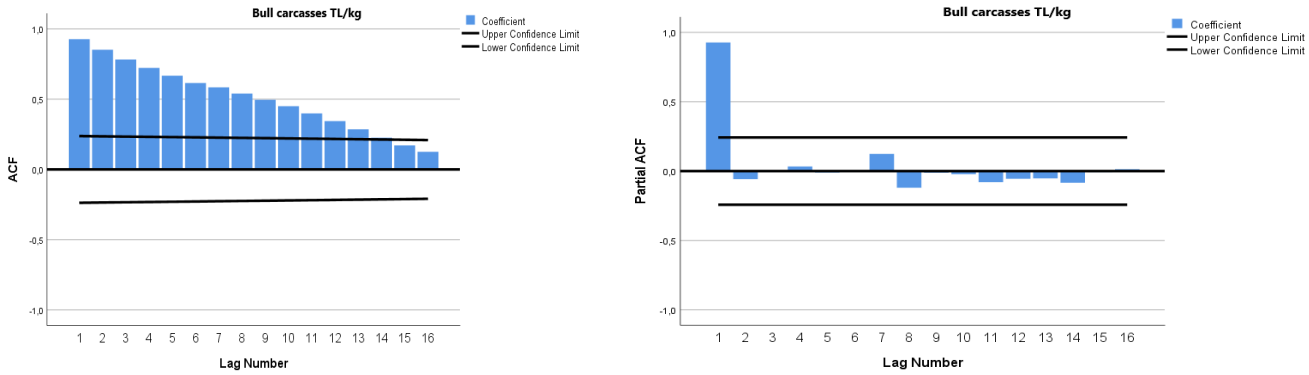

Figure 2. ACF and PACF graph of the series of bull carcass prices

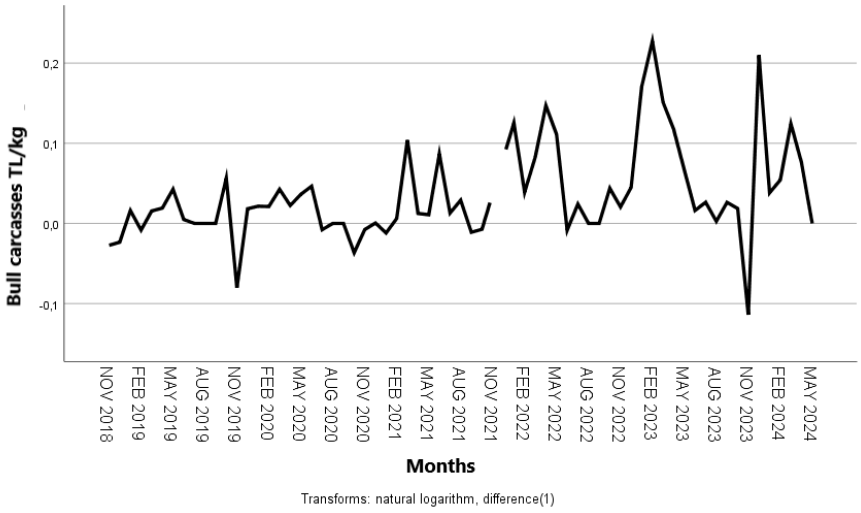

Figure 3. Graph of the series with bull carcass price difference

ACF and PACF graphs of bullock carcass prices (TL/kg) as a result of the difference process are given in Figure 4.

Examining the ACF and PACF graphs in Figure 4 reveals that the values exceed the confidence limit for the first three lags but remain below it from the fourth lag onward, suggesting that the time series is stationary. Several models were tested to identify the most suitable model for the time series, and it was determined that the ARIMA(0,1,0)(1,1,0) model is the best fit for forecasting beef carcass prices.

The predictions created by the selected model for the bull carcass prices in the following months are presented in Table 2.

When Table 2 is examined, in the analysis made on beef carcass price values, the Mean Absolute Percentage Error (MAPE) value was obtained as 8.624 and the R^2 value as 0.984. The ARIMA (0,1,0) (1,1,0) model gives the optimum result.

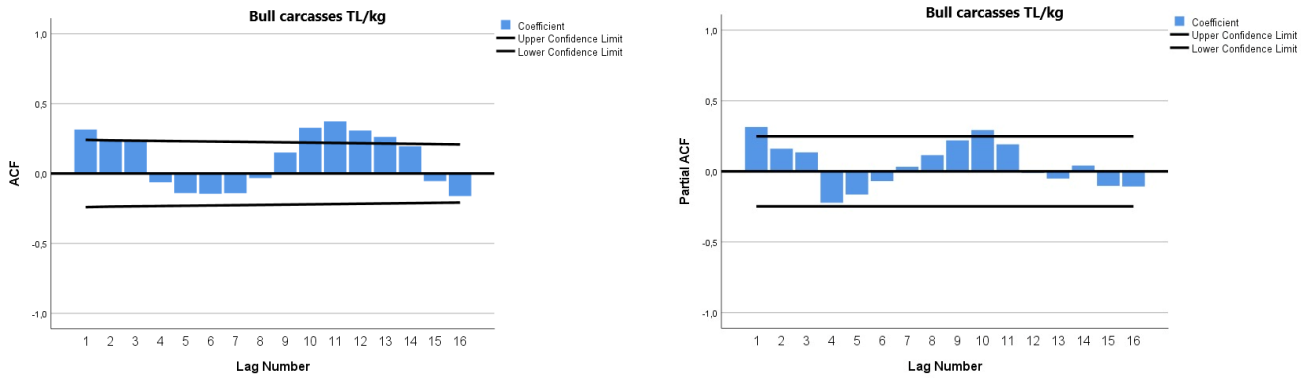

Figure 4. ACF and PACF graph of the series with bull carcass price difference

Table 2. Estimation of bull carcass price values for the future

Period	Forecast	UCL	LCL	MAPE	R^2	Model
June 2024	367.19	392.50	341.89			
July 2024	375.58	411.36	339.80			
August 2024	380.51	424.34	336.69			
September 2024	387.49	438.09	336.88			
October 2024	396.60	453.18	340.03			
November 2024	393.09	455.07	331.12			
December 2024	419.30	486.25	352.36	8.624	0.984	ARIMA (0,1,0) (1,1,0)
January 2025	440.63	512.19	369.06			
February 2025	471.80	547.71	395.90			
March 2025	507.38	587.39	427.37			
April 2025	536.45	620.36	452.53			
May 2025	550.37	638.01	462.72			

When Figure 5 is examined, it is estimated that carcass prices will decrease in November 2024, increase again from December, and the average beef carcass price will be 550.37 TL/kg in May 2025.

The compliance criteria for the price prediction models used in the time series analysis are presented in Table 3.

The goodness-of-fit criteria for the obtained models are evaluated in comparison with one another. R^2, often referred to as the coefficient of determination, is a widely used criterion that measures the goodness of fit for linear models. This coefficient ranges from 0 to 1, with lower values indicating a poor fit to the data. Stationary R^2 is another criterion that compares the stationary component of the model with the baseline model, making it useful when a trend or seasonal pattern is present. The Root Mean Squared Error (RMSE) is the root mean square error and indicates how much the model's estimates deviate from the dependent series; low values indicate better model estimates.

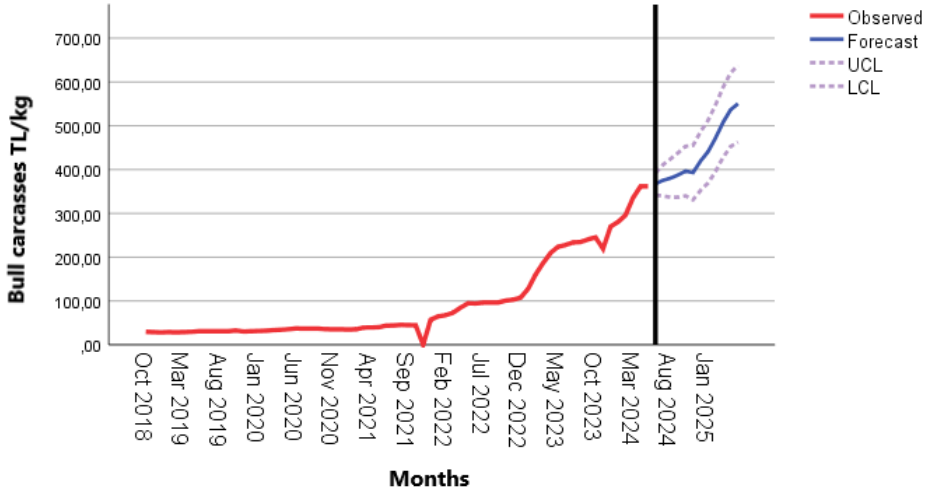

Figure 5. Bull carcass price value prediction graph for the future

Table 3. Fit criteria for bull carcasses price prediction models

Fit Criteria	ARIMA (0,1,0) (1,1,0)
Stationary R-squared	0.459
R-squared	0.984
RMSE	12.651
MAPE	8.624
MaxAPE	91.148
MAE	7.438
MaxAE	52.255
Normalized BIC	5.221
Statistics	17.977
DF	17
Sig.	<0.001

MAPE shows the average absolute percentage error and is used in comparing different series since it is independent of the units of the series. Models with this value below 10 are considered to be “very good”, models between 10 and 20 are considered to be “good”, models between 20 and 50 are considered to be “acceptable” and models above 50 are considered to be “incorrect and faulty”.

Mean Absolute Error (MAE) represents the average absolute error and is expressed in the units of the series. Maximum Absolute Percentage Error (MaxAPE) measures the highest percentage error between the estimated values and actual values, making it unit-independent and useful for analyzing worst-case scenarios in forecasts. Maximum Absolute Error (MaxAE) indicates the largest absolute error and is presented in the same units as the dependent series. The Normalized Bayesian Information Criterion (BIC) assesses the overall model fit, aiding in model comparison for the same series, with lower values indicating a more effective model.In our study, the Box-Jenkins models created for beef carcass prices are statistically significant ($p<0.001$). The MAPE value shows that the series of prices contain quite usable estimates.

Discussion

In Turkey, 2,384,047 tons of red meat will be produced in 2023, with 70.07% of the production coming from cattle, 23.87% from sheep, 5.41% from goats and 0.65% from buffalo, and the demand for beef is increasing every year (TurkStat, 2024). Red meat production in Turkey, at 1,651,650 tons, is insufficient to meet the consumption of 2,146,000 tons, causing a supply deficit of approximately 490,255 tons (TEPGE, 2023). The supply deficit in red meat in Turkey is met through imports. Although attempts are made to balance prices by increasing supply in the domestic market through carcass meat import decisions, these decisions put pressure on local producers and are not a sustainable solution in the long term in stabilizing prices (Akın et al., 2020). However, studies indicate that increasing the supply of red meat through imports is insufficient to regulate red meat prices in domestic markets (Aktaş, 2020). On the other hand, it has been reported that state interventions in the red meat market have been insufficient to stabilize prices (Arıkan et al., 2019).

In this study, price data of bull carcasses slaughtered in slaughterhouses and combined in Tokat province in Turkey between 2018 and 2024 were used. Since the time series created using the price data did not show stationarity, they were made stationary after taking their first differences. As a result of the time series analysis, it was determined that the most suitable ARIMA (0,1,0) (1,1,0) model for bull carcass prices.

In line with the determined ARIMA models, carcass prices were estimated until May 2025. According to the estimation results, it was estimated that bull carcass prices in Turkey would reach an average of 550.37 TL/kg in the May 2025 period.

It has been stated that red meat prices in Turkey have experienced mild price fluctuations in the long term (Ayyıldız, 2017). In order to protect consumers from these fluctuations, products are sold below market prices through the Meat and Milk Board. However, it has been stated that the primary purpose of this practice is to meet the demand for red meat by low-income

consumers rather than ensuring price stability and that it does not have an increasing effect on the market for red meat (Çiçek et al., 2020).

Conclusion

In conclusion, time series analysis is used as an effective tool for predicting future trends in carcass meat prices. In the case study conducted on bullock meat prices in Tokat province, results obtained through ARIMA models revealed that prices follow a particular trend and exhibit seasonal fluctuations. This analysis method provides forecasts on how prices may change in the future by considering trends, seasonality, and other patterns within the time series of past data, thereby increasing price predictability in the short term. As a result, both red meat producers and consumers can be better prepared for market uncertainties and engage in strategic planning. Particularly in countries like Turkey, where inflationary pressures are high, the findings of this study contribute to the development of policy recommendations aimed at stabilizing the red meat market and provide crucial insights for determining strategies to minimize price fluctuations.

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Ethical Statement

This study was approved by the Fırat University Non-Interventional Research Ethics Committee (27.07.2023/10-35).

Author Contributions

Investigation: M.Ç. and M.S.A; Material and Methodology: M.Ç. and M.S.A; Supervision: M.Ç. and M.S.A; Visualization: M.Ç and M.S.A; Writing-Original Draft: M.Ç. and M.S.A; Writing-review & Editing: M.Ç. and M.S.A.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Effects of Different Temperature and Humidity Regimes on Reproduction and Development of *Lucilia sericata* (Meigen,1826) Female Populations

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Abstract

Aim to study: The development of animals such as *Lucilia sericata* (Diptera: Calliphoridae), which are unable to regulate their body temperature metabolically and instead maintain a constant temperature by absorbing heat from their surroundings (i.e. poikilotherms), has been extensively described using the temperature collection model. This study aimed to investigate oviposition tendency and oviposition development times and ideal temperature and humidity values for mass rearing of the green bottle fly *L. sericata*, which was studied in the laboratory at three different constant temperatures (25 °C, 30 °C, and 35 °C) and three different constant humidities (35% relative humidity (R.H.), 50% R.H., and 65% R.H.).

Material and methods: The humidity was fixed at each experimental temperature to determine the maximum egg- laying and development time at different temperatures, and the number of days and degrees required to complete each stage was determined. The temperature was fixed in the different “”””””” “humidity experiments, and the insectarium was examined under controlled conditions in a 12:12 (L:D) photoperiod cycle.

Results: A significant difference was obtained between the number of *L. sericata* eggs laying at different temperature values ($\chi 2=21.143$, $p<0.05$). A significant difference was found between the number of *L. sericata* eggs laying at different humidity values ($\chi 2=17.913$, $p<0.05$).

Conclusion: A temperature of 35°C and 50% humidity were identified as the optimal breeding conditions for the oviposition tendency of *L. sericata* flies in a specific insect house under laboratory conditions.

Keywords: Egg-laying, Growth and development, Humidity, *Lucilia sericata*, Temperature.

Farklı Sıcaklık ve Nem Rejimlerinin *Lucilia sericata* (Meigen,1826) Dişi Popülasyonlarının Üreme ve Gelişimi Üzerine Etkileri

Öz

Çalışmanın amacı: *Lucilia sericata* (Diptera: Calliphoridae) gibi vücut sıcaklığını metabolik olarak düzenleyemeyen ve bunun yerine çevrelerinden ısı emerek sabit bir sıcaklığı koruyan hayvanların (yani poikilotermler) gelişimi, sıcaklık toplama modeli kullanılarak kapsamlı bir şekilde tanımlanmıştır. Bu çalışmanın amacı, laboratuvarda üç farklı sabit sıcaklıkta (25 °C, 30 °C ve 35 °C) ve üç farklı sabit nemde (%35 R.H., %50 R.H. ve %65 R.H.) çalışılan yeşil şişe sineği *L. sericata*'nın yumurtlama eğilimini ve yumurtlama gelişim sürelerini ve kitle yetiştiriciliği için ideal sıcaklık ve nem değerlerini araştırmaktır.

Materyal ve yöntemler: Farklı sıcaklıklarda maksimum yumurtlama ve gelişme süresini belirlemek için her bir deney sıcaklığında nem sabitlenmiş ve her bir aşamanın tamamlanması için gereken gün sayısı ve dereceler belirlenmiştir. Farklı nem deneylerinde sıcaklık sabit tutulmuş ve insektaryum 12:12 (L:D) fotoperiyot döngüsünde kontrollü koşullar altında incelenmiştir.

Bulgular: Farklı sıcaklık değerlerinde yumurtlayan *L. sericata* yumurta sayıları arasında anlamlı bir fark bulunmuştur ($\chi 2=21.143$, $p<0.05$). Farklı nem değerlerinde yumurtlayan *L. sericata* yumurta sayıları arasında anlamlı bir fark bulunmuştur ($\chi 2=17.913$, $p<0.05$).

Sonuç: 35°C sıcaklık ve %50 nemin, laboratuvar koşullarında belirli bir böcek barınağında *L. sericata* sineklerinin yumurtlama eğilimi için en uygun üreme koşullarını oluşturduğu tespit edilmiştir.

Anahtar kelimeler: Yumurtlama, Büyüme ve gelişme, Nem, *Lucilia sericata*, Sıcaklık.

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Introduction

One of the main themes in biology is the understanding of how ecological conditions affect the evolution of an organism's body size and development time (Oudman et al., 1991; Plaistow et al., 2004; Gomez-Mestre & Buchholz, 2006). From a developmental point of view, it is difficult for organisms, especially during metamorphosis, to survive to adulthood with limited resources. According to their biological life cycles, their ability to maintain the developmental rate balance in their periodic phases has been a subject of curiosity and research (Wilbur & Collins, 1973). The balance between body size relative to age is an important criterion in the physiological process of the development of holo-metabolic insects. Physiological differences or some parameters involved in development are influential on the evolution of life histories. Minimum survival, weight and critical weight are examples of threshold parameters for development (Mirth & Riddiford, 2007). The distribution and characteristics of Calliphoridae species vary among populations worldwide, with variable body sizes and developmental times related to ecological conditions and responses. *Lucilia sericata* (Diptera: Calliphoridae), which is dependent on nutrient-rich substrates such as carrion and biological waste, can be stretched by rearing under laboratory conditions to achieve superiority *L. sericata* is sensitive to environmental factor changes in laboratory rearing conditions such as temperature, humidity, photoperiod, and food type (Clark et al., 2006; Tarone & Foran, 2006).

Lucilia sericata, also known as green bottle fly and green meat fly, is also known as myiasis flies because *L. sericata* is not a parasite of humans in its adult stage. In their larval stages, they settle in different anatomical regions of humans (Faulde et al., 2001) and animals such as sheeps, cats, dogs and carrion crows (Crystal and Ramirez, 1975; East and Eisemann, 1993; Dik et al., 2012) and cause myiasis. It causes traumatic Myiasis in livestock in many European, African and Asian countries (Dik et al., 2012). It is reported to be a serious problem in Hungary (Farkas et al.,1997). In our country, it is reported that miyasis cases caused by *L. sericata* are mostly encountered in Konya and Kırıkkal in Central Anatolia (Dik et al., 2012). *Lucilia sericata* is typically one of the first flies to reach the corpse after death. It is a biological marker in forensic entomology and entomotoxicology Forensic entomologists use blow fly development to estimate a postmortem interval (Byrd & Castner, 2010; Tomberlin et al., 2011a).

At the same time, in the treatment of chronic wounds that are slow to heal, facultative *L. sericata* larvae are used medicinally in the first and second stages of development to perform micro debridement as an alternative to surgical intervention (Gazi et al., 2021). In addition to the use of live larvae of this fly in wound treatment, secretions obtained from larvae are also employed in wound treatment. With regard to this, the antibacterial activities of secretions obtained from larvae have been investigated in both in vitro and in vivo environments (Zerek et al. 2019). Myiasis is associated with Post Mortem Interval (PMI) predictions and the development of success in a non-healing wound. Researchers have noted that members of Calliphoridae have different minimum developmental ranges between species and within species (Kamal, 1958; Greenberg, 1991; Grassberger & Reiter, 2001). This may result from genetic variation or environmental factors in laboratory rearing conditions (Clark et al., 2006; Tarone & Foran, 2006). Variation in minimum development times implies variation in age estimates and requires species-specific optimization. In this regard, studies on *L. sericata* larval stage development show that the minimum development time varies depending on the

variability of the rearing temperature or relative humidity factor (Tarone & Foran, 2011).

Temperature is an important factor not only for adults but also for all developmental stages in the life cycle. However, for egg laying, hatching, survival and the ability to transition to the next larval stage, humidity, light, nutrient amount and variety are also essential environmental factors along with temperature (Lefebvre & Pasqueraul, 2004). Temperature, humidity, and nutrient content are the main factors, but population density in laboratory-rearing conditions also regulates development. For example, when larval density is high and food is limited, larvae rapidly pass through three distinct stages due to food competition and pupate before the normal process. However, due to the larvae's excessive feeding activities and rapid metabolism within the aggregate, it has been determined that the ambient temperature rises further, and the development time is shortened to the extent that it exceeds the maximum life temperature (Campobasso et al. 2001; Kotze et al., 2016; Pruna et al., 2019).

According to Campobasso et al. (2001) and Ash and Greenberg, (1975), some Calliphoridae species enter larval or pupal diapause in response to seasonal changes, and their development stops. Pupal development is faster at high temperatures than at low temperatures (West, 1951). In a study on the effect of different temperatures on the development of *L. sericata*, it was reported that pupal entry was not observed at 15 °C, pupal stage constituted about 46% of the total developmental stage at 20 ºC, 42% at 25 ºC and 44% at 30 ºC (Grassberg & Reiter, 2001). Adult emergence occurs early at low temperatures, and small adult individuals with underdeveloped wings and smaller-than-normal appearances are formed. Therefore, the optimum temperature is also essential during the pupation period, and typical development occurs at a controlled optimum temperature (Grassberg & Reiter, 2001; Danks, 2013).

Humidity acts as a stimulus for the oviposition of Calliphorid adult females (Ashworth & Wall, 1994). It has been reported that Calliphorid females do not lay eggs unless their ankles are in contact with water and moist areas. The eggs dry up without moisture (Demirsoy, 2001). A suitable humidity environment is essential for egg laying and egg hatching. An environment with high humidity and high temperature decreases the activity of the flies and prepares for a faster death. Therefore, an environment with the possibility of evaporation increases resistance to high temperatures (Merrit, 1980). Calliphorids are typically very sensitive to ambient humidity levels at all life cycle stages, but when humidity is too high, the larvae leave the food medium, and larval development stops (Payne, 1965). Optimum humidity about temperature is essential in determining a precise developmental time. Between certain humidity limits, the growth rate of the larvae varies linearly with humidity. For larvae applied to the wound to treat non-healing wounds, the moist wound environment is ideal for the larvae to develop (Merrit, 1980).

The objective of this study is to identify the optimal temperature and humidity values for the development of *L. sericata*, a green bottle fly of forensic and medical importance, under laboratory conditions. Furthermore, the study aims to develop a standardisation protocol that can be applied consistently across different laboratories. The results of this study will ensure the continuity of the culture of *L. sericata* under laboratory conditions.

Material and Methods

Culture Supply with *Lucilia sericata* Colonies

A colony of *L. sericata* was established from pupae provided by the Biotherapy Laboratory of

Istanbul Cerrahpaşa University in October 2021. The obtained *L. sericata* pupae were transformed into adult individuals within 7-10 days in specially prepared 45x45x45 cm wire cages surrounded with tulle. Adult individuals were fed *ad libitum* with 20% sugar water medium and *ad libitum* food in 250 ml Erlenmeyer flasks. To feed the flies, one end of the cage was covered with cotton wool to reach the bottom of the bottle. The number of flies in a cage was maintained at approximately 500 to 1000 flies per cage under a pre-optimised day/night photoperiod consisting of 28ºC ambient temperature, 50% RH humidity and a 12:12 (L:D) hour light cycle. On the fifth day after the emergence of adult flies, 30 g of chicken liver per day in petri dishes was added to the cages for ovary development. One week later, 50 g of chicken liver was fed twice weekly for egg laying on the 12th or 13th day. Following the laying eggs of the female flies, the collected eggs were sterilized and incubated on liver agar medium prepared in petri dishes. After two days, the larvae in these petri dishes were transferred to the nutrient-agar medium in larger, approximately 15 cm diameter petri dishes to eliminate food competition by providing the massively growing larvae with a more extensive habitat and the required amount of nutrients. One part of the collected eggs was reserved for the experimental plan and targeted studies, and the other was used for colony maintenance. The larvae require a dry, calm, and shaded environment during the transition from the prepupal to the pupal stage of their development. This environment was created using plastic or glass jars (15x10x25 cm) and sawdust. During metamorphosis, a hole was made in the lid of the container or glass jar, and this part was covered with gauze to allow the air necessary for the larvae's life to enter and prevent the larvae from getting out of the container. Following a 3-5 day incubation period, petri dishes containing sterilized larvae at maximum feeding stage 3 were placed in the plastic container or jar on the sawdust. The larvae were expected to move away from the nutrient medium into the sawdust, and the petri dish was removed when they left the medium. The larvae were incubated in the fly-rearing room until they appeared as pupae, and the pupae were removed from the sawdust and placed in a new fly cage. The formation of the working groups was based on the selection of groups comprising 100-200 individuals, which had remained intact throughout the larval-rearing phase. This approach was deemed necessary to ensure the accurate evaluation of the targeted data. For each value of all studies, two repetitions were performed. In each repetition, the numerical data of the findings obtained from the determined number of groups (different numbers of study groups were formed depending on the target study) were collected, then divided by the number of groups, and the average obtained was recorded as the finding of that repetition study. The findings obtained from 2 repetitions were summed and divided by 2, averages were taken, and standard deviations were calculated.

Experimental Design

Determination of differences in egg laying of *Lucilia sericata* at various temperature regimes

In order to ascertain the egg-laying differences of *L. sericata* at varying temperature values, a standardisation was established in accordance with the reproductive potential, with experiments conducted at three distinct temperatures: 25 °C, 30 °C and 35 °C. Due to the adaptation of the flies to the laboratory, the temperature to be studied was fixed at °C, and the humidity value was fixed at the favorable humidity value, considering the possibility of humidity change depending on the temperature. A total of 6 study groups (100 females and 100 males in each study group) were formed, 2 for each temperature value and optimum humidity corresponding to this temperature. In order to stimulate egg and sperm formation in adults, they were fed with sugar

water in the first two days after emerging from pupae, and on the third day, the liver was renewed at 12 hours and fed with 50 g of liver for 24 hours. The days of oviposition, maximum oviposition days, and total number of eggs per cage were determined. The regular liver release process was carefully followed to avoid deviating from the experimental result data. In order to separate and count the eggs adhered to each other after spawning, the packets were placed in sterile falcon tubes filled with 9-10 ml distilled water and shaken gently by hand for 1-2 minutes until the separation process was realized. After separating the eggs, they were filtered through filter paper and counted directly if they were less dense or photographed if they were more dense and counted on the photograph using "AutoCAD Select Similar Programme" on the computer, and their numbers were determined. To calculate the average per cage, 6 study cages were divided into groups of 2 and labeled as L1, L2, and L3 for *L. sericata*. At the end of 12 hours in the first feeding, the total number of eggs per cage of *L. sericata* in the L1 group was recorded. Three or four feedings were made for the continuation of the cycle. At the end of a study repetition, the sums of the data obtained from the L1, L2, and L3 groups during the repetition were divided by the number of data taken (number of days of data obtained), and the number obtained was recorded as the total number of eggs per cage in one repetition. At the end of 2 study replicates, the means of the replicate findings were calculated with their standard deviations and recorded as the total number of eggs for the temperature value studied.

Determination of egg-laying differences of *Lucilia sericata* at various humidity regimes

Three different humidity values of 35%, 50%, and 65% were selected to determine the egg-laying differences of *L. sericata* at different humidity values, and the temperature of the laboratory environment was fixed at a pre-optimized temperature of 35°C. A total of 6 study group cages (100 females and 100 males in each cage), 2 for each humidity value, were established. The first spawning days, minimum and maximum spawning days, average of all eggs laid in one repetition per cage, and average life span were determined separately for *L. sericata* at the determined humidity values. The mathematical calculation of the target studies planned to obtain the study data was carried out by applying the same methods and mathematical calculations we followed in different temperature parameters.

Statistical Analyses

IBM SPSS Statistics 24.0 package program was used for statistical analysis of the data obtained in the study. Friedman's test method, which is a non-parametric method and an alternative to the repeated measures ANOVA method, was used to compare the egg-laying numbers at temperature and humidity values. This method had a significant difference in temperature or humidity values analyzed with the Wilcoxon signed-rank test, a non-parametric method, and an alternative to the dependent group's t-test method. A comparison was made at the $P<0.05$ significance level for statistical analyses.

Results

The present study was evaluated to develop a standardization of *in vitro* temperature and humidity parameters for *L. sericata* (Diptera: Calliphoridae) species used in larval wound treatment under laboratory cultivation conditions, and the following results were obtained.

It was concluded that abiotic factors influenced the larval culture, growth, and development of *L. sericata* under laboratory conditions. At different temperatures of 25 ºC, 30 ºC, and 35ºC and constant humidity of 50%, adult life spans of *L. sericata* range between 18-30 days, first egg-

laying days between the 8th and 14th days and maximum egg-laying days between the 12th and 20th days. Days and the highest number of eggs laid between the 12th and 20th days showed that temperature is an essential criterion in spawning ability and fertility, and at 35%, 50%, and 65% different humidity values, 35 ^{o}C constant temperature, the adult life span of *L. sericata* ranging between 24-30 days, first egg laying days ranging between the 8th and 15th days, first egg laying days ranging between the 14th and 22nd days.

It was concluded that the humidity factor is a second important criterion in regular reproduction and reproductive potential; in order to provide medical larvae of specific standards in laboratory conditions, the ambient humidity should not be above 60-65% if the ambient temperature is fixed at 35 ^{o}C in order to prevent pathogen-induced contamination. Suppose it is desired to increase the reproductive potential of adults according to medical needs and to obtain medical larvae in a shorter time. In that case, choosing the temperature at 35^{o}C is appropriate by fixing the ambient humidity at 50%.

Effects of 25 ^{o}C, 30 ^{o}C, and 35 ^{o}C Temperature Regimes on Reproduction and Development in *Lucilia sericata* Female Populations

Understanding how ecological conditions drive the evolution of body size and development time is a significant theme in biology (Gomez-Mestre & Buchholz, 2006). Based on the literature we reviewed before the study, we have analyzed the reproduction of Diptera: Calliphorid flies in the insectarium. The temperature conditions for reproduction and development are generally 25^{o} C and above, and the potential for reproduction and development decreases at lower temperatures (Davies & Hobson, 1935; Mumcuoğlu et al., 2001; Blystone & Hansen, 2014). In our targeted study, depending on the physical characteristics of the insectarium in laboratory conditions, the temperatures suitable for insectarium conditions where reproductive behavior is observed in species-specific breeding and regular reproduction is observed were studied. In light of the literature data (Mumcuoğlu et al., 2001; Tachibana & Numata, 2001; Mohd et al., 2005; Polat et al., 2010; Barnes & Gennard, 2013), the ambient constant humidity value was taken as 45% during the study in order to determine the efficiency in oviposition rates at the determined critical base temperatures.

Whether there is a significant difference between *Lucilia sericata* egg-laying numbers at different temperature values was compared with the Friedman test, a non-parametric method. As a result of this analysis, the temperature values between which there was a significant difference were compared pairwise using the Wilcoxon signed-rank test, a non-parametric method.

A significant difference was found between the number of *L. sericata* eggs laying at different temperature values ($\chi 2$=21.143, P<0.05). Accordingly, when it was analyzed between which temperature values there was a difference, there was a difference between the number of eggs laying at all temperature values. The mean number of egg-laying at 35 °C was higher than the mean number of egg-laying at 30 °C and 25 °C, and the mean number of egg-laying at 30 °C was higher than the mean number of egg-laying at 25 °C (Figure 1). When the reproductive activity at all three temperatures studied under controlled conditions for *L. sericata* species was evaluated, eggs were obtained with regular reproduction in general. In the laboratory environment, stress factors (loudness, high population of flies in the cages, the density of objects around the cages, aromatic odor, for example) were encountered, negatively affecting the egglaying of flies during the egg-laying period. As a result of the trials at all three temperatures determined for the target

study, it was observed that egg-laying efficiency was low in the presence of these stress factors. In order to ensure laboratory line continuity and to establish standardization, all stress factors in the environment were eliminated. At 25 °C, spawning occurred 5-6 hours after the Petri dishes with chicken or beef liver were placed in the cages, while at 30 °C, the first spawning occurred within 2-3 hours, and at 35 °C, the first spawning occurred 1 hour after the Petri dishes with food were placed in the cages. On the other hand, it was observed that the adults were more mobile in the cages as the temperature values increased (Figure 1).

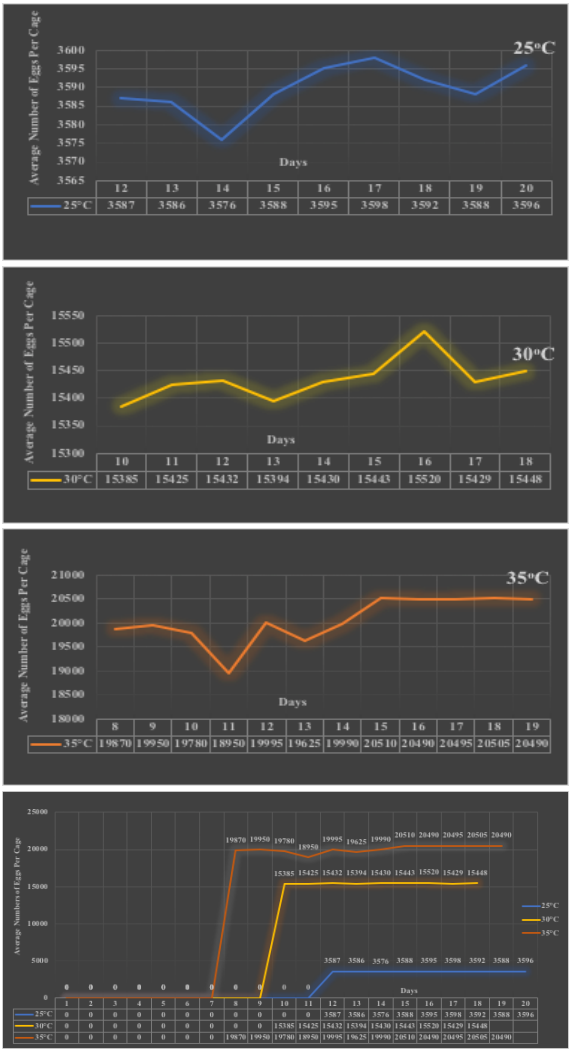

Figure 1. Time-dependent mean egg-laying trend of *L. sericata* at different temperatures

It was observed that *L. sericata* females usually clustered in the surface area of the lungs in the cage for reproduction, chose to lay eggs between the pieces of lungs, in the moist parts of the upper parts of the liver, and did not lay eggs in the veined and oily parts. It was observed that egg laying slowed down with drying due to the decay of the liver, so the liver was moistened by wetting from time to time. When the egg-laying potential of fresh liver and rotten lider was examined, it was determined that egg-laying was higher in moist areas where the amount of liquid was dense in rotten or near-rotten liver.

Effects of 35%, 50%, and 65% Humidity Regimes on Reproduction and Development in *Lucilia sericata* Female Populations

The study was carried out at three different humidity values: 35% (±2), 50% (±2) and 65% (±2). Based on the literature information that we reviewed before the study, the insectarium was used for the reproduction and development of Diptera: Calliphorid flies in the insectarium for reproduction, development, and colonization Davies and Hobson (1935); Mumcuoğlu et al. (2001); Tachibana and Numata (2001); Mohd et al. (2005); Barnes and Gennard, (2013); Shefa et al. (2013); Thyssen et al. (2013); Blystone and Hansen, (2014) and Evans (1935), which showed that development is faster and eggs hatch faster in the range of *L. sericata* > 50% relative humidity and that higher relative humidity conditions are beneficial. The reproductive potential was evaluated at humidity levels below 45% relative humidity to determine the lower limit of humidity at which oviposition occurs in *L. sericata* species, and humidity levels below 35% relative humidity, which was determined to be the lower limit, was not studied. The maximum limit humidity value was determined according to our pre-study experiments to ensure the adaptation of the flies to the climate chamber (insectarium). According to the literature, most Calliphorid species are

reported to perform optimally between 80-90% relative humidity (Engelmann, 1970) for oviposition and oviposition rates. However, due to the high level of condensation in the environment when the humidity value is above 70% in the laboratory environment, the contamination situation related to the formation of fungi in the adult cages and the mortality of the cultures due to this, the maximum limit humidity value was determined as 65% and higher humidity values were not studied. In order to determine the effects of 35%, 50%, and 65% humidity regimes on the reproduction and development of *L. sericata* female populations, the temperature value at which we fixed the laboratory environment at different temperature values of 25 ºC, 30 ºC, and 35 ºC, determined according to our findings obtained from our study.

Whether there is a significant difference between *L. sericata* egg-laying numbers at different humidity values was compared with the Friedman test, a non-parametric method. As a result of this analysis, the difference between the humidity values was compared pairwise using the Wilcoxon signed-rank test, a non-parametric method.

A significant difference was found between the number of *L. sericata* eggs laying at different humidity values ($\chi 2=17.913$, $p<0.05$). Accordingly, when the humidity values were analyzed, there was a difference between the number of eggs laying at 35% humidity and those laying at 50% and 65% humidity values. The average number of eggs laying at 50% and 65% humidity values was higher than 35%. However, there is no significant difference between the number of egg-laying larvae at 50% and 65% humidity values ($p>0.05$).

Discussion

The literature reported that the developmental stages of fly species should be graded in hours, as in Greenberg's study in 1985. Because insects are cold-blooded, they cannot keep their body temperature constant. Their activity, growth, and development rates depend on the environment's temperature, which must be at or above the minimum life temperature for insects to grow. Insects require a certain amount of heat to move from one stage of development to the next. There is usually a threshold temperature for each species, which is reported to be 15ºC for necrophagous species, and there is little data available for larval development below this temperature (Maria & Queiroz, 1996). Another study reported that carrion flies' reproduction and development is generally slower at lower temperatures (Byrd & Butler, 1996; Anderson & Cervenka, 2001). *L. sericata* eggs are often laid in areas 2-4 cm above the skin where the temperature is 28-34 °C (Cragg, 1956; Wall et al., 1992). At these temperatures in sheep fleece, the eggs hatch within 10-12 hours and the larvae feed in 2-3 days in three developmental stages (Wall et al., 1992). The critical abiotic factor limiting hatching success and the initiation of myiasis in the host is humidity (Davies & Hobson, 1935). In accordance with the results of previous studies and the data outputs obtained from our own study, the number of eggs decreased with increasing ambient humidity (Table 1 and Figure 2).

Short days and low temperatures were synergistically effective in inducing diapause in larval generations of *L. sericata* (Tachibana & Numata, 2004). In a larval culture study, Anderson (2001) found that larvae entered diapause at the prepupal stage under laboratory conditions at 15.8 ºC. Niedeeregger et al., (2010) showed that larvae developed from *L. sericata* at 13 ºC did not pupate similarly. In their study, Grassberg & Reiter (2001) noted that *L. sericata*

adults did not emerge at temperatures below 15 °C. Our study's findings, in which we examined the reproductive potential of *L. sericata* species at 25 °C, 30 °C, and 35 °C temperature regimes, parallel these literature data. It was determined that the egg-laying efficiency and egg density of *L. sericata* species increased in direct proportion to the temperature, and they showed more reproductive potential at high-temperature values than at low-temperature values (Table 2).

Table 1. The egg-laying tendency of *Lucilia sericata* at different humidity values

Humidity range (%)	Time of first ovulation	Maximum ovulation days range	Average number of eggs in each cage	Average life
35%	Days 13-15	Days 19 to 22	4935 pieces	Days 24 to 26
50%	Days 10-11	Days 18 to 22	17495 pieces	Days 28 to 30
65%	Days 8-9	Days 14 to 19	11324 pieces	Days 29 to 31

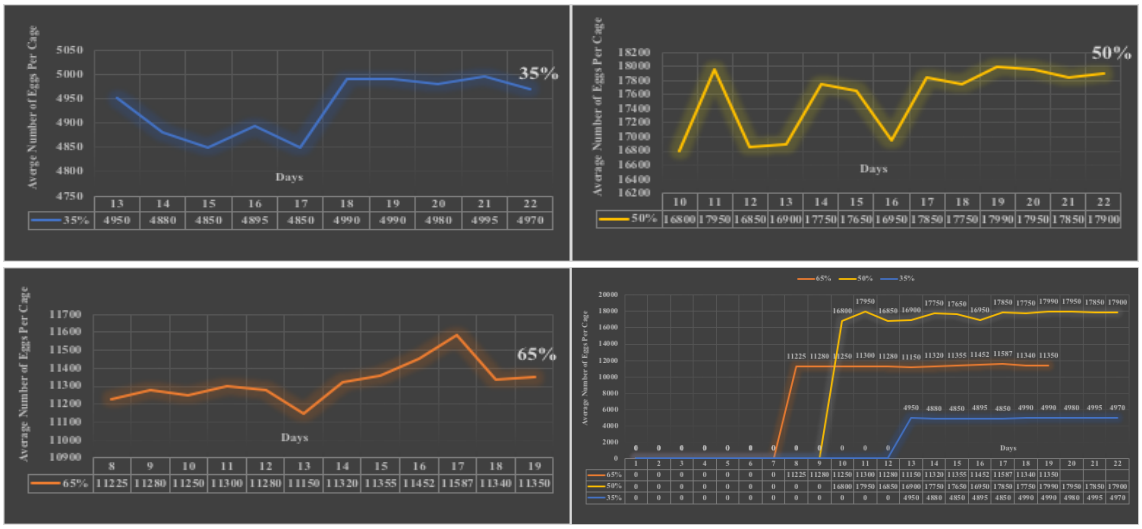

Figure 2. Time-dependent mean egg-laying trend of *L. sericata* at different humidity values

Table 2. The egg-laying tendency of *L. sericata* at different temperatures

Temperture	Time of first ovulation	Maximum ovulation days range	Average number of eggs in each cage	The end of the culture
25 °C	Days 12-14	Days 16 to 20	3490 pieces	Days 22 to 24
30 °C	Days 10-12	Days 15 to 19	15319 pieces	Days 26 to 28
35 °C	Days 8-9	Days 12 to 19	20561 pieces	Days 28 to 30

Cragg (1956) noted that *L. sericata* prefers warmed surfaces and does not lay eggs below 30 °C and on shaded surfaces. Niedeeregger et al., (2010) found that rearing at fluctuating temperatures between 5 ºC and 29 ºC did not improve *L. sericata* because periods of 5 ºC in the climate chamber prevented larvae from hatching. In our study, *L. sericata* was found to lay eggs at 25 ºC, which is not consistent with Niedeeregger et al., (2010) and Cragg (1956). However, when the number of eggs laying at 25 ºC, 30 ºC, and 35ºC was evaluated according to the results of the Friedman test ($p<0.05$), the temperature of 30 ºC and above at 35 ºC (Table 3) indicates a high level of reproductive potential in parallel with the increase in temperature and this respect, it is by Cragg (1956).

According to Rognes, (1991), the life span of Calliphoridae adults was reported to be between 14-21 days. Our research on *L. sericata* species found that adults lived 22-24 days at 25 ºC with 45% constant humidity, 26-28 days at 30 ºC, and 28-30 days at 35 ºC. Our findings for *L. sericata* species are parallel with those of Rognes (1991). Unlike the literature, *L. sericata* adult life span was one week longer at 30 ºC and 35 ºC, and the long life span of the adults at these temperature values is due to the consistent constancy of temperature and humidity values, which are among the environmental factors preferred by the species, as well as the regular food substrate. Insects are exposed to multiple abiotic factors in laboratory environments as well as in natural environments. Our literature review before the study revealed that humidity's effects are related to temperature, that changes in humidity accompany temperature changes, and that they can affect insect physiology, affecting the development, life span, and oviposition of many insects. (Ludwig, 1945; Engelmann, 1970; Norhisham et al., 2013). For this reason, the ambient temperature was fixed at 35 ºC for the humidity values to be determined.

Table 3. Friedman Test Table between *L. sericata* Egg Laying Numbers at Different Temperature Values

Temperture	N	X±ss	Rank Mean	Chi-Square	p	Difference**
(1)25 °C	18	1395,67±1800,29	1,44	21,143	,000*	1 with 2,3
(2)30 °C	18	7717±7940,77	1,94			2 with 3
(3)35 °C	18	12231,11±10046,68	2,61			

It has been reported that low relative humidity (R.H.) conditions can cause detrimental effects such as decreased egg production, egg drying, and even egg mortality (Buxton, 1932; Guarneri et al., 2002) and that flies lay eggs in the direction of clustering to withstand low humidity (Davies, 1948; Hans, 2016). Norhisham et al, (2013) observed a high egg mortality rate at 20% relative humidity and found that dehydration at low R.H. caused the egg's chorion and embryo to shrink.
In determining the upper and lower humidity limit values in our study, the literature information (Davies & Hobson, 1935; Mumcuoğlu et al., 2001; Tachibana & Numata, 2001; Mohd et al., 2005; Barnes & Gennard, 2013; Shefa et al., 2013; Thyssen et al., 2013; Blystone and Hansen, 2014) was taken into consideration that the optimum humidity in the environment varies between 45% and 60% on average. The lower humidity value limit was determined to be 35%, with the idea that egg production would occur due to the high temperature fixed in the study. The upper limit of the humidity value in our study was not studied at higher humidity values due to

mortality in cultures due to increased contamination when the humidity value was 65-70% in the laboratory environment in our preliminary experiments.

In the laboratory environment with 35% humidity, it was found that the mating efficiency of the adults in the cage decreased and caused mortality. At the same time, it was determined that the low humidity in the laboratory environment also affected the food left in the cages, and the surfaces of the lungs started to dry rapidly. It was observed that there was spawning when released into the cages, but spawning decreased with drying. It was determined that the first spawning activity started later than the other humidity values studied.

According to Holmes et al, (2012), humidity is essential for ovarian maturation and egg eclosion in Diptera. In *L. sericata*, Davies & Hobson, (1935) stated that the moisture value of the environment and food are essential criteria affecting oviposition. Uvarov, (1931) states that the reproductive rate increases at higher relative humidity percentages. It was observed that spawning started earlier at 50% humidity, and reproductive potential was maximum at this value. The earliest spawning was observed at 60% humidity, but a decrease in reproductive potential was detected. Our findings regarding humidity values determined that reproductive potential did not increase directly with humidity values. We think the decrease in reproductive potential at 60% humidity is due to the formation of fungus in the cages according to the insectarium area due to the constant temperature and death of the cultures with contamination.

According to our findings (Table 4), there is a difference between the number of egg-laying at 35% humidity and the number of egg-laying at 50% and 60% humidity ($\chi 2=17.\ 913$, $p<0.05$). The average number of eggs laying at 50% and 60% humidity exceeds 35%. However, there was no significant difference between the number of egg-laying larvae at 50% and 60% humidity values ($p>0.05$).

Table 4. Friedman Test Table between *L. sericata* Egg Laying Numbers at Different Humidity Values

Humidity (%)	N	X±ss	Rank Mean	Chi-Square	p	Difference **
(1)%35	18	1810,79±2436,1	1,42	17,913	,000*	1 with 2,3
(2)%50	18	9181,05±8955,58	2,47			
(3) %65	18	7152,05±5612,84	2,11			

It was observed that the females of the species were also sensitive to relative humidity saturation. When there was not enough humidity in the environment for reproduction, they waited to lay their eggs, and the eggs laid were found to hatch due to dehydration. Humidity changes an organism's oviposition rate and total egg production (Ludwig, 1945). In our study, our findings (Table 4) on egg-laying rates at constant temperature and different humidity values within the species indicate that humidity affects reproductive potential and are in parallel with the literature data.

Each species has a humidity range considered optimal for specific physiological processes such as oviposition (Ludwig, 1945). This range is

reported to vary between 45% and 60% for *L. sericata* females to lay their eggs (Davies &Hobson, 1935; Mumcuoğlu et al., 2001; Tachibana & Numata, 2001; Mohd et al., 2005; Polat et al., 2010; Barnes & Gennard, 2013; Shefa et al., 2013; Thyssen et al., 2013; Blystone & Hansen, 2014). Our study observed that *L. sericata* reproduced at three humidity values of 35%, 50%, and 65%. We believe that oviposition was observed, albeit low, at 35% humidity, which does not comply with the literature, which we determined as the lower limit because we fixed the temperature, another influential factor in oviposition, at the appropriate value.

Conclusion

In conclusion, this research on the mass rearing of *L. sericata* flies may be an alternative method to estimate the relationship between environmental factors of temperature and humidity and time in fecundity and the development of necrophagous species under laboratory-rearing conditions. However, parameters such as photoperiod (Nabity et al., 2006) and tissue type of rearing substrate (Day and Wallman, 2007) may affect the development time of fly fecundity and oviposition. In addition, model research for rearing the species under laboratory conditions that cover the extreme limits of the temperature and humidity range, taking into account parameters such as insectarium (fly rearing room) area, internal and external factors inside the insectarium, and fly population density, may contribute. Our evaluations of our limited study are promising. However, optimized temperature and humidity ranges can be determined with further research in wide ranges to ensure colony culture continuity and mass production under laboratory conditions.

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Ethical Statement

This study does not present any ethical concerns.

Author Contributions

Writing-Original Draft: N.P., Writing- review & Editing: N.P., S.M., M.K.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

No datasets were generated or analysed during the current study.

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Quail Meat Under Threat: Hidden Microplastics Pose Risks to Public Health and Environment

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Abstract

Aim of study: This study aimed to determine the presence of microplastics in tissues and organs of quails (*Coturnix coturnix*) and to evaluate the potential risks of microplastic contamination in terms of human consumption and environmental impacts.

Materials and Methods: Organ and tissue samples were analyzed from five laying quails that had died naturally. Samples were treated with 10% KOH and filtered in a laminar flow cabinet. Microplastics were identified using light microscopy and FTIR spectroscopy.

Results: Microplastics in filament, fragment, and film forms were detected in quail tissues and digestive system contents. Polyethylene and polyvinyl stearate polymers were the most common types of microplastics. The highest microplastic density was found in intestinal contents. The presence of microplastics in edible tissues (breast and leg meat) was identified, posing potential risks for human consumption.

Conclusion: The presence of microplastics in quail meat and tissues poses potential risks for human consumption and highlights the prevalence of environmental pollution.

Keywords: Environmental pollution, microplastic, microplastics contamination, public health, quail.

Bıldırcın Eti Tehdit Altında: Gizli Mikroplastikler Halk Sağlığı ve Çevre İçin Risk Oluşturuyor

Öz

Çalışmanın Amacı: Bu çalışma, bıldırcınlarda (*Coturnix coturnix*) doku ve organlarda mikroplastik varlığını belirleyerek, insan tüketimi ve çevresel etkiler açısından mikroplastik kirliliğinin olası risklerini değerlendirmeyi amaçlamaktadır.

Materyal ve yöntemler: Ölü olarak temin edilmiş beş yumurtacı bıldırcından alınan organ ve doku örnekleri analiz edildi. Örnekler 10% KOH ile muamele edilip, laminar akış kabininde filtrelendi. Mikroplastikler ışık mikroskobu ve FTIR spektroskopisiyle tanımlandı.

Bulgular: Çalışmada bıldırcın dokularında ve sindirim organları içeriğinde filament, fragment ve film formunda mikroplastikler tespit edildi ve polietilen ile polivinil stearat polimerleri en yaygın mikroplastik çeşitleri olarak tespit edildi. En yüksek mikroplastik yoğunluğu bağırsak içeriğinde görüldü. Yenilebilir dokularda (göğüs ve but eti) mikroplastik varlığı saptandı, insan tüketimi için risk oluşturabileceği belirlendi.

Sonuç: Bıldırcın eti ve dokularında mikroplastik varlığı insan tüketimi için potansiyel risk oluşturmakta, çevresel kirliliğin yaygınlığını göstermektedir.

Anahtar Kelimler: Çevresel Kirlilik, Mikroplastik, Mikroplastik Kontaminasyonu, Halk Sağlığı, Bıldırcın.

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Introduction

Microplastics, defined as plastic particles smaller than 5 mm, are recognized as a pervasive pollutant that affects not only aquatic ecosystems but also terrestrial environments and their inhabitants (Cole et al., 2015; Olubusoye et al., 2023; Sun et al., 2019). Studies have shown that microplastic can disrupt the ecosystem health through breaking the food chain (Bhusare et al., 2024; Olubusoye et al., 2023). The bioaccumulation of microplastics throughout ecosystems, along with their cascading effects across the food web, impacts not only individual species but also extends beyond aquatic environments to terrestrial ecosystems, thereby influencing broader ecosystem dynamics (Cole et al., 2015; Davis & Raja, 2020). The accumulation of microplastics in terrestrial environments is concerning, as they can be transported into aquatic systems via runoff, perpetuating the pollution cycle (Lehner et al., 2019; Paudel et al., 2024). Moreover, ingesting microplastics by terrestrial organisms, including mammals, raises significant concerns regarding bioaccumulation and toxicity (Bhusare et al., 2024; Jeong et al., 2024; Dong et al., 2023). The ingestion of microplastics by terrestrial organisms occurs through contaminated food, water, and air. Studies have demonstrated that microplastics can accumulate in the tissues of mammals and poultry, leading to potential risks such as reproductive toxicity and metabolic disorders (Prata et al., 2021; Bhusare et al., 2024; Paudel et al., 2024).

Microplastics in animals living in terrestrial ecosystems can disrupt various physiological parameters, thereby posing a significant threat to animal health. The presence of microplastics in the food chain constitutes a direct risk to the health of terrestrial organisms, as these particles can carry toxic substances capable of impairing growth, reproduction, and overall well-being (Jeong, et al., 2024; Paudel et al., 2024; Bhusare et al., 2024; Prata et al., 2021). Moreover, the long-term effects of microplastic exposure in mammals and poultry are still under investigation; however, existing evidence suggests that it may lead to chronic health issues, including inflammation and oxidative stress (Mahmud et al., 2024; Li et al., 2024). Terrestrial mammals and poultry are increasingly exposed to microplastics, which have become ubiquitous across various ecosystems. The primary exposure routes include ingestion via the digestive tract and, to a lesser extent, inhalation of particles through the respiratory system. Studies have demonstrated that these particles can accumulate in various tissues, including the liver, kidneys, and gastrointestinal system (Deng et al., 2017; Salikova et al., 2024; Yong et al., 2020).

The ingestion of microplastics in animals typically occurs through the consumption of contaminated feeds and water. Once ingested, microplastics can translocate from the intestine to the lymphatic and circulatory systems, reaching various organs and tissues (Palaniappan et al., 2021; Smith et al., 2018). Studies have reported the presence of microplastics, such as polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), etc., in animal tissues, raising concerns about their potential toxicity and long-term health impacts. For instance, microplastics have been associated with oxidative stress, inflammation, and disruptions in metabolic processes (He & Yin, 2023; Roman et al., 2024; Salikova et al., 2024).

Additionally, evidence is growing that microplastics may affect reproductive health, with potential transgenerational effects observed in mammalian models (He & Yin, 2023; Mills et al., 2023). Beyond mammals, the presence of microplastics in poultry feed and manure has been documented, heightening concerns about their accumulation in edible poultry tissues,

particularly from a public health perspective. Research has indicated that microplastics can be found in poultry meat, posing a risk of human exposure through the consumption of contaminated products (Kadac-Czapka et al., 2023; Ma & Li, 2023).

Despite the expanding literature on microplastics in poultry, significant research gaps remain, and the full extent of their health effects on poultry, as well as their implications for food safety and human health, are not yet fully understood (Lackner & Branka, 2024; Lu et al., 2022; Ma & Li, 2023). Recent studies on microplastics in poultry have predominantly focused on chickens, with limited research on other avian species that are consumed by humans and integral to natural food chains, such as wild birds (Cusworth et al., 2023; Jasińska et al., 2023; Lackner & Branka, 2024; Lu et al., 2022).

This study highlights the potential for microplastics to contribute to broader health issues, mainly through wildlife contamination and the potential bioaccumulation in the food chain, ultimately impacting humans (Blackburn & Green, 2021). Consequently, this study addresses the overlooked issue of microplastic contamination in the organs and tissues of quails (Coturnix coturnix), which both humans and wildlife consume. We have investigated the presence of microplastics in various tissues and organs, including the liver, spleen, ovaries, pancreas, heart, gizzard, intestines, intestinal contents, gizzard contents, breast and leg meat, and visceral fat, providing valuable insight into microplastic contamination in quails.

Material and Methods

Animals

This study was conducted on five laying quails (*Coturnix coturnix*) in the egg-laying period, which had died naturally or due to disease and were obtained from the same farm in Kastamonu Province, Türkiye. To prevent environmental microplastic contamination, necropsy procedures were performed under a fume hood, and the necessary tissues and contents were collected.

The following organs and tissues were sampled for the study: gizzard and its contents, intestine (including the duodenum, jejunum, and ileum), intestinal contents, liver, spleen, visceral fat, breast muscle, leg muscle, heart muscle, ovary, and pancreas.

Tissue Extraction

To extract potential microplastics from the tissues, an alkaline digestion process was applied using filtered 10% KOH (w/v) (Rani et al., 2023). Tissues were finely chopped into small pieces with a knife and placed in 500 mL glass beakers.

All glassware used during the extraction process was washed sequentially with filtered distilled water, filtered ethanol (Absolute for Analysis; CAS No: 64-17-5, Merck, Germany), filtered acetone (CAS No: 67-64-1, Isolab, Germany), and again filtered distilled water. To prevent contamination, the glassware was covered with aluminum foil.

Filtered 10% KOH (200 mL) was added to the tissue samples, then incubated at 60°C in an oven for 24 hours with periodic gentle shaking. After the incubation, the samples were vacuum-filtered under a Laminar Flow cabinet with only one individual present to prevent overcrowding and air movement. In this study, glass fiber with a pore size of 1.2 μm, preheated at 300°C, was used (Filter-Lab MFV3-047).

Gastrointestinal Content Extraction

Gizzard and intestinal contents collected from the animals were treated with 10% KOH at 60°C for 24 hours to break down organic materials. The extract was then transferred to glass tubes and centrifuged at 1000 rpm for 5 minutes to remove

coarse particles, such as feed materials. The liquid fractions were subsequently vacuum-filtered under a Laminar Flow cabinet using filters preheated at 300°C.

Contamination Control

To monitor potential airborne contamination of the liquids used in the study, filters obtained under a Laminar Flow cabinet were used as negative controls.

Quantification and Classification of Microplastics

After drying, the filters were examined under a light microscope (Leica DM500). Suspected microplastic particles were classified as filaments, fragments, and films. All particles count was recorded. Each observed particle was photographed and scaled (Leica ICC50W). For further microplastic characterization, Fourier-transform infrared (FTIR) spectroscopic analysis was performed. FTIR analyses (ATR-FTIR, Perkin Elmer, Spectrum-two, USA) were carried out in the 600-4000 cm^{-1} range with a resolution of 4 cm^{-1} and 32 scans in absorption mode. The spectra were compared with the library (Fiveash Data Management, Inc. 2006-2008) database for validation. A match rate of 70% or higher was used as the criterion for polymer identification.

Statistical Analysis

Statistical analyses were performed to evaluate the differences in microplastic density across tissues and animals. To test for statistically significant differences, One-Way ANOVA was applied for both tissue- and animal-level comparisons. For post-hoc comparisons, Tukey's HSD test was applied to identify specific contributing to significant differences when ANOVA results indicated significance. All statistical analyses were conducted using IBM SPSS Statistics 23, with a significance level of $p \leq 0.05$.

Results

No contamination was detected in the control filters from the materials used in the study. The Petri dishes used were sequentially washed with distilled water, ethanol, acetone, and distilled water, covered with aluminum foil, and confirmed to be free from airborne contamination under microscopic examination.

Analyses of the filters obtained after the extraction of tissue and content samples from the animals revealed the presence of microplastics (Tables 1 and 3). The results variability in microplastic levels among animals from the same facility. Microscopic examination of tissue and content extract identified microplastic particles in the forms of filament, fragment, and film (Figure 1). Total microplastic amounts for the animals (Q1, Q2, Q3, Q4, and Q4) were 61, 44, 37, 28, and 34, respectively (Figure 2). However, there was no statistically significant difference in the microplastic amounts among the quails ($p = 0.351$).

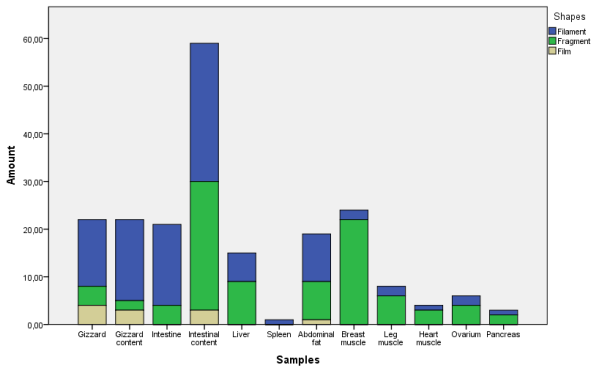


Figure 1. The distribution of microplastic shapes by tissues (particle count/sample).

Significant differences in microplastic presence were observed among tissue and content samples ($p < 0.05$). Among potential contamination routes, gastrointestinal contents (gizzard and intestinal contents) exhibited the highest microplastic presence. Notably, the small intestine contents had the highest density, 11.8±3.27 (Table 2).

Table 1. Microplastic counts in animals. All particles classified as filament, fragment, and film. Groups sharing the same superscript letter indicate no statistically significant differences ($p > 0.05$). Different superscripts denote statistically significant differences ($p < 0.05$).

	Filament					Fragment					Film					**Total**
Quail Samples	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
Gizzard	3	4	3	3	1	1	2	0	0	1	1	0	0	3	0	22
Gizzard content	3	3	5	3	3	1	0	0	1	0	0	2	0	0	1	22
Intestine	6	2	3	2	4	0	2	0	0	2	0	0	0	0	0	21
Intestinal content	6	5	8	4	6	7	5	5	3	7	2	0	0	0	1	59
Liver	2	1	0	0	3	2	2	1	2	2	0	0	0	0	0	15
Spleen	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Abdominal fat	2	4	2	1	1	2	2	2	1	1	1	0	0	0	0	19
Breast muscle	1	0	1	0	0	8	5	4	4	1	0	0	0	0	0	24
Leg muscle	1	0	1	0	0	2	4	0	0	0	0	0	0	0	0	8
Heart muscle	1	0	0	0	0	2	1	0	0	0	0	0	0	0	0	4
Ovarium	1	0	1	0	0	3	0	1	0	0	0	0	0	0	0	6
Pancreas	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	3
Total	28	19	24	13	18	29	23	13	12	14	4	2	0	3	2	204
	Microplastic Amounts (Mean ±S.D)															
Quail	**Q1**		**Q2**		**Q3**		**Q4**		**Q5**							
Amounts	5.08 ± 3.27^{a}		3.66 ± 3.05^{a}		3.08 ± 3.62^{a}		2.33 ± 2.42^{a}		2.83 ± 4.10^{a}							

Table 2. Microplastic densities by tissues (particle count/sample), classified by density group. Groups sharing the same superscript letter indicate no statistically significant differences ($p > 0.05$). Different superscripts denote statistically significant differences ($p < 0.05$).

Sample	Groups based on microplastic density	Microplastic Amounts (Mean ±S.D)
Intestinal content	**Group 1 (Highest Density)**	11.8±3.27^{a}
Gizzard	**Group 2 (Moderate Density)**	4.40±1.81^{b}
Gizzard content		4.40±0.54^{b}
Breast muscle		4.80±2.86^{b}
Liver		3±1.58^{b}
Abdominal fat		3.8±1.78^{b}
Intestine		4.20±1.79^{b}
Pancreas	**Group 3 (Low Density)**	0.60±0.89^{b}
Heart muscle		0.80±1.30^{b}
Ovarium		1.20±1.78^{b}
Leg muscle		1.60±1.81^{b}
Spleen	**Group 4 (Lowest Density)**	0.20±0.45^{c}

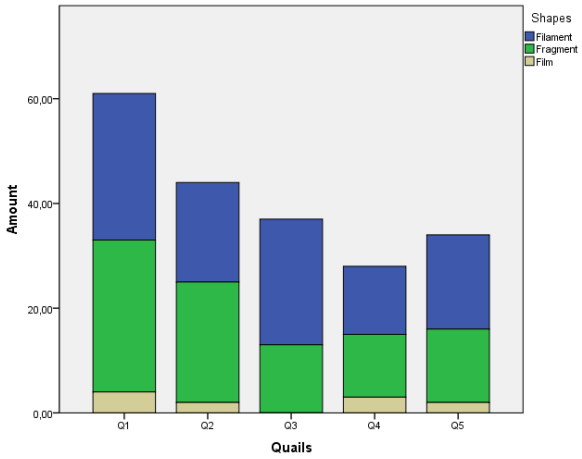


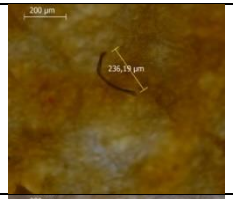
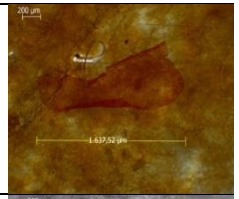
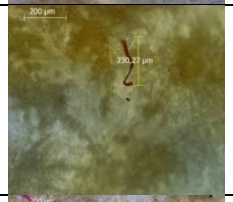
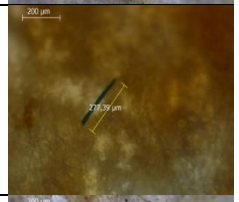
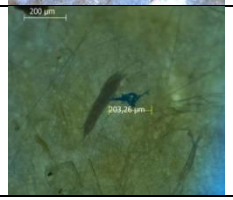
Figure 2. The distribution of microplastic shapes by animals (particle count/animal).

Microplastic analysis revealed that the majority of the microplastics were in filament and fragment forms. Microplastic levels in gizzard tissue were found to be similar to those in gizzard contents, averaging 4.40±1.81. The average microplastic presence in intestinal tissue (including duodenum, jejunum, and ileum) was notably lower than that in intestinal contents, at 4.20±1.79.

In edible tissues, such as breast and leg muscles, average microplastic amounts were 4.80±2.86 and 1.60±1.81, respectively ($p= 0.324$). Among the other tissues, the spleen exhibited the lowest microplastic presence, with an average value of 0.20±0.45 (Table 2). The size of microplastic particles detected across tissues and contents ranged from 30 to 1600 µm.

When microplastic presence was grouped by tissue type, intestinal contents had the highest density, followed by the gizzard, gizzard contents, and breast muscle. The liver, abdominal fat, and intestines formed the third group, while the spleen, pancreas, and heart muscle exhibited the lowest levels. Microscopic examination of green-colored fragments with average length of 67.07±29.89 µm observed in the intestinal content, liver, abdominal fat, breast muscle, leg muscle, heart muscle, and ovaries, was followed by FTIR analysis. FTIR scans confirmed that these fragments were composed of PE (Table 3-4). Filament and film-like particles identified in gizzard and gizzard contents, as well as filament and fragment particles in the liver, heart muscle, intestinal content, and abdominal fat tissues, were also classified as PE (Table 5). Additionally, other microplastic particles were predominantly identified as polyvinyl stearate (PVS) based on FTIR spectra.

Table 3: Microplastic particle images in filament, fragment, and film shapes by tissue.

Sample	Microplastic Images		
Gizzard			
Gizzard content			
Intestine			
Intestinal content			
Liver			
Spleen			

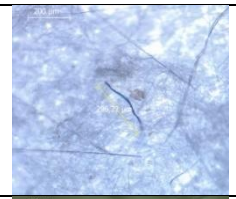
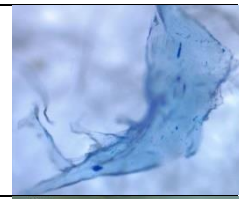
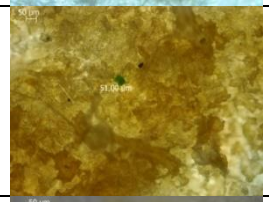
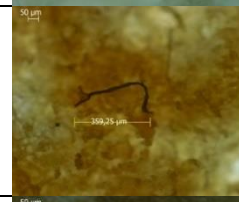
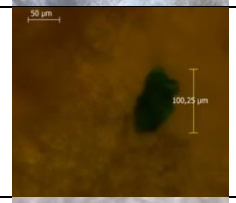
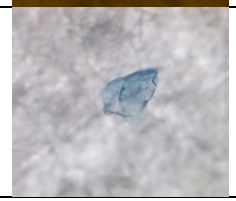
Abdominal fat			
Breast muscle			
Leg muscle			
Heart muscle			
Ovarium			
Pancreas			

Table 4: Microplastics, green-colored and in fragment shapes in different tissues were confirmed as PE by FTIR.

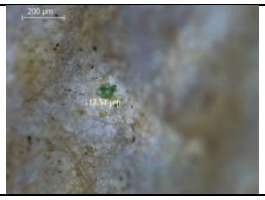
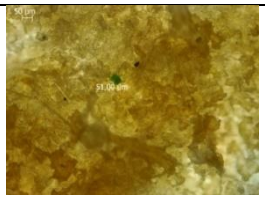
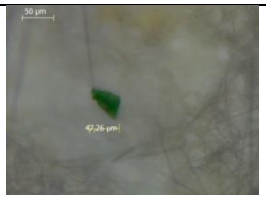
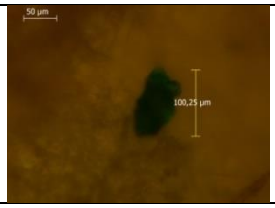
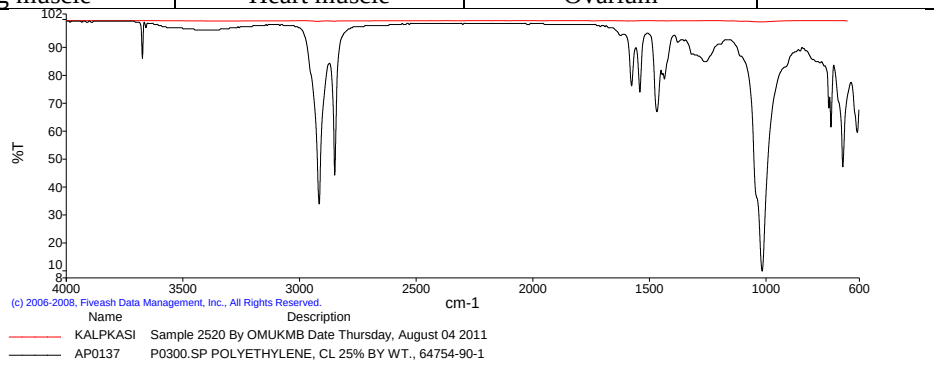
			
Intestinal content	Liver	Abdominal fat	Breast muscle
			
Leg muscle	Heart muscle	Ovarium	
			
FTIR Image			

Table 5. Microplastics in filament and film shapes in different tissues were confirmed as PE by FTIR.

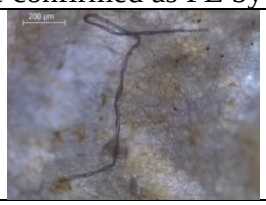
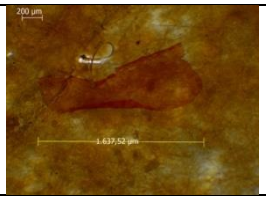
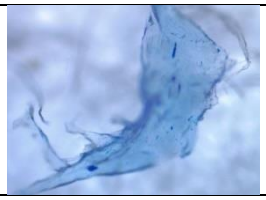
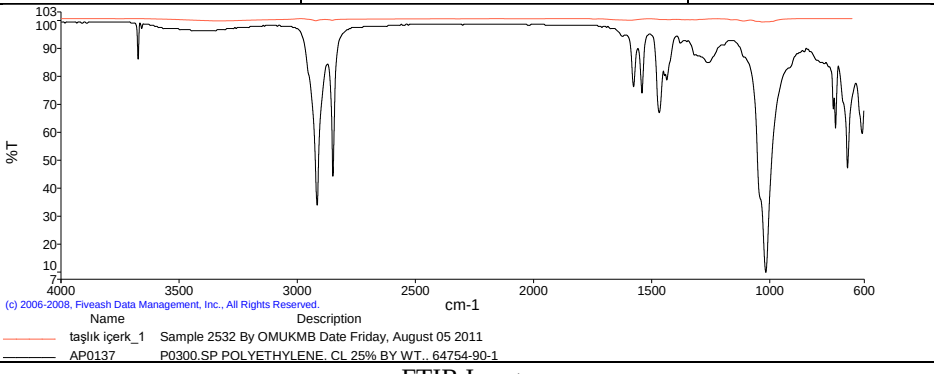
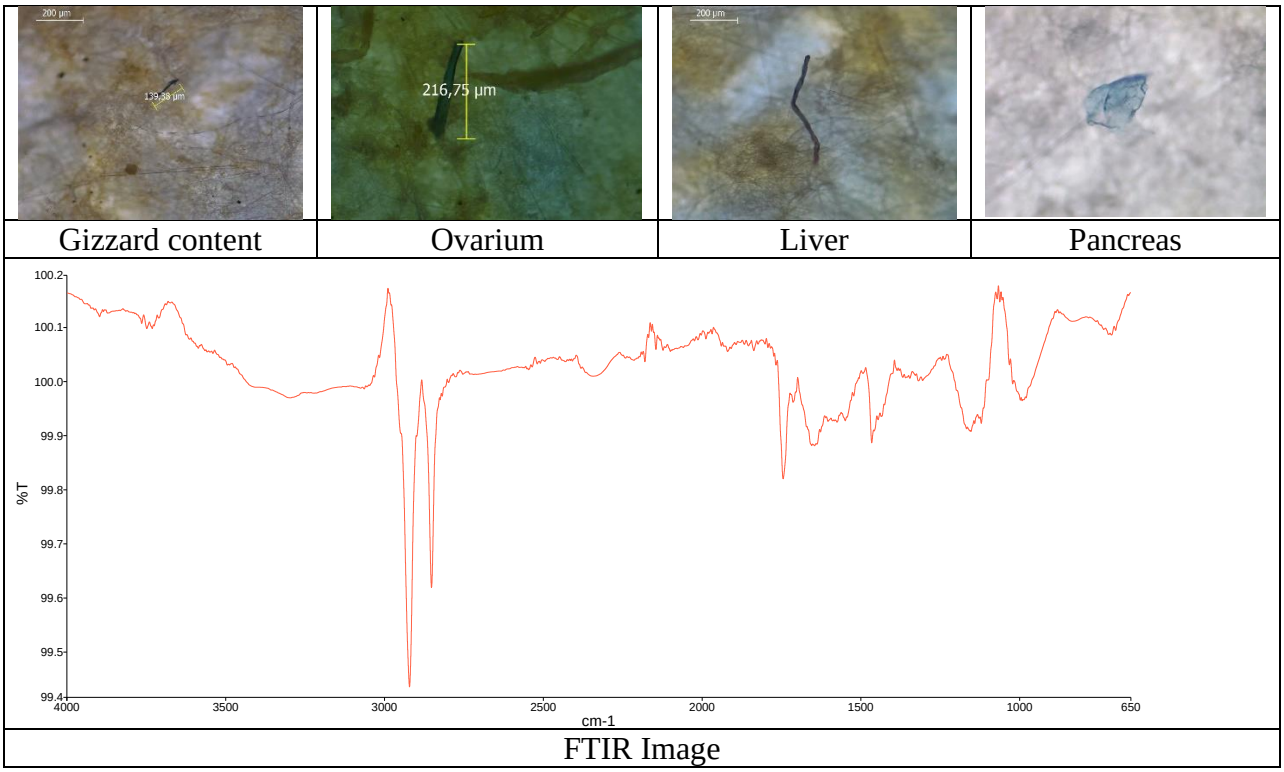
		
Heart muscle	Liver	Gizzard content
		
Gizzard	Intestinal content	Abdominal fat
		
FTIR Image		

Table 6. Microplastics in filament and fragment-like shapes in different tissues were confirmed as PVS by FTIR.



The presence of microplastics in edible tissues such as breast and leg muscles raises concerns about human exposure through consumption. Based on the findings, estimated microplastic exposure through breast muscle was calculated as 0.48 particles/gram, while leg muscle exposure was 0.16 particles/gram.

Discussion

Previous studies have reported microplastic contamination in edible seafood, primarily resulting from environmental sources (Bergami et al., 2016). This raised interest in the presence of microplastics in terrestrial ecosystems, especially in animals farmed for human consumption. Recent research has confirmed microplastic contamination in feces and tissue samples of domestic animals (Susanti et al., 2021; Beriot et al., 2021; Wu et al., 2021). In this study, microplastic presence was confirmed in five laying quails (*Coturnix coturnix*) obtained from the same farm and raised for human consumption. Microplastics in filament, fragment, and film shapes were identified in the gastrointestinal contents and various tissues of the animals.

These findings, consistent with previous studies, indicate that microplastic contamination is not confined to aquatic ecosystems but is rapidly and extensively spreading in terrestrial ecosystems as well. Considering these findings, terrestrial products, like aquatic products, are significantly contributor to human exposure to microplastics and potential health risks.

Quails are widely used for meat and egg production (Lukanov & Pavlova, 2020). They are classified into three types: laying (light), dual-purpose, and meat (heavy). This study did not analyze egg samples from the laying quails due to the potential presence of other animals in the same cage. However, the detection of PE polymers in the ovaries, with particle sizes ranging from 51-100.25 µm, suggest that such particles could potentially be transferred via eggs developing in

the female reproductive tract. Meat-type quails are predominantly farmed in countries such as Spain, France, Italy, and Portugal, as well as the USA and China (Dalle Zotte & Cullere, 2024). In Türkiye, quail meat production was reported as 103 tons in 2019 (TUIK, 2019), although its consumption is less common compared to other countries.

The average microplastic levels in edible tissues such as breast and leg muscles were found to be 4.80±2.86 and 1.60±1.81, respectively ($p = 0.324$). These values correspond to potential human exposure rates of 0.48 particles/gram for breast muscle and 0.16 particles/gram for leg muscle (Domenech & Marcos, 2021). Consequently, this study is the first to report potential microplastic exposure through quail meat, highlighting the risks associated with their consumption in regions where quail products are popular, such as European countries.

Microplastic exposure in intensively farmed animals is likely caused by air, water, and feed contamination (Walkinshaw et al., 2022; Beriot et al., 2021; Chen et al., 2020). Wu et al. (2021) identified filament and fragment-shaped microplastics, such as PE and PP, larger than 1000 µm in chicken feces. They suggested that possible sources of PE in feces might include the inner linings of feed bags and drinker equipment. In this study, the similarity of microplastic types (PE, PSA) in the gastrointestinal system contents further support the notion that these are likely contamination sources. The transfer of microplastics through the intestinal wall is largely size-dependent (Li et al., 2024). Particles smaller than 150 µm are reported to be more likely absorbed via endocytosis by intestinal epithelial cells (Yong & Du, 2023). Based on measurements, PE particles averaging 67.07±29.89 µm in length, found in intestinal contents, liver, abdominal fat, breast and leg muscle, heart muscle, and ovaries, are likely transported from feed to other tissues through gastrointestinal system. Although the lengths of other particles suggest a limited passage through the gastrointestinal wall, their diameters might allow transit under intestinal movement. PVS particles are also presumed to follow similar pathways to spread throughout the body.

Based on the study findings, filament-shaped particles were the most prevalent, followed by fragments and films ($p < 0.000$). The presence of filament-shaped particles strengthens the assumption that they might originate from plastic packaging products (Ivleva et al., 2017). The detection of PE and PVS polymers predominantly suggests that the feed and watering materials used in the poultry industry are primary contributors to microplastic contamination. However, air and water contamination cannot be disregarded.

Detecting microplastics in farmed quails, which are consumed as human food, indicated an additional potential route for human exposure to microplastics. These findings emphasize the need for strategies to reduce plastic use and contamination, which could have broader implications for environmental and public health policies. Furthermore, the presence of microplastics in the gastrointestinal system and edible tissues suggests that greater caution is needed regarding feed contamination with microplastics. Stakeholders in animal farming should adopt stricter measures to minimize plastic use. Additional efforts to prevent microplastic contamination, such as implementing methods to degrade microplastics during feed preparation or within the gastrointestinal system, are becoming essential.

Financial Support
This study did not receive a grant by any financial institution or sector.

Ethical Statement

This study was approved by the Kastamonu University Animal Experiments Local Ethics Committee (E-16498365-605-2400143033).

Author Contributions

Investigation: V.D., Ç.S. and S.G.; Material and Methodology: V.D., Ç.S. and S.G.; Supervision: V.D.; Visualization: M.S.A.; Writing-Original Draft: V.D. and Ç.S..; Writing- review & Editing: V.D., Ç.S., S.G. and M.S.A.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Clinical Manifestation of A Cat With Acute Acetaminophen Toxicity

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Abstract
This case report presents diagnostic methods used for a 2.5 kg, 1-year-old female British Shorthair cat admitted to the veterinary hospital with non-specific symptoms, including anorexia, lethargy, jaundice and dehydration. After a comprehensive evaluation of the anamnesis, clinical findings, laboratory results, imaging and the exclusion of similar diseases, a diagnosis of iatrogenic acetaminophen toxicity was established. While numerous case reports and studies address acetaminophen toxicity, this report emphasizes how the employed diagnostic methods contribute to accurate diagnosis. Ultimately, thorough evaluation of anamnesis data, combined with these examination techniques, can facilitate precise disease identification and enhance awareness of its characteristics. Moreover, case reports like this one can increase awareness and help reduce fatalities associated with acetaminophen intoxication.
Keywords: Acetaminophen, Cat, Toxicity.

Akut Asetaminofen Toksikasyonlu Bir Kedinin Klinik Görünümü

Öz
Bu vaka raporu, anoreksi, letharji, ikterus ve dehidrasyon gibi spesifik olmayan semptomlarla hayvan hastanesine başvuran 2.5 kg, 1 yaşında dişi bir British Shorthair kedisi için kullanılan tanı yöntemlerini sunmaktadır. Anamnez, klinik bulgular, laboratuvar sonuçları, görüntüleme ve benzer hastalıkların dışlanmasının kapsamlı bir değerlendirilmesinin ardından, iatrojenik asetaminofen toksisitesi tanısı konulmuştur. Asetaminofen toksisitesini ele alan çok sayıda vaka raporu ve çalışma bulunmasına rağmen, bu rapor kullanılan tanı yöntemlerinin doğru tanıya nasıl katkıda bulunduğunu vurgulamaktadır. Nihayetinde, anamnez verilerinin detaylı değerlendirilmesi ve bu muayene tekniklerinin bir araya getirilmesi, doğru hastalık tanısını kolaylaştırabilir ve hastalığın özellikleri hakkında farkındalığı artırabilir. Ayrıca, bu gibi vaka raporları, asetaminofen zehirlenmesi ile ilişkili ölümleri azaltmaya yardımcı olmak ve farkındalığı artırmak için önemlidir.
Anahtar kelimeler: Asetominofen, Kedi, Toksikasyon.

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Introduction

A wide range of over-the-counter (OTC) medications is available in veterinary practice, many of which carry risks of toxicity, and their use is becoming more widespread. These OTC drugs are easily obtained from the market, are often inexpensive, and can be purchased without a prescription or professional guidance. Presently, the deliberate misuse of OTC medications is a significant issue in both veterinary and human healthcare (Gülersoy et al., 2021). For example, acetaminophen, also called paracetamol, is a widely used and effective analgesic and antipyretic in humans. However, it is closely linked to severe complications in companion animals, with toxicity cases frequently reported. (Özkan, 2017). The primary cause of acetaminophen intoxication is the owner's improper administration of the drug without first consulting a veterinarian (Denzoin Vulcano et al., 2013). In mammals acetaminophen is biotransformed to nontoxic products in the liver via conjugation with glucuronic acid and excreted by the kidneys. A small portion of acetaminophen is metabolized through the cytochrome P-450 enzyme pathway producing a metabolite, N-acetyl-para-benzoquinoneimine (NAPQI) which is highly toxic. Acetaminophen exposure becomes toxic, when glucuronidation and sulfation pathways become saturated. NAPQI binds to cellular proteins and membranes and leads to cellular injury and death, particularly hepatocytes. Cats are extremely sensitive to the toxic effects of acetaminophen, since the conjugation of glucuronides with many toxic compounds occurs slowly in cats. This is due to little possession of glucuronyl transferases. Deficiency of the glucuronide conjugation pathway results in more drug being conjugated to sulfates; however, the sulfation pathway is also lower in cats than other species (Allen, 2003; Sidhu et al., 2021). Acetaminophen poisoning is commonly presented with clinical signs of anorexia, dullness, facial and paw edema, muddy mucous membranes, respiratory distress and hematuria (Sidhu et al., 2021). There is no safe dose of acetaminophen for cats, making its toxicity more prevalent in felines than in canines. The toxic dose is reported to be between 50-100 mg/kg body weight; however, signs of toxicity and even death can occur at doses as low as 10 mg/kg body weight (Aronson & Drobatz, 1996; Sidhu et al., 2021). This case presentation aims to raise awareness of the clinical signs and symptoms associated with acetaminophen toxicity, a condition with a high mortality rate due to misuse. It focuses specifically on the clinical manifestations of acetaminophen overdose in cats.

Case Description

A 2.5 kg, 1-year-old female British Shorthair cat was brought to Harran University Veterinary Faculty Animal Hospital showing anorexia, lethargy, severe jaundice, and hematemesis. The owner reported administering 120 mg of acetaminophen (Calpol®, Abdi Ibrahim, Türkiye) orally to the cat once daily for two consecutive days, without prior consultation. During this time, the patient started vomiting, showed persistent anorexia, and exhibited symptoms such as hematemesis and abdominal swelling. Before the onset of illness, the cat was fed a commercial dry cat food and was an intact indoor cat with no previous medical history. It was noted that antiparasitic treatments and vaccinations were administered regularly. After evaluating the anamnestic data, physical and laboratory examinations were conducted. The physical examination included measurements of respiratory rate (RR), heart rate (HR), an assessment of palpable lymph nodes, auscultation of the lungs and heart, and a rectal temperature check. At this time, a fecal sample was collected directly from the rectum using a sterile swab for examination. The physical assessment revealed a

skin turgor of 4 seconds, indicating an 8% dehydration level, accompanied by enophthalmos and prolonged skin elasticity. Additionally, the gingival and conjunctival mucosa appeared anemic, with the gingival capillary refill time exceeding 2 seconds. HR and RR were measured at 45 beats per minute (reference range: 16-40/min) and 60 breaths per minute (reference range: 120-140/min), respectively. The rectal temperature was recorded at 39.8 °C (reference range: 37.5-39.1 °C) (Klaasen, 1999). Lung auscultation indicated bradypnea, with mild crackles noted in the cranial lobes (16 breaths per minute). The fecal sample (2 to 3 g) was mixed with 15 mL of pre-made zinc sulfate solution ($ZnSO_4$, specific gravity 1.18) in a 15 mL conical tube. If necessary, more $ZnSO_4$ was added to maintain the volume at 15 mL, and the mixture was centrifuged at 500 to 600 × g for 5 minutes. After centrifugation, additional $ZnSO_4$ was added to create a positive meniscus, onto which a coverslip (22 mm × 22 mm) was placed for 5 minutes. The coverslip was then removed and examined under a light microscope (×40 magnification, Olympus, Japan). The fecal analysis revealed no parasites or parasite eggs. After the physical examination, venous blood samples (3-5 mL) were collected via venipuncture of the cephalic vein for a complete blood count (CBC, using K_3EDTA tubes) and serum biochemistry (using tubes without anticoagulant), following proper aseptic protocols. Imaging studies, including radiographic and ultrasonographic examinations, were then performed for further investigation

Table 1. Haematological findings

Parameters	Result	Reference values*
WBC (/µL)	13.4	3.0- 14.8
Lymp (x10^9/L)	4.06	1.2 – 8.0
Mono (x10^9/L)	0.99	0- 600
Neu (x10^9/L)	8.00	2.5- 8.5
RBC (x10^6/µL)	2.18	5.92- 9.93
HGB (g/dL)	6.30	9.3- 15.9
HCT (%)	22.4	29- 48
MCV (fL)	102.7	37- 61
MCH (pg)	28.7	11- 21
MCHC (%)	47	30- 38
PLT (x10^3/µL)	350	200- 500

WBC; Leukocyte, Lymp; Lymphocyte, Mono; Monocyte, Neu; Neutrophil, RBC; Red blood cells, HGB; Hemoglobin, HCT; Hematocrit, MCV; Mean cellular volume, MCH; Mean corpuscular hemoglobin, MCHC; Mean corpuscular hemoglobin concentration, PLT; Platelet, *(Klaasen, 1999).

The CBC analysis showed elevated mean cellular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) compared to reference values, while erythrocyte (RBC), hematocrit (HCT), and hemoglobin (HGB) levels were below normal (Table 1). Comparison of the serum biochemical analysis results with reference values revealed increased mean levels of cholesterol (CHOL), total protein (TP), gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL) along with decreased albumin (ALB). (Table 2). Imaging investigations comprised radiographic examinations (in lateral and ventrodorsal positions, DR, Fujifilm, Japan) and ultrasonography (using a 7.5-11 MHz microconvex probe, Mindray Z60, China) of the thorax and abdomen. The radiographic examination revealed renomegaly and a loss of visceral detail, while the ultrasound showed renal cortical hyperechogenicity, free fluid, decreased corticomedullary separation, structural deterioration, and hyperechogenicity in the liver.

Table 2. Biochemical findings

Parameters	Result	Reference values*
TP (g/dL)	8.5	6.0- 7.9
ALB (g/dL)	2.1	2.5- 3.9
ALP (U/L)	65	10- 50
GLU (mg/dL)	100	50- 170
TBIL (mg/dL)	1.2	0.1- 0.4
CHOL (mg/dL)	250	75- 220
GGT (U/L)	20	1-10
ALT (U/L)	131	10- 100
Ca (mg/dL)	10.8	8.2- 10.8
CRE (mg/dL)	1.11	0.6- 2.4
BUN (mg/dL)	29.2	14- 36
AST (U/L)	33	10- 100

TP; Total protein, ALB; Albumin, ALP; Alkaline phosphatase, GLU; Glucose TBIL; Total bilirubin, CHOL; Cholesterol GGT; Gamma glutamyl transferase ALT; Alanine aminotransferase, Ca; Calcium, CRE; Creatinine, BUN; Blood urea nitrogen AST; Aspartate aminotransferase, *(Klaasen, 1999).

Based on the clinical, laboratory, and imaging abnormalities, the differential diagnosis included Feline Cholangitis/Cholangiohepatitis Syndrome, Feline Calicivirus (FCV), Feline Herpesvirus (FHV), Feline Immunodeficiency Virus (FIV), Feline Leukemia Virus (FeLV), Feline Parvovirus (FPV), and Toxoplasmosis. To rule out diseases with similar clinical manifestations, the following diagnostic assays were performed: FCV antigen (Asan Pharm®, Korea; sensitivity 96%, specificity 98%), FHV antigen (Asan Pharm®, Korea; sensitivity 96.5%, specificity 98%), FIV antibodies/FeLV antigen (Asan Pharm®, Korea; sensitivity 98%, specificity 98.7%), FPV antigen (Asan Pharm®, Korea; sensitivity 97.8%, specificity 98.8%), and Toxoplasma antibodies (Anigen, China; sensitivity 100%, specificity 99%). Following negative results, the Biopanda Feline Coronavirus Antigen Rapid Test (relative sensitivity 92.54%, relative specificity 97.09%) was conducted for FIP diagnosis to detect Feline Coronavirus (FCoV) antigen, and all tests returned negative. In conclusion, the diagnosis of iatrogenic acute acetaminophen toxicity was confirmed through anamnesis, physical examination, and laboratory and imaging findings.

Discussion

Acetaminophen toxicity is relatively common due to oral administration by owners or accidental ingestion by animals. Acetaminophen has no safe dose for cats, which leads to its toxicity being more common in felines than in canines. The toxic dose is reported to range between 50 and 100 mg/kg of body weight; however, toxicity and even death can occur at doses as low as 10 mg/kg of body weight (Aronson & Drobatz, 1996; Sidhu et al., 2021). In this case, the administration of 120 mg of acetaminophen once daily for two days resulted in findings in the cat, including hemolytic anemia, pigmenturia, depression, and icterus. Since symptoms such as bradycardia, respiratory depression, and dehydration are non-specific, they must be assessed alongside anamnestic data consistent with acetaminophen intoxication. Recognizing the clinical manifestations aids in diagnosing acetaminophen toxicity and helps alert pet owners to this serious condition. Also, case reports like the present one can help raise awareness and reduce fatalities related to this condition.

The CBC is one of the most frequently conducted laboratory tests in medicine, offering insights into the size and quantity of circulating blood cells. Within the CBC, the red cell indices are

calculated parameters that include RDW, MCV, MCH, and MCHC. These indices are derived from measured HGB, HCT, and RBC counts, and they aid in identifying the etiology of anemia (El Brihi & Pathak, 2024). A previous study reported changes in hematological values, including WBC, MCV, MCH, PCV, and MCHC, as a result of acetaminophen toxicity, which were associated with induced liver injury. Given that hemolytic anemia is a key clinical symptom of acetaminophen intoxication, the low levels of RBC and HGB observed in this case are likely due to excessive accumulation of methemoglobin. This accumulation may cause hemoglobin denaturation, Heinz body formation, increased osmotic fragility of RBCs, and subsequent hemolytic anemia, leading to icterus, hemoglobinemia, tissue anoxia, and cyanosis (Juma et al., 2015). Thus, the low RBC, HGB, and HCT levels observed in this case may be explained by increased osmotic fragility of RBCs and induced hepatotoxicity (Juma et al., 2015; Özkan, 2017). Therefore, although serum biochemistry is more useful for assessing the degree of hepatotoxicity, the evaluation of hematological values can also provide important information for prognosis and treatment planning (Juma et al., 2015).

In all species, acetaminophen is metabolized in the liver through glucuronidation, oxidation, and sulfation. The resulting glucuronide and sulfate conjugates are non-toxic and are excreted in urine and bile. In most species, the oxidation pathway plays a minor role, while glucuronidation is the primary route of acetaminophen metabolism. However, cats have a limited capacity to conjugate with glucuronic acid due to low levels of glucuronyl transferase, the enzyme responsible for the final step of the glucuronidation pathway. As a result, cats have a restricted ability to metabolize acetaminophen into non-toxic metabolites. These inactive metabolites are eliminated by the kidneys (Bates, 2013). When the glucuronidation and sulfation pathways become saturated, acetaminophen is alternatively metabolized through the cytochrome P-450 enzyme pathway, leading to the formation of a toxic metabolite known as N-acetyl-p-benzoquinone imine (NAPQI). Normally, the toxic effects of NAPQI are mitigated by its conjugation with glutathione. However, when exposure to acetaminophen exceeds the capacity of these pathways, NAPQI can bind to cellular proteins and membranes, resulting in cellular injury and death, primarily in hepatocytes (Allen, 2003). Thus, laboratory findings of hepatotoxicity generally develops 24-36 hours post-ingestion. It was highlighted that in cases of acetaminophen intoxication, hepatic enzymes should be monitored carefully (Richardson, 2000). In this case, notable serum biochemistry abnormalities included elevated levels of ALT, TP, ALP, GGT, CHOL and TBIL along with decreased ALB. The increased liver enzymes observed align with findings from previous reports (Sidhu et al., 2021). The elevated hepatic enzymes such as ALT, ALP, GGT were likely due to liver damage. ALT activity is the most frequently used biomarker for hepatotoxicity. Elevated levels of this enzyme are released during liver damage. Its measurement is a more specific test for detecting liver abnormalities, as it is primarily found in the liver. ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST. ALP is predominantly found in the cells lining the biliary ducts of the liver. Its levels may be elevated if bile excretion is inhibited due to liver damage. GGT is an enzyme found in the liver, kidneys, and pancreatic tissues, with its concentration being lower in the liver compared to the kidneys. It is more clinically useful than ALP. While ALP is more sensitive, GGT is much more specific. The elevation of either of the two enzymes helps determine the occurrence of liver injury (Singh et al., 2011). Liver cells are involved in many pathways of lipid metabolism, such as oxidizing triglycerides to produce energy,

lipoprotein production, conversion of excess carbohydrates and proteins into fatty acids and triglyceride, or synthesis of cholesterol and phospholipids. Acetaminophen has been demonstrated to irreversibly inhibit fatty acid β-oxidation, disrupt lipid metabolism and increase triglyceride levels in the serum and liver (Suciu et al., 2015). The increase in bilirubin was attributed to accelerated RBC destruction. Additionally, the elevated bilirubin levels may be linked to reduced hepatic clearance and liver dysfunction (Ettinger & Feldman, 2010). Serum TP levels generally do not increase in acetaminophen toxicity and often remain within normal ranges. However, in rare cases, conditions such as hemoconcentration (e.g., dehydration caused by vomiting) or an acute-phase inflammatory response can lead to slight increases in specific protein fractions, particularly globulins. In the present case, the low ALB level, coupled with elevated TP, may suggest an increase in globulin levels. While elevated TP is not a hallmark of acetaminophen toxicity, serum biochemical parameters can provide valuable insight into concurrent hemoconcentration or inflammatory processes (Juma et al., 2015; Sidhu et al., 2021).

Ultrasonography is a key and sustainable imaging method for monitoring hepatic diseases. It is an inexpensive, real-time, and noninvasive technique for detection (Tanaka, 2020). As previously noted, the primary toxic effect of acetaminophen is hepatotoxicity, leading to damage to hepatic cells. In the acute stages of acetaminophen toxicity, liver tissue may appear less dense or more radiolucent on radiographs due to necrosis, edema, or fatty infiltration. However, these changes are often subtle and may not be as obvious as on ultrasound or computed tomography scan (Ettinger & Feldman, 2010; Tanaka, 2020). Consequently, ultrasonography is recommended, with the most significant ultrasonographic finding being perirenal fluid leakage, which is thought to be caused by anoxia induced by methemoglobin, leading to increased capillary wall permeability (Juma et al., 2015). Therefore, the abnormal ultrasonographic findings in this case were associated with elevated capillary permeability. Radiographic imaging findings in this case, including altered liver density, were consistent with the loss of visceral detail typically observed in visceral vascular emergencies (Figure 1). Also, previous reports have documented subcutaneous edema related to hypoalbuminemia (Soeters et al., 2019). In this case presentation, free fluid was observed in Morison's pouch probably due to low ALB level (Figure 2). Assessing free fluid in Morison's pouch during acetaminophen toxicity can offer valuable insights into the severity of the condition and aid in prognostic estimation.

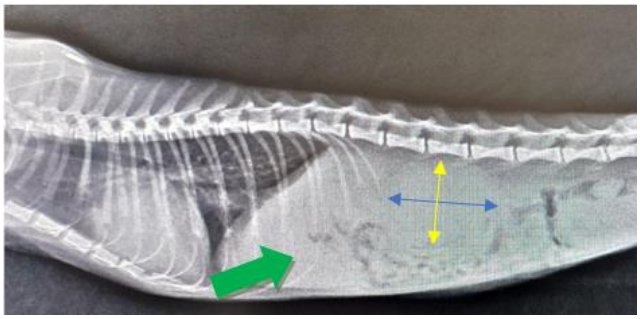

Figure 1. Loss of visceral detail (green arrow), Renomegaly (indicated by blue arrow; 6.68 cm, yellow arrow; 6.39 cm)

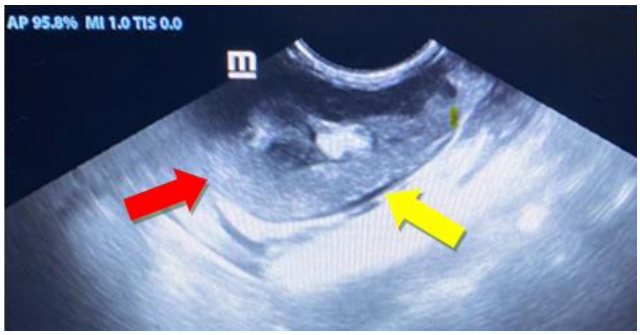

Figure 2. Free fluid in Morison's pouch (yellow arrow) and renal cortical hyperechogenity (red arrow)

Conclusion

In conclusion, the identified toxicity is commonly encountered in clinical practice. Assessing the mentioned parameters is crucial for early suspicion of toxicity and prompt initiation of

treatment protocols. Alongside CBC and serum biochemistry, imaging techniques particularly ultrasonography play a vital role in detecting complications and predicting prognosis. A thorough evaluation of anamnesis data, combined with these examination methods, can aid in accurate disease diagnosis and enhance awareness of its characteristics. Additionally, case reports like the present one can enhance awareness and help reduce fatalities associated with acetaminophen intoxication.

Financial Support

This study did not receive a support by any financial institution/sector.

Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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None.

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Surgical Treatment of Cranioschisis and Meningocele in a Newborn Calf

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Abstract
Congenital cranial diseases can be life-threatening and fatal in all animals. This case report aims to provide information about a meningocele case associated with cranioschisis observed in a 1-day-old Simmental male calf. Following clinical and radiographic examinations, the calf was diagnosed with cranioschisis and meningocele. After completing preoperative procedures, the patient was prepared for surgery. During the operation, an elliptical incision was made to remove the skin segment forming the swelling on the skull, and the cranial defect, measuring 4 cm in diameter, was closed by approximating the connective tissue in the area. No intraoperative complications were encountered. Postoperative evaluations conducted at the 2nd week and 2nd month revealed no neurological symptoms, and the calf's overall health status was good. In conclusion, this case report demonstrates that early intervention in small-scale defects and repair through tissue suturing can be successful. It also emphasizes the importance of considering surgical intervention over euthanasia in similar cases with a poor prognosis.
Keywords: Calf, cranial defect, cranioschisis, meningocele, surgery.

Yenidoğan Bir Buzağıda Kraniyoşizis ve Meningoselin Cerrahi Tedavisi

Öz
Konjenital kafatası hastalıkları tüm hayvanlarda yaşamsal riskler taşıyarak ölümle sonuçlanabilir. Bu olgu sunumu 1 günlük simental erkek buzağıda karşılaşılan kraniyoşizise bağlı meningosel olgusu hakkında bilgi verilmesi amaçlanmıştır. Yapılan klinik ve radyografik muayeneler sonucunda buzağıya kraniyoşizis ve meningosel tanısı konuldu. Preoperatif işlemlerin ardından hasta operasyon için hazırlandı. Operatif işlemde kafatası üzerindeki şişliği oluşturan deri parçası eliptik bir enzisyon ile uzaklaştırılarak 4cm çaplarındaki kraniyal defekt bölgedeki bağ dokusu daraltılarak kapatıldı. İntraoperatif herhangi bir komplikasyon ile karşılaşılmadı. Postoperatif 2. hafta ve 2. ay kontrollerinde buzağının herhangi bir nörolojik bulgu göstermeyip genel sağlık durumunun iyi olduğu görüldü. Sonuç olarak, bu olgu sunumunda küçük çaplı defektlerde erken müdahale ve defektin doku dikişleri ile sağaltımında başarılı olunabileceği ve bunun gibi prognozu kötü hastalıklarda ötenazi yerine operatif işlemin değerlendirilmesinin önemli olabileceği sonucuna varıldı.
Anahtar kelimeler: Buzağı, kranial defekt, kraniyoşizis, meningosel, cerrahi.

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Introduction

Congenital deformities, such as meningoencephalocele, meningocele, encephalocele, encephalocele, anencephaly, hydrocephalus, and spina bifida, are defined as morphological and functional disorders resulting from neural tube defects during embryonic development. These anomalies can be observed in all animal species and may be hereditary or caused by various factors such as the consumption of toxic plants, viral infections, nutritional deficiencies, mycotoxins, and pesticides (Dantas et al., 2010; Santos et al., 2012; Boscarato et al., 2020; Cutler et al., 2006). These rare anomalies have been treated with different surgical approaches in calves and other animal species (Ahmadnejad et al., 2022; Krishna & Palli, 2016; Oliveira et al., 2014; Silva et al., 2023; Alonso et al., 2019).

Cranioshisis is a malformation characterized by incomplete closure of the skull during embryological development and protrusion of the brain tissue through this opening (Cho et al., 2015). Meningocele occurs when the meninges, which contain cerebrospinal fluid (CSF), herniate through a defect in the skull (Boscarato et al., 2020). This anomaly has been reported in calves, lambs, foals, piglets, cats, dogs, and humans (Ahmadnejad et al. 2022). The lesions formed by herniated structures are mostly covered with skin and are approximately 2-10 cm in diameter. It has also been reported that the size of the hernia is always larger than that of the skull defect. (Cantile & Youssef, 2016). While meningoceles are mostly reported in the occipital and frontal regions (Cho et al., 2015), this condition has been reported differently in cattle in the parietal and frontal regions (Boscarato et al., 2020). The diagnostic process involves clinical examination and characteristic anatomopathological findings, revealing a deficiency in skull bones (Boscarato et al., 2020; Oliveira et al., 2009). Furthermore, radiography and imaging modalities, such as tomography and magnetic resonance, can be used as complementary examinations to support the diagnosis (Boscarato et al., 2020; Nogueira et al., 2017).

The aim of this case report was to provide information about the diagnosis and treatment of meningocele associated with cranioschisis observed in a calf.

Case Description

Our case involved a 1-day-old male Simmental calf weighing 45 kilograms. The calf was brought to the Harran University Animal Hospital Surgery Clinic with the complaint of a large fluid-filled swelling on the forehead. According to the anamnesis, the owner stated that the swelling was congenital; however, it did not affect the calf's nutrition or general condition, and the mass had remained the same size since birth. Clinical examination of the mass revealed fluctuating swelling following the median line from the supraorbital curve of the frontal bone to the midline of the nasal bones (Figure 1). When cranial and spinal reflexes were examined, it was determined that the swelling did not cause any neurological issues. The swelling was approximately 10x8x13.5 cm in diameter. In addition, a circular defect in the skull was detected on palpation of the root swelling.

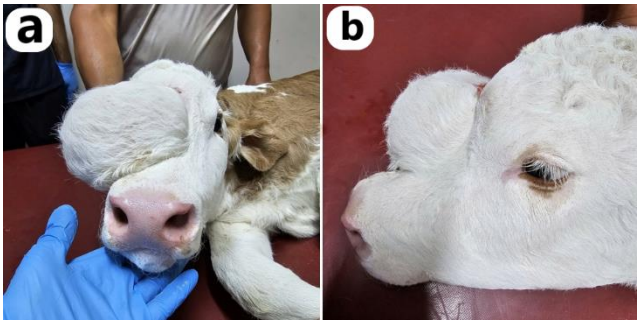

Figure 1. In a 1-day-old calf, there is an increase in volume in the frontal region (a), characterized by a large mass extending along the median line from the supraorbital curve of the frontal bone to the midline of the nasal bones (b).

The radiographic images of the calf were taken in the laterolateral and dorsoventral positions to detect the presence of any other pathological findings. In the images obtained, a round and clearly demarcated mass with soft tissue-specific radiopacity was observed in the frontal bone near the skull (Figure 2). Approximately 120 ml of colloquio serosanguine-like transparent content was drained using a puncture procedure. It was observed that the mass shrunk as the fluid drained, and only an extra piece of skin remained. The contents were sent to the laboratory for analysis, and the presence of CSF was confirmed. At the end of all procedures, it was diagnosed that the calf's skull was swollen due to meningocele caused by a congenital cranioshisis defect.

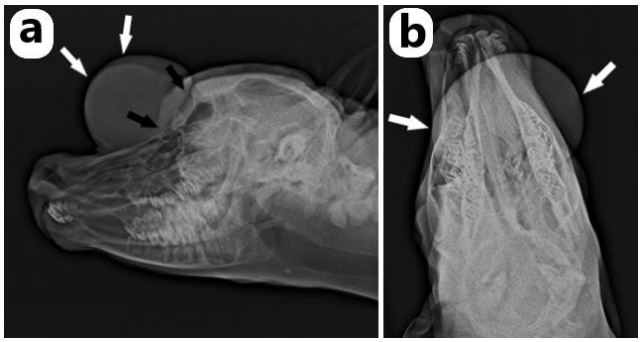

Figure 2. Lateral skull plain radiograph (a), dorsoventral radiograph (b). Showing the cranial defect (black arrows) and the hernial sac (white arrows).

Necessary information was provided to the patient owners, and the decision for the operative procedure was made. Accordingly, electrocardiogram (ECG), pulse rate (120), SpO2 (92), etCO2 (33), rectal temperature (38.7), and blood pressure (124/85) values were recorded using a mind-ray bedside monitor. Then, 3 ml of blood was collected from the vena jugularis separately for blood gas-electrolyte, hematologic, and biochemical evaluations. Blood gas electrolyte and hematochemical values were within the normal reference range. In addition, intraocular pressure (IOP) was measured using a tonovetplus (right eye IOP: 23 mmHg, left eye IOP: 25 mmHg). All values were within the reference range. Amoxicillin+clavulanic acid (140+35 mg/ml, Synulox®, Pfizer, USA) was administered intramuscularly at a dose of 8.75 mg/kg body weight for prophylaxis during the preoperative period. For analgesic effect, meloxicam (20 mg/ml, Metacam®, Boehringer Ingelheim, Germany) was administered subcutaneously at a dose of 0.5 mg/kg body weight. Both preoperatively and during the operation, fluid support was provided via physiological saline through the jugular vein. Accordingly, the calf was pre-anesthetized with midazolam (0.1 mg/kg, intravenous (IV)) (15 mg/3 ml, Zolamid®, Vem İlaç, Turkey), and propofol (1.5 mg/kg, IV) (20 mg/mL, Propofol-PF MCT %2®, Polifarma, Turkey) was administered for induction. The calf was anesthetized with 2.5-3% sevofloran (Sevones®, Polifarma, Turkey) through endetracheal intubation and continued with 100% oxygen. After preparation for anesthesia, the calf was placed in the sternal position. After the aseptic application of the frontal region and sac, the skin was opened using a linear incision. After the skin was separated, a meningeal membrane protruding from the frontal opening, with a diameter of 4 cm, was observed in the cranium. This membrane, which looked similar to the internal hernial sac, was separated from the skin by blunt dissection. The meningeal membrane was resected, leaving sufficient tissue to allow for the closure of the inferior frontal defect later. The frontal defect was closed with polyglycolic acid (PGA) No. 1 suture using resected tissue with simple separate sutures. The skin was sutured with No.1 silk using a simple suture technique according to standard procedures (Figure 3). In the postoperative period, amoxicillin+clavulanic acid (140+35 mg/ml, Synulox®, Pfizer, USA) was administered as an intramuscular injection in body weight dose (8.75 mg/kg) once daily for 5 days, and meloxicam (20 mg/ml, Metacam®, Boehringer Ingelheim, Germany) was administered subcutaneously in body weight dose (0.5 mg/kg) once daily for 3 days. The calf showed uneventful

recovery in the 2nd week after surgery and it was observed that the 2nd and 3rd week follow-ups were satisfactory (Figure 4). Two months later, the calf showed a good nutritional status and no neurological signs. Postoperative evaluations at the 2nd week and 2nd month showed that the blood gas, electrolyte, and hematochemical values were within the normal reference range.

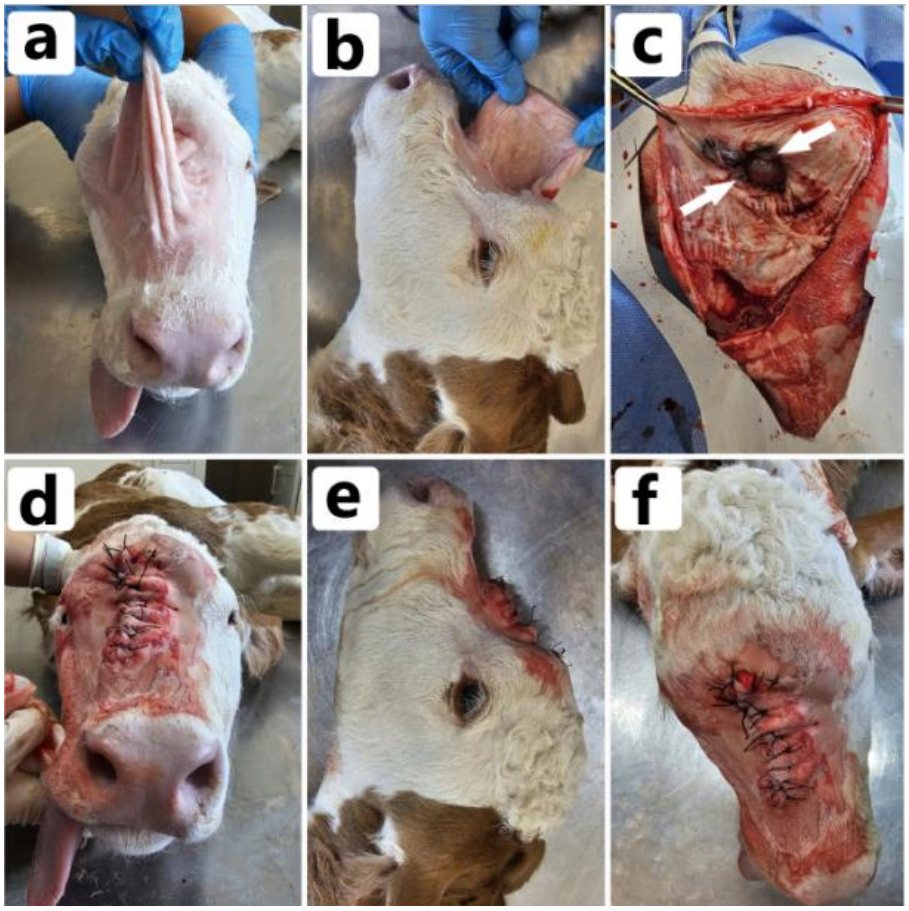

Figure 3. Preparation of the area for surgery after drainage of the sac (a. b), a circular defect (white arrows) in the frontal bone (c), postoperative images of the suture area (d. e. c.).

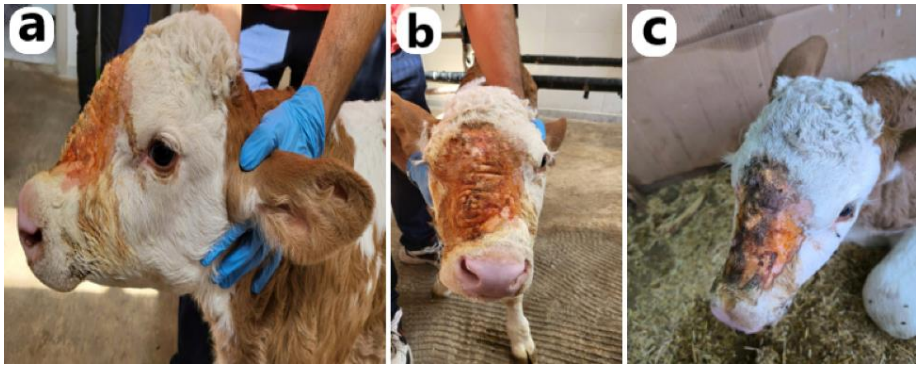

Figure 4. Control images of the postoperative process for the 2nd (a. b) and 3rd weeks (c).

Discussion

Congenital anomalies of the skull have been reported in different species using various surgical procedures. However, in many cases, it has been reported to result in the death of the animal within a short period of time (Alonso et al., 2019; Oliveira et al., 2014). In the present case report, surgical intervention for meningocele due to congenital cranioschisis in a calf yielded successful results. No complications were observed during follow-up. It has been reported that the use of ultrasound, X-ray and computed tomography for the diagnosis of meningoencephalocele and meningocele, which are congenital anomalies observed in the skull, is useful for the definitive diagnosis of the lesion (Back et al, 1991; Kohli & Naddaf, 1998; Ohba et al., 2008). In this case report, X-rays were used to detail the diagnosis of the lesion, and the observation of the defect in the frontal region and the radiopaque bulge arising from this defect were useful in finalizing the diagnosis.

In studies involving meningocele cases, it was reported that fluctuating swelling filled with CSF was drained before the surgical procedure in some studies, and in some studies, the procedures were performed without drainage. Additionally, the studies do not report any complications caused by fluid-filled masses during surgical procedures. (Boscarato et al., 2020; Krishna & Palli, 2016). In the present case, the operative procedure was performed by draining the CSF via puncture, and no complications were encountered.

In many studies on meningoceles, it has been reported that animals that were healthy in the preoperative period died in a short time due to neurological and septic complications in postoperative follow-up, and were even euthanized in some cases. For example, one study reported that in a case of meningocele observed in a foal, the animal was euthanized on the 13th postoperative day owing to neurological complications (Alonso et al., 2019). In another case study, it was reported that the blood values of the lamb deteriorated significantly in the postoperative period, and the animal showed a general disorder and died on the 5th postoperative day (Ahmadnejad et al., 2022). In the present case report, all clinical and laboratory calf values were within the preoperative reference range. In the postoperative follow-up for 2 months, no complications were found in the calf, and the patient continued to live in a healthy manner. In light of these results, regular postoperative follow-up in cases with meningoencephalocele may prevent complications that may occur in the animal and increase the chance of survival. A calf with meningoencephalocele has been reported to have a circular defect with a diameter of 6 cm in the upper part of the median frontal line of the skull, and successful results were obtained without complications from skin suturing (Ohba et al., 2008). In another study, the aim was to create a new bone matrix using a cartilage graft to repair the defect in the parietal bone; however, apathy and seizures were reported to begin on the 13th day, ultimately resulting in death on the 15th day (Oliveira et al., 2014). In another case, cranioplasty using a polymethylmethacrylate plate was applied to a 5 cm diameter frontal opening in the treatment of meningocele-related cranioschisis, and satisfactory results were obtained 19 months after surgery (Silva et al., 2023). In another study, although the prognosis of the animal was not good, a 4.5 cm diameter frontal opening was surgically closed by suturing the edges of the meninges using a simple continuous suture method. This study did not yield the expected results, and resulted in euthanasia (Boscarato et al. 2020). From this study, it can be concluded that the general condition of animals with these anomalies is important to obtain favorable results in the postoperative period. A calf with a 7 cm diameter frontal defect closed without the use of a graft was

discharged 39 days later in a healthy condition without recurrence of deformity or CSF accumulation. These findings show that surgical treatment is effective and its use is recommended in similar cases (Nogueira et al., 2017). In our study, no material was used for the repair of a 4 cm diameter defect; the closure was achieved by suturing the meningeal membrane directly to the subcutaneous tissues. The sutures were removed in the 2nd week of the postoperative period, and no abnormalities were detected in the general examination. The nutritional status of the animals was quite good, and no neurological symptoms were observed. Similar findings were observed in controls performed two months later. In conclusion, closure of the defect with the surrounding tissues showed uncomplicated and short-term healing. The method of closure plays a significant role in prognosis, but the general health condition of the animal also greatly influences the healing process. Simple suture techniques usually provide positive results for smaller defects, while complex techniques involving grafts or synthetic materials may not always be successful, especially in animals with compromised health. This highlights the critical importance of carefully assessing and stabilizing the animal's overall condition before surgery to improve the chances of recovery.

Conclusion

Despite the unfavorable prognosis of the disease current literature includes cases with good prognosis following surgical intervention. The preference for surgical intervention in the treatment of meningocele-associated cranioschisis has provided satisfactory results in terms of calf's quality of life and livestock sustainability. The cause of meningocele and cranioschisis in calves is not fully understood and further research is needed.

Financial Support

This study did not receive a grant by any financial institution/sector.

Ethical Statement

This study does not present any ethical concerns.

Author Contributions

Investigation: K.Y. and M.S.K.; Material and Methodology: K.Y. and K.D.İ.; Supervision: M.S.K. and M.S.H.; Visualization: K.D.İ.; Writing-Original Draft: K.Y. and M.H.; Writing-review & Editing: K.Y.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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