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RESEARCH ARTICLE

Clinical, Biochemical, Radiographic and Thermographic Evaluation of Extremity Fractures in Calves

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ABSTRACT

This study aim to assess extremity fractures in calves aged 0-6 months by clinical, thermographic, and radiographic methods. A total of 26 patients were assessed clinically, thermographically, and radiographically. Thermographic assessments were conducted using a thermography apparatus. Radiographic evaluations of fracture cases were conducted, and the fractures were categorized. Serum calcium levels in the control and fracture groups were 10.60±0.25 and 11.67±0.23 mg.dl-1, respectively, with the increase in fractures being statistically significant ($p<0.05$). The TNF-α measurement levels were recorded as 0.11±0.01 and 0.15±0.05 pg.ml-1 in the respective groups, with the increase in fractures being statistically significant ($p<0.05$). The IL-1β measurement levels were recorded as 18.67±4.71 and 30.69±7.53 pg.ml-1, respectively, with the increase in fractures being statistically significant ($p<0.05$). The IL-6 measurement levels were recorded as 61.79±5.52 and 98.29±31.85 pg.ml-1, respectively, with the increase in fractures being statistically significant ($p<0.05$). Cortisol measurement values were established at 3.36±0.54 and 4.93±0.97 mcg.dl-1, with a statistically significant increase in fracture cases ($p<0.05$). A thermographic assessment of fracture cases revealed an elevation of 4.14±2.2 °C along the fracture line. Fractures resulting from dystocia and trauma in calves are significant among calf surgical conditions. It was determined that thermography may serve as a diagnostic tool in fracture cases, and further comprehensive investigations are required for its application in the postoperative period.

Keywords: Calf, Fracture, Radiography, Thermography

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Buzağılarda Ekstremite Kırıklarının Klinik, Biyokimyasal, Radyografik ve Termografik Olarak Değerlendirilmesi

ÖZ

Bu çalışmada 0-6 aylık yaş aralığındaki buzağılarda ekstremite kırıklarının klinik, biyokimyasal, termografik ve radyografik olarak değerlendirilmesi amaçlanmıştır. Toplam 26 olgu klinik, termografik ve radyografik olarak değerlendirilmiştir. Termografi cihazı ile termografik incelemeler gerçekleştirilmiş ve kırık olgularının radyografik incelemeleri yapılarak kırıklar sınıflandırılmıştır. Serum kalsiyum ölçüm değerleri kontrol ve kırık gruplarında sırasıyla; 10.60±0.25, 11.67±0.23 mg.dl-1 olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0.05$). TNF-α ölçüm değerleri gruplarda sırasıyla 0.11±0.01, 0.15±0.05 pg/ml olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0.05$). IL-1β ölçüm değerleri sırasıyla 18,67±4,71, 30,69±7,53 pg.ml-1 olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0,05$). IL-6 ölçüm değerleri sırasıyla 61,79±5,52, 98,29±31,85 pg.ml-1 olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0,05$). Kortizol ölçüm değerleri sırasıyla; 3,36±0,54, 4,93±0,97 mcg.dl-1 olarak belirlenmiş ve kırık olgularındaki artış istatistiksel olarak anlamlı bulunmuştur ($p<0,05$). Kırık olgularının termografik incelemesinde kırık hattında 4,14±2,2 ℃'lik istatistiksel olarak anlamlı artış kaydedilmiştir. Buzağılarda distosi ve travmaya bağlı gelişen kırıklar, buzağı cerrahi hastalıkları arasında önemli bir yere sahiptir. Termografinin kırık olgularında da tanı yöntemi olarak kullanılabileceği ve termografik muayenenin cerrahi sağaltımı izleyen dönemde kullanımına ilişkin daha ayrıntılı çalışmalara ihtiyaç olduğu sonucuna varılmıştır.

Anahtar kelimeler: Buzağı, Kırık, Radyografi, Termografi.

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INTRODUCTION

Improper manipulation or the application of manual and mechanical forces by owners and occasionally veterinarians during delivery can result in traumatic injuries to muscles, bones, joints, nerves, and other soft tissues, frequently leading to fetal demise during delivery or the postpartum period (Aksoy et al., 2009). Fractures of the extremities are commonly observed in calves (Aksoy et al., 2009; Belge et al., 2016). Fractures in calves commonly occur in the femur (14%), metacarpus (50%), metatarsus (50%) (Bilgili et al., 2008; Nichols et al., 2010; Rodrigues et al., 2012), radius-ulna (7%), and infrequently in the vertebra (7%). Fractures of the metacarpus occur with double the frequency of metatarsal fractures (Arıcan et al., 2014). X-ray offers essential insights for diagnosis, treatment, and prognosis of vital information. It induces several positions, particularly from lateromedial and caudo-cranial orientations (Ewoldt et al, 2003). Fractures of the metacarpals in calves predominantly occur at the distal epiphysis and metaphysis. Salter-Harris Type I fractures frequently occur in the distal epiphysis and metaphysis of cattle. The cortex considerably narrows at the transition from diaphysis to metaphysis, resulting in restricted axial strength in this region of the metacarpus (Belge et al, 2016). The management of fractures in calves holds significant relevance in veterinary orthopedics (Durmuş et al, 2009). The genetic and economic value of the animal, its weight, the type and location of the fracture, and the veterinarian's experience are crucial factors in the treatment of fractures in farm animals (Arıcan et al, 2024; ElShafaey et al, 2014).
This study aim to assess extremities fractures in calves aged 0-6 months by clinical, thermographic, and radiographic methods.

MATERIALS and METHODS

This research was performed in accordance with the Afyon Kocatepe University Experimental Animals Local Ethics Committee (No: 88-19, protocol approval dated 17.09.2020).

Material

The study involved 26 calves aged 0-6 months, presented to the Afyon Kocatepe University Veterinary Faculty Surgery Department Large Animal Clinic from 2019 to 2020 with lameness complaints, subsequently diagnosed with fractures through clinical and radiological assessments. The control group comprised 7 healthy calves of the same age range. A complete blood count was conducted using a hemogram instrument (Human, Humancount-80, Wiesbaden, Germany). A centrifuge apparatus (Nüve, Nüve 300, Turkey) was employed to isolate the serum from the blood specimens. Analyses of serum biochemical parameters were conducted using a biochemistry instrument (Human Humastar-180, Wiesbaden, Germany). Radiographic assessments were conducted using an X-ray apparatus (ATS CMP 200 DR 1000 mA). A/P and M/L images were acquired using a CR device (Fujifilm CR IR 392, Turkey). Thermographic assessments were conducted on instances with suspected fractures using a thermography instrument (LW-EAA-STC-IOS SEEK). ELISA test kits were conducted using an ELISA reading instrument (Biotek Instruments, MWGt Lambda Scan 200, Winooski, VT, USA). The biochemical investigation utilized test kits from the YLBiont brand (Shanghai YL Biotech Co. Ltd., No. 5588, Caoan Road, Jiading District, Shanghai, China).

Method

Initially, clinical tests were conducted on calves with fractures of the metacarpus, metatarsus, femur, antebrachium, and tibia included in the thesis study. Thermographic assessments were conducted on suspected fracture cases using a thermography device (LW-EAA-STC-IOS SEEK Thermal Camera - IOS); alterations in the color scale and the resulting findings were documented, and the acquired images were archived. Radiographic examinations were conducted using the ATS CMP 200 DR 1000 mA X-ray equipment, obtaining A/P and M/L pictures, which were analyzed on the Fujifilm CR IR 392 CR device, and fracture cases were assessed. Incoming fracture patients were categorized and assessed. Based on the acquired findings, a diagnosis was established, and the corresponding treatment procedure was implemented accordingly. Hematological and biochemical analyses were conducted using blood samples obtained from the jugular veins of the calves.

Biochemical Measurements

Routine Biochemical Analyses

Blood collected in gel tubes devoid of anticoagulant was centrifuged at 5000 rpm for 3 minutes (Nüve, Nüve 300, Turkey), and the resultant serums were aliquoted and preserved at -20 °C until analysis. The serum samples were analyzed for creatinine, potassium, total protein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, calcium, urea, magnesium, gamma-glutamyl transferase, and glucose parameters.

Assessment of Serum Concentrations of TNF-α, IL-1β, IL-6, Cortisol, TSH, T4, MDA (Malondialdehyde), and AOA (Antioxidant Activity) in Blood Serum

The following parameters were examined in these tests: serum TNF-Alpha (Bovine Tumor Necrosis Factor, TNF ELISA Kit, Catalog No: E0133b, BO), serum Interleukin 1β (IL-1β) (Bovine Interleukin-1IL-1β, ELISA Kit, Catalog No: YLA0051 BO), serum

Interleukin 6 (IL-6) (Bovine Interleukin-6 IL-6, ELISA Kit, Catalog No: E0079b, BO), serum Cortisol (Bovine cortisol ELISA kit, Catalog No: YLA0060BO), serum T4 (Bovine Thyroxine ELISA kit, Catalog No: YLA0079BO), and serum TSH (Bovine Thyroid Stimulating Hormone ELISA kit, Catalog No: YLA0132BO) receptor antagonist. Furthermore, malondialdehyde (MDA) and antioxidant activity (AOA) levels were analyzed in blood serum samples.

Assessment of MDA (Malondialdehyde) and AOA (Antioxidant Activity) Levels in Serum

The MDA level in samples was assessed using a twofold boiling method, which is a version of the Drapper and Hadley (1990) technique. During the initial boiling, MDA associated with the materials is liberated from the proteins, resulting in protein precipitation. In the subsequent boiling, the total MDA interacts with TBA, and the absorbance of the resulting colored complex is quantified at 532 nm. The concentration is determined using the molar absorption coefficient of MDA.

The usual Fe-EDTA complex solution reacts with hydrogen peroxide via the Fenton reaction, facilitating the generation of hydroxyl radicals. Reactive oxygen radicals breakdown benzoate, leading to the release of TBARS. The incorporation of antioxidants into human fluids results in the inhibition of TBARS formation. The reaction is quantified colorimetrically, with the inhibition of color development assessed as AOA (Koracevic et al., 2001).

Statistical Analysis

The findings derived from the research were analyzed utilizing One-Way ANOVA and the Student's T-test for paired groups within the SPSS 16.0 statistical software. The Duncan test was utilized for the results exhibiting statistical differences, and the data were presented as "Mean ± standard deviation." A P value of less than 0.05 was deemed statistically significant**.**

RESULTS

This study involved clinical, thermographic, and radiographic assessments of 18 metacarpal fractures, 3 femoral fractures, 3 metatarsal fractures, 1 antebrachial fracture, 1 tibial fracture, and 7 healthy cases in calves aged 0-6 years, presented at the surgical big animal clinic from 2019 to 2020.

The clinical examination results, comprehensive blood and biochemical analysis findings, radiographic examination results, and thermographic examination findings for 18 metacarpal, 3 femoral, 3 metatarsal, 1 antebrachial, and 1 tibial fractures are presented below.

Results of Clinical Examination

69.24% of the fractures involved the metacarpus, 11.53% the femur, 11.53% the metatarsus, 3.85% the tibia, and 3.85% the antebrachium. It was established that 84.62% of the fracture cases were attributable to dystocia, whereas 15.38% were due to various injuries occurring in the postnatal period. 61.54% of the fracture cases were male calves, while 38.46% involved female calves. It was established that 26.92% of the fracture cases were left-sided, 65.38% right-sided, and 7.7% bilateral. The average age of the fracture cases was established as 7.61±2.12 days, and the average weight was 45.38±3.53 kg. The average pulse rate for fracture cases is 123.07 ± 8.48 beats per minute, and the average respiratory rate is 51 ± 5.65 breaths per minute. In fracture cases, 76.92% of the mucosa is normal, 15.39% is pale, and 7.69% is hyperemic. In the assessment of lymph nodes in fracture cases, 88.46% were normal, 7.69% exhibited minor swelling, and 3.84% were swollen. In fracture instances, 76.92% exhibited normal hunger, while 23.04% experienced diminished appetite.

Results of Complete Blood Count

This study conducted statistical analyses of complete blood results from blood samples obtained from the jugular vein of both the control and fracture groups. Upon comparison of the control group with the fracture group, LYM (%), GRA (%, 109.l-1), Hb, RBC, MCV, and HCT values were statistically significant ($p<0.05$), although WBC, MID (%, 109.l-1), LYM (109.l-1), and PLT results were not significant ($p>0.05$) (Table 1).

Results of Serum Biochemistry Measurements

This study conducted statistical analysis of complete blood results from blood samples collected from the jugular vein of both the control and fracture groups. The comparison of the control group with the fracture group revealed statistically significant differences in CRE, TP, AST, Ca++, and GGT values ($p<0.05$), although K+, ALT, ALP, URE, Mg++, and GLU results were not statistically significant ($p>0.05$) (Table 2).

Biochemical tests revealed statistically significant differences ($p<0.05$) in the values of TNF-α (pg.ml-1), IL-1β (pg.ml-1), IL-6 (pg.ml-1), Cortisol (mcg.dl-1), MDA (µmol.l-1), and AOA (µmol.l-1) when comparing the control group to the fracture group. Table 3.

Table 1: Complete blood count results in the control and fracture groups

Group Parameters	Control	Fracture	Reference Range (Roadknight et al, 2021)	P
WBC (10^9.l-1)	9.88	12.86	4.0-21.2	0.255
LYM (%)	78.08	44.91	45-75	**0.000**
MID (%)	4.94	2.95	2.0-7.0	0.127
GRA (%)	17.00	52.11	15-65	**0.000**
LYM (10^9.l-1)	8.03	5.01	0.1-10.3	0.127
MID (10^9.l-1)	0.49	0.40	0.0-2.3	0.651
GRA (10^9.l-1)	1.36	7.44	0.6-6.70	**0.000**
Hb (g.dl-1)	10.61	8.76	6.4-15.1	**0.014**
RBC (10^{12}.l-1)	13.78	7.24	6.8-14.6	**0.0604**
MCV (fl)	30.31	35.53	26.5-44.5	**0.002**
HCT (%)	44.19	25.50	19-47	**0.026**
PLT (10^9.l-1)	264.71	277.00	161-1313	0.713

(WBC: White blood cells, LYM%: Lymphocyte percentage, MID%: Monocyte, GRA%: Granulocyte, LYM: Lymphocyte count, MID: Monocyte count, GRA: Granulocyte count, Hb: Hemoglobin, RBC: Red blood cells, MCV: Mean Erythrocyte Volume, HCT: Hematocrit percentage, PLT: Platelet count)

Table 2: Serum biochemistry results in the control and fracture groups

Group Parameters	Control	Fracture	Reference Range (Roadknight et al, 2021)	P
CRE (mg.dl-1)	1.01	2.02	0.5-2.2	**0.010**
K^+ (mEq.l-1)	4.81	5.12	4.2-7.2	0.132
TP (g.dl-1)	6.53	5.64	4.4-8.4	**0.018**
AST (U.l-1)	69.27	116.69	60-125	**0.034**
ALT (U.l-1)	23.70	22.65	6.8- 22.3	0.848
ALP (IU.ml-1)	257.85	373.50	123-738	0.183
Ca^{++} (mg.dl-1)	10.60	11.67	8-11.7	**0.006**
URE (mg.dl-1)	21.77	28.41	10-25	0.143
Mg^{++} (g.dl-1)	2.26	2.34	1.5-2.9	0.596
GGT (U.l-1)	29.42	659.53	26-1379	**0.000**
GLU (mmol.l-1)	89.41	90.85	40-100	0.822

(CRE: Creatinine, K+: Potassium, TP: Total protein, AST: Aspartate Aminotransferase test, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, Ca++: Calcium, URE: Urea, Mg++: Magnesium, GGT: Gamma Glutamine Transtera, GLU: Glucose)

Table 3: ELISA results in the control and fracture groups

Parametres	Control	Fracture	P
TNF-α **(**pg.ml-1)	0.11	0.15	**0.002**
IL-1β **(**pg.ml-1)	18.67	30.69	**0.000**
IL-6 **(**pg.ml-1)	61. 79	98.29	**0.000**
Cortizol (mcg.dl-1)	3.36	4.93	**0.000**
MDA (µmol.l-1)	3.77	5.00	**0.000**
AOA (µmol.l-1)	6.80	4.95	**0.000**

(TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1 beta, IL-6: Interleukin-6, MDA: Malondialdehyde, AOA: Antioxidant activity)

Findings from Radiographic Examination

Based on the relationship between the fracture ends and the external environment, 88.47% of the cases are classified as closed fractures, whereas 11.53% are categorized as open fractures. Based on the quantity of fracture fragments, 73.08% are classified as two-fragmented, whereas 26.92% are categorized as comminuted fractures. 42.3% of fracture cases are classified as displaced fractures, whereas 57.7% are categorized as non-displaced fractures.

Based on the anatomical position of the fracture instances, 96.15% are diaphyseal, while 3.85% are epiphyseal. Diaphyseal fractures occur in 3.85% of the proximal one-third and 96.15% in the distal one-third. Based on the fracture line, 52% of diaphyseal fractures are transverse, whereas 48% are short oblique fractures. 69.23% of transversal fractures and 11.66% of short oblique fractures exhibited displacement (Table 4).

Results of Thermographic Examination

Thermographic and body temperature assessments of the animals in both the control and fracture groups were conducted. The thermographic assessments conducted in the fracture region of the fracture group yielded an average temperature of 30.85±4.33 °C, while the average body temperature was 37.92±0.83 °C. The comparison of thermographic measures with body temperature yielded statistically significant results ($p<0.05$). The control group had an average temperature of 26.71±2.13 °C, while the average body temperature was recorded at 38.47±0.44 °C. The comparison of thermographic measures with body temperature yielded statistically significant results ($p<0.05$) (Table 5).

In the fracture group, the mean temperature was 30.85±4.33 °C in the thermographic assessments conducted in the fracture region, while in the control group, the mean temperature was 26.71±2.13 °C. The thermographic temperature disparity between the fracture group and the control group was shown to be statistically significant ($p<0.05$) (Table 5).

Table 4: Radiographic findings of the fracture group

Case	Bone	Side	Localization
C1	Tibia	Right	Distal 1/3 transversal displaced
C2	Metacarpus	Bilateral	Distal 1/3 short oblique
C3	Metacarpus	Right	Distal 1/3 short oblique
C4	Metatarsus	Left	Distal 1/3 short oblique
C5	Metacarpus	Right	Salter Harris Tip II
C6	Femur	Right	Distal 1/3 transversal displaced
C7	Metacarpus	Right	Distal 1/3 kısa oblik deplase fragmentary
C8	Metatarsus	Left	Proksimal 1/3 short oblique displaced
C9	Metacarpus	Left	Distal 1/3 transversal
C10	Metacarpus	Right	Distal 1/3 short oblique
C11	Metacarpus	Left	Distal 1/3 short oblique
C12	Metacarpus	Right	Distal 1/3 short oblique fragmentary
C13	Metatarsus	Right	Distal 1/3 transversal fragmentary
C14	Antebrachium	Right	Distal 1/3 transversal displaced
C15	Metacarpus	Right	Distal 1/3 short oblique
C16	Metacarpus	Bilateral	Distal 1/3 transversal displaced
C17	Metacarpus	Right	Distal 1/3 short oblique
C18	Femur	Left	Distal 1/3 transversal displaced
C19	Femur	Right	Distal 1/3 transversal displaced
C20	Metacarpus	Right	Distal 1/3 transversal
C21	Metacarpus	Left	Distal 1/3 short oblique
C22	Metacarpus	Right	Distal 1/3 transversal
C23	Metacarpus	Right	Distal 1/3 transversal displaced
C24	Metacarpus	Right	Distal 1/3 short oblique
C25	Metacarpus	Right	Distal 1/3 transversal displaced
C26	Metacarpus	Left	Distal 1/3 transversal displaced

Table 5: Thermography and Body Temperature Averages

Group	Thermography	Body Temperature	p
Fracture(°C)	30.85	37.92	0.000
Control (°C)	26.71	38.47	0.000
p	0.024	0.112	

DISCUSSION

Görgül et al. (2004) indicated that 93.5% of fracture cases in calves were Holstein and 6.5% were Simmental, however Gangl et al. (2006) showed that 90.91% were Belgian Blue/White, 6.06% were dairy, 2.02% were Blonde d'Aquitaine, and 1.01% were Charolais. Yanmaz et al. (2014) determined that 52.3% of fractures occurred in crossbreds, 20% in Brown Swiss, 18.5% in East Anatolian Red (EAR), 4.6% in Holstein, and 4.6% in Simmental. This study found that 73.08% of the fracture instances involved Simmental cattle, while 26.92% involved Holstein cattle. This rate is attributed to the increased feeding of Simmental breed livestock in the Afyonkarahisar region.

Gangl et al. (2006) reported that 58.59% of the fracture cases in calves were in the change and 41.41% were in males. However, Arıcan et al. (2024) reported that 80% of the fracture cases were in females and 20% were in female calves. In addition, Alam et al. (2014) reported that 66.67% of the fracture cases in calves were in males and 33.33% were in females.Akın (2017) found that 65.62% were male, attributing the increased fracture susceptibility in male calves to dystocia resulting from their greater birth weight compared to females. This study found that, consistent with existing literature, 61.54% of fracture cases were male and 38.46% were female.

Aksoy et al. (2009) indicated that the ages of calves with limb fractures varied from 1 to 30 days, with the majority being 1 to 3 days old. Feist et al. (2019) observed that 67.5% of extremities fractures in calves occurred in individuals younger than two weeks, with 60.8% occurring during parturition or postnatal handling. This study found that fractures occurred in calves with an average age of 7.61±2.12 days. The results are consistent with the existing literature.

Fractures during birth predominantly occur in the metacarpal/metatarsal regions, as birth aids are typically utilized in these areas (Aksoy et al, 2009; Yanmaz et al, 2014). Numerous researches have indicated that improper or excessive traction during challenging deliveries predominantly results in traumatic injuries to the muscles, bones, joints, nerves, and other soft tissues in the metacarpus and metatarsus (Akın, 2017). Arıcan et al. (2014) highlighted the etiology of fracture cases in their study, indicating that 32.5% of calf fractures resulted from excessive force and improper help during parturition, whilst the remaining 67.4% were attributed to trauma and inadequate treatment. Yanmaz et al. (2014) indicated that 20 fracture cases (30.8%) resulted from disproportionate and inadvertent force applications during delivery, whereas 45 cases (69.2%) were attributed to traumatic incidents such as slipping, falling, and impact. In the fracture cases included in this study, 84.62% were attributed to dystocia, while 15.38% resulted from various traumas during the postpartum period. This outcome aligns with the conclusions of Arıcan et al. (2014) and Yanmaz et al. (2014).

The inflammatory phase of normal bone healing occurs within the initial 24-48 hours. It persists until the development of new blood vessels, granulation tissue, and cartilage. Currently, the fracture site is predominantly infiltrated by polymorphonuclear leukocytes, macrophages, and mast cells (Paskalev, 2009). This study detected an elevation in WBC numbers in the fracture group (12.86±1.69 10^9.l^-1) compared to the control group (9.88±1.87 10^9.l^-1), although this difference was not statistically significant ($p>0.05$).

The elevation in creatinine levels may result from muscular injury and physical exertion. Reductions in creatinine levels may result from diminished creatine synthesis in the liver and a sluggish metabolism in muscle tissue. Nikolaevna and Uygunovich (2021) documented in their study involving rabbits with experimental fractures that serum creatinine levels increased arithmetically on the 7th day relative to the 0th day and subsequently decreased by the 90th day, attributing this variation to the equilibrium between creatinine synthesis and metabolism. In this investigation, the creatinine level of the control group animals was measured at 1.01±0.08 mg.dl-1, which was found to be comparable to the findings of Rassel et al. (2021) in calves (1.303±0.0009). The creatinine level in fracture cases was significantly elevated compared to the control group (2.02±0.35 mg.dl-1) with statistical significance ($p<0.05$). The elevation of creatinine levels in the fracture group is believed to stem from heightened protein catabolism due to soft tissue injury.

The activity of transaminases is utilized to assess the extent of impairment in the activities of the liver, heart, and skeletal muscles. The elevation of these enzymes in blood serum correlates with their release from injured organs and tissues (Nikolaevna and Uygunovich, 2021).

Serum AST levels elevate in cases of skeletal muscle injury. Pearson et al. (2019) assessed AST levels in calves subjected to trauma from dystocia and found an elevation in blood AST levels. This study found AST levels to be 116.69±20.34 U.l-1 in fracture patients and 69.27±6.40 U.l-1 in the control group, with this increase being statistically significant ($p<0.05$). The elevated serum AST levels in calves with fractures, in comparison to the control group, may result from damage to adjacent soft tissues, and this finding aligns with existing research.

Glutamyl transferase (GGT) serves as a marker for colostrum consumption in calves (Wolf et al, 2021). GGT levels have been seen to rise in cases of hepatocellular and biliary tract damage (Clark et al, 1987). In this investigation, serum GGT levels were measured at 29.42±11.98 U.l-1 in the control group and 659.53±151.76 U.L-1 in the fracture group, both falling within reference ranges. Despite the GGT value

being statistically considerably elevated in the fracture group ($p<0.05$), it lacks clinical significance.

The concentration of Serum Total Protein (TP) in neonatal calves is approximately 4-7 g/dl (Wood and Quiroz-Rocha, 2010). In their investigation on calves with femur fractures, Bellon and Mulon (2011) reported an average blood total protein level of 5.57 g.dl-1. Steiner et al. (1996) assessed the total protein level in a calf with a metacarpal fracture and reported that it fell within the reference range. In this investigation, serum total protein concentrations were measured at 6.53±0.26 g.dl-1 in the control group and 5.64±0.19 g.dl-1 in fracture cases, both falling within the reference range. Despite a statistically significant difference in TP levels across the groups ($p<0.05$), the difference lacked clinical significance.

Antioxidant activity (AOA) denotes the overall antioxidant capacity of blood (Chirase et al., 2004). The heightened generation of reactive species may lead to a reduction in in-vivo antioxidant activity values (Aengwanich et al., 2011). Chirase et al. (2004) showed a decline in AOA levels in calves subjected to transportation stress. In this investigation, serum AOA levels were quantified at 6.80±0.64 µmol.l-1 in the control group and 4.95±0.92 µmol.l-1 in fracture cases, demonstrating statistical significance ($p<0.05$). The reduced AOA values in fracture cases relative to the control group are attributable to oxidative stress. These findings align with the existing research.

Sheweita and Khoshhal (2007) indicated that ALP levels rose subsequent to fracture development. This investigation revealed elevated ALP levels in the fracture group (373.50±76.38 IU.ml-1) relative to the control group (257.85±37.20 IU.ml-1); however, the difference was not statistically significant ($p>0.05$). Conversely, the ALP level in the fracture group rose by almost 50% compared to the control group. This corroborates the literature.

Bone tissue markers are utilized to assess the impacts on bone production, osteoporosis, or both conditions. Calcium serves as a marker for bone loss, whereas alkaline phosphatase (ALP) indicates bone growth and osteoblastic activity (Bozukluhan et al, 2018). Calcium is essential for the mineralization of the callus, vital to the fracture healing process. As dietary calcium is insufficient to fulfill this requirement, the calcium necessary for callus mineralization is predominantly sourced from bone tissue, which serves as a calcium reservoir, to facilitate proper bone healing (Fischer et al, 2018). Chaurasia et al. (2019) revealed in their study on dogs that serum calcium levels diminished on the 15th postoperative day, attributing this decline to excessive calcium deposition at the fracture site. Furthermore, Mohuiddin et al. (2018) assessed the blood calcium levels in calves on the day of fracture (day 0) and again on the 21st day, noting that the calcium level was normal on day 0 but diminished by day 21. The reduction in serum calcium levels may be attributed to increased calcium mobilization during callus development and accelerated healing (Deka et al., 1994). This investigation determined the calcium level in the fracture group to be (11.67±0.23 mg.dl-1), which was significantly higher than the control group (10.60±0.25 mg.dl-1), with statistical significance ($p<0.05$). Despite the statistical significance of this variation, it lacks clinical relevance as the calcium measurements remain within the reference range.

The acute phase response is a multifaceted systemic defensive mechanism activated by trauma, infection, stress, neoplasia, and inflammation (Cray et al, 2009). The three proinflammatory cytokines, TNF-α, IL-1β, and IL-6, are regarded as the primary activators of the systemic inflammatory response. These cytokines serve as the primary mediators of the acute phase response. Inflammation, infection, or tissue injury prompts cytokine secretion by immune cells. Consequently, the synthesis of acute phase proteins is stimulated. TNF-α is a significant mediator of osteopenia (Sheweita and Khoshhal, 2007). TNF-α concentration reportedly peaks within the initial 24 hours post-trauma and diminishes after 72 hours (Gerstenfeld et al, 2003). TNF-α is synthesized by macrophages and other inflammatory cells, functioning as a chemotactic agent by eliciting secondary inflammatory signals. It is also recognized for inducing the development of mesenchymal stem cells (MSCs) into osteogenic cells in vitro (Marsell and Einhorn, 2011). IL-1β and IL-6 are particularly crucial for the process of fracture healing. IL-1β expression coincides with that of TNF-α in a biphasic manner. Macrophages create it during the acute phase of inflammation, and osteoblasts also stimulate IL-6 production. They facilitate the formation of primary cartilaginous callus. They promote angiogenesis, induce vascular endothelial growth factor (VEGF) production, and contribute to the development of osteoblasts and osteoclasts (Marsell and Einhorn, 2011). It is well acknowledged that physical and psychological stress elevates plasma IL-6 and acute phase protein concentrations in people and experimental animals. Evidence exists that physical stress can stimulate the production of acute phase proteins in cattle (Murata et al, 2004). Korkmaz et al. (2015) showed in their study that TNF-α, IL-1β, and IL-6 concentrations increased 15 minutes post-cauterization operation. The investigation revealed that concentrations of TNF-α, IL-1β, and IL-6 were significantly elevated in fracture patients compared to the control group ($p<0.05$). The elevation of inflammatory mediators during the acute phase of fractures aligns with existing studies.

The assessment of alterations in blood cortisol levels is typically utilized as a marker for the immediate response to stress. Korkmaz et al. (2015) indicated that the cortisol levels in calves with cauterized horns elevated. Our investigation revealed that cortisol levels in fracture cases (4.93±0.97 mcg.dl-1) were statistically substantially elevated compared to the control group (3.36±0.54 mcg.dl-1) ($p<0.05$). The elevation of serum cortisol levels correlates with the fracture occurrence

resulting from the trauma's intensity. Fractures arise following significant mechanical trauma that concurrently inflicts harm on adjacent soft tissues. To rectify this damage, both local and systemic responses are initiated, evidenced by the activation of cellular and humoral components (Keel and Trentz 2005). Numerous studies have indicated a correlation between bone healing and elevated reactive oxygen species (ROS) in the fracture site. Lipid peroxidation byproducts serve as dependable markers of oxidative stress at the fracture site, in blood serum, and in urine (Paskalev, 2009). Following a bone fracture, the generation and buildup of reactive oxygen species (ROS) at the fracture site and in the peripheral circulation arise due to the disruption of blood flow at the fracture ends. This is a localized indicator of oxidative stress that impacts the entire organism and can significantly modify the bone healing process (Paskalev, 2009). Oxidative stress has been shown in both spontaneous and artificially induced fractures in rats (Yeler et al., 2005) as well as in experimental osteotomies conducted on rats (Tasatargil et al., 2007). Trauma stimulates the hypothalamic-pituitary-adrenal axis, the primary mechanism for stress response (Pacák and Palkovits, 2001). Cytokines are chemicals released by the pituitary gland that stimulate the adrenal cortex to respond to stress (John and Buckingham, 2003). Diverse traumas can induce oxidative stress by the enhancement of lipid peroxidation and oxidation or by eliciting an inflammatory response in the tissue (Baines and Shenkin, 2002). Lipid peroxidation and plasma MDA concentrations are directly correlated with the extent of tissue damage (Prasad et al, 2003). Korkmaz et al. (2015) documented an elevation in MDA levels in calves subjected to surgery in their study. The investigation revealed that MDA levels were elevated in calves with fractures (5±0.73 μmol.l-1) compared to the control group (3.77±0.39 μmol.l-1), with statistical significance ($p<0.05$). This outcome parallels that of Korkmaz et al. (2015). Fractures of the extremities in calves comprise 21-50% metacarpal and metatarsal injuries, followed by 15-32% femoral fractures and 12-15% tibial fractures (Gangl et al, 2006). Arıcan et al. (2014) indicated that fractures in calves predominantly occurred in the metacarpal bones (60.6%), followed by the femur (14.9%), metatarsal bones (7.1%), tibia (8.8%), antebrachium (6%), and humerus (3.2%). Mohuiddin et al. (2018) documented fractures as follows: 40% metacarpal, 20% metatarsal, 20% radial, 10% humeral, and 10% femoral. This study revealed that 69.24% of fracture cases in calves involved the metacarpus, 11.53% the femur, 11.53% the metatarsus, 3.85% the tibia, and 3.85% the antebrachium. The findings were found to be consistent with the literature.

Research indicates that the anatomical distribution of fracture lines comprises 36.36% distal epiphyseal, 63.64% diaphyseal (Tulleners, 1986), 84.5% distal diaphyseal, 10.4% proximal diaphyseal, 2.2% epiphyseal, 1.6% metaphyseal, and 1.1% comminuted fractures (Arıcan et al, 2014), along with 60.86% diaphyseal, 28.98% metaphyseal, 5.80% distal epiphyseal, and 4.36% metaphyseal fractures with epiphyseal separation (Belge et al, 2016). Akın (2017) discovered that the predominant occurrence of calf extremity fractures (68.75%) takes place at the distal diaphysis in a study. This study found that 96.15% of fractures were diaphyseal, whereas 3.85% were epiphyseal, based on the anatomical placement of the fracture line. It was established that 3.85% of the diaphyseal fractures occurred in the proximal one-third, whereas 96.15% were located in the distal one-third. The findings were found to be consistent with the existing literature. Belge et al. (2016) indicated that all fracture instances were closed in their research. Tullener (1986) indicated that 69.70% of fracture cases were closed and 30.30% were open fractures in his study, but Mohuiddin et al. (2018) found that 70% were closed and 30% were open fractures. In our study, 88.47% of the fractures were classified as closed, while 11.53% were classified as open, based on the relationship of the fracture ends to the external environment. 42.3% of the fracture cases were classified as displaced fractures, whereas 57.7% were categorized as non-displaced fractures. The results are incongruent with Belge et al. (2016), although analogous to Tullener (1986) and Mohuiddin et al. (2018).

Inflammation leads to heightened circulation in the affected area, resulting in an elevation of temperature as a primary symptom. Any trauma or illness invariably induces alterations in circulation. Thermography identifies a "hot spot" linked to localized inflammation (Redaelli et al, 2014). A 1 °C discrepancy between two anatomically symmetrical regions signifies inflammation in that location. Yanmaz et al. (2007); Soroko et al. (2013); Alsaaod et al. (2015). Thermography may serve as an adjunctive diagnostic method for identifying lameness in cattle. The assessment of local temperature variation is a crucial signal for the early identification of inflammation linked to lameness in inflammatory diseases (Alsaaod and Buscher, 2012). Redaelli et al. (2009) conducted thermographic assessments in both healthy and lame cows, establishing a correlation between thermography and the diagnosis of foot ailments. Whay et al. (2004) assessed the temperature of the metatarsal joint, lateral hoof wall, mid-tarsus, and lateral capsule ungula in the abaxial direction, concluding that lesioned feet exhibited higher temperatures than healthy feet. Renn et al. (2014) indicated that temperature was elevated in lame cows by thermography and proposed that thermography could serve as an alternate diagnostic instrument for lameness assessment. Alsaaod and Buscher (2012) indicated an elevation in the surface temperature of the coronary band of the affected foot relative to the healthy contralateral foot in lame cattle. Furthermore, the researchers determined that the disparities observed between healthy and lesioned feet, as assessed by thermography, could facilitate the

identification of hoof lesions in dairy cows without necessitating a clinical foot examination.

Cockcroft et al. (2000) employed thermography to detect septic arthritis of the metatarsophalangeal joint in a two-year-old Friesian heifer. They observed elevated temperatures in all lateral, medial, plantar, and dorsal projections of the inflamed metatarsophalangeal joint compared to the healthy contralateral joint, concluding that thermography can serve as a tool for localizing the inflamed area.

Stromberg (1974) validated the efficacy of thermography in identifying pathological alterations in the superficial digital flexor tendon (SDFT) prior to the manifestation of clinical inflammatory symptoms.

Vaden et al. (1980) employed thermography to identify subclinical arthritis. Doğan et al. (2016) employed thermography to diagnose septic arthritis in calves, identifying hot patches in affected joints.

Turner (1991) documented the application of continuous temperature monitoring in the foot for diagnosing subclinical inflammation up to two weeks prior to the manifestation of clinical lameness symptoms. The quality of thermograms is influenced by exercise, perspiration, body posture and angle, body coverings, systemic and topical pharmaceuticals, regional and local anesthetic blocks, sedatives, tranquilizers, anesthetics, vasoactive agents, and skin lesions such as scars (Alsaaod et al, 2015). No pharmacological intervention was administered in this study that could influence the outcomes.

This study revealed an elevation in temperature near the fracture line and its vicinity when juxtaposed with the control group. Thermographic investigations in the fracture group revealed an average temperature of 30.85±4.33 °C, whereas the control group exhibited an average temperature of 26.71±2.13 °C. The thermographic temperature disparity between the fracture group and the control group was determined to be statistically significant ($p<0.05$). The temperature increase was determined to be associated with the inflammatory phase, the initial stage of fracture healing. The literature analysis revealed an absence of evidence concerning the application of thermography in fracture cases involving calves. Consequently, thermographic assessment may be utilized to monitor the inflammatory process in fracture situations.

CONCLUSION

Fracture cases resulting from dystocia and trauma in calves hold significant relevance among surgical disorders affecting calves. The conclusion was reached that thermography can serve as a diagnostic tool in fracture cases, and that implementing the appropriate treatment option will significantly benefit the national economy. Furthermore, more comprehensive studies are required regarding the application of thermographic examination in the postoperative period alongside fracture diagnosis.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: YK and ZKS contributed to the project idea, design and execution of the study. YK and ZKS contributed to the acquisition of data. YK and ZKS analysed the data. YK and ZKS drafted and wrote the manuscript. YK and ZKS reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study was carried out at Afyon Kocatepe University Veterinary Health Application and Research Center. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Afyon Kocatepe University (AKUHADYEK, Ref No: 109, Tarih: 18/09/2019)

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