TURKISH JOURNAL OF VETERINARY RESEARCH

Vol: 8 No:2
Year 2024
E-ISSN: 2602-3695

TJVR
http://dergipark.gov.tr/tjvr

TURKISH JOURNAL OF VETERINARY RESEARCH

E-ISSN:2602-3695

Year	Volume	Issue / Number
2024	8	2

All articles in this journal are available free of charge from
https://dergipark.org.tr/en/pub/tjvr

TJVR is published biannually

Indexing:

The Turkish Journal of Veterinary Research (TJVR) is indexed and abstracted by the following indexes and platforms (Alphabetical order); *Academic Keys, Asos Index, BASE, CABI Abstract, Crossref, EBSCO, Entire WEB, EuroPub, Genamics, Google Scholar, Ideal Online, Index Copernicus, IP Indexing, J-Gate, Journal TOCs, Open Access Library, Open AIRE, Pmi to Cite, Research BIB, Scholar Index, TRDizin, Turkiye Atıf Dizini, Udl Edge, Ulrichs Web, World Cat.*

TURKISH JOURNAL OF VETERINARY RESEARCH
E-ISSN:2602-3695

Year	Volume	Issue / Number
2024	8	2

ORIGINAL ARTICLES

REVIEW ARTICLES

Original Article https://doi.org/10.47748/tjvr.1438078

TJVR 2024; 8 (2): 89-94

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

A review of surgical cases of newborn calves

Nihan Avcı[1]* Fehmiye Gümüş Özdil[1] Başak Boztok Özgermen[1] Mehmet Sağlam[2]

[1] *Department of Surgery, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Türkiye*
[2] *Department of Surgery, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye.*

Correspondence: *Nihan Avcı (nihanavci26@gmail.com)*

Received: 16.02.2024 Accepted: 06.03.2024

ABSTRACT

Objective: This study aimed to retrospectively evaluate the diseases of calves. Surgically encountered diseases, causes, indicated treatment methods, and possible complications were evaluated and it was thought to contribute to scientific and clinical studies on this subject.

Material-Methods: The material of this study consisted of a total of 150 calves of different breeds, ages, and sexes brought to Aksaray University Faculty of Veterinary Medicine Animal Hospital Surgery Clinic between December 2017 and 2023.

Results: In terms of surgical diseases, 37.3% of 150 calves were soft tissue, 56.6% orthopedics, 2% ophthalmology, and 4% neurology cases. Congenital anomalies were found in 44.65% of 56 patients with soft tissue disease. Orthopedic examination revealed, 57.1% of the cases had fractures, 23.8% had arthritis and 19% had tendon diseases. In addition to soft tissue diseases, there were 5 ophthalmologic cases, including orbital mass (n=2) and dermoid cyst (n=3)

Conclusions: Finally, while cattle breeding has great economic importance in our country, calf losses occur due to economic conditions such as artificial insemination errors, irregular registration systems, lack of standardization of care and housing, incorrect intervention of the patient owner, and treatment costs. It is envisaged that economic losses will be minimized with early diagnosis, early intervention, appropriate medical treatment, and surgical treatment options.

Keywords: *Calf diseases, Congenital Atresia ani, Fracture, Cyst Dermoid*

INTRODUCTION

According to Turkish Statistical Institute (TurkStat) data for June 2023, the number of cattle in Türkiye was 16,688,000. This rate decreased by 2.0% compared to December of the previous year (Anonymous, 2023). This decreases, even at a low rate, has a serious negative impact on the country's economy and animal husbandry. In Türkiye, most losses in cattle breeding are caused by newborn calves. Faulty interventions cause calf losses during pregnancy examinations and delivery, inadequate care and feeding conditions, and failure to prevent contamination. These losses have a negative impact on the economy of cattle farms and consequently on the national economy due to complications encountered during treatment as well as treatment costs.

Umbilical lesions are a common problem in newborn calves. The rate of infections and complications that cause it varies between 1.3-29.9% (Wielan et al., 2016). Short-cutting or rupture of the umbilical cord after birth, lack of proper wound care, unhygienic housing conditions, and hereditary diseases are the causes of umbilical

lesions. Omphalitis, omphalophlebitis, omphaloarteritis, umbilical abscess, eventration umbilicalis, and hernia umbilicalis are among the umbilical lesions encountered in newborn calves (Yurdakul et al., 2021).

Umbilical lesions can also cause infection in the joints via a hematogenous route (Hosie, 2007). Infection carried hematogenously via the portal vein usually causes septic arthritis in the joints (Marchionatti et al., 2016; Alkan et al., 2019). Arthritis, which is characterized by pain, swelling, and temperature increase in the joint area, is a common disease resulting in lameness (Goodarzi et al., 2015; Jesse et al., 2017).

Calves need to receive colostrum in the neonatal period. Colostrum has a laxative effect in neonatal calves and facilitates meconium excretion by accelerating bowel movement (Mejer, 2015). In cases where colostrum cannot be received sufficiently, obstructions may be seen due to the inability to expel meconium. Another reason for failure to expel meconium is atresia cases. It occurs as a result of congenital complete or partial closure of the intestinal lumen or the intestinal lumen is not formed at all. Atresia can occur in the colon, rectum, and anus. This condition causes calf losses and economic losses. Atresia cases are also known to be hereditary (Belge et al., 2000; Azizi et al., 2010). In addition to these, another problem that affects both the patient owner and the country's economy is extremity fractures. As a result of faulty interventions or blunt trauma during birth, fractures of the extremities, especially the metatarsus and metacarpus, are frequently encountered (Arıcan et al., 2014; Yurdakul et al., 2021).

This study aimed to retrospectively evaluate the diseases of calves brought to Aksaray University Faculty of Veterinary Medicine Animal Hospital Surgery Clinic between 2017 and 2023. Surgically encountered diseases, causes, indicated treatment methods, and possible complications were evaluated and it was thought to contribute to scientific and clinical studies on this subject. In addition, it is aimed to be a guide for our colleagues in the development of clinical services and treatment methods by evaluating calf losses caused by care and nutrition errors.

MATERIALS and METHODS

Permission for sample collection and the conduct of the study was obtained from Aksaray University Experimental Animals Local Ethics Committee (Letter date 19.12.2023 and approval no: 2023/11/63).

The material of this study consisted of a total of 150 calves of different breeds, ages, and sexes brought to Aksaray University Faculty of Veterinary Medicine Animal Hospital Surgery Clinic between December 2017 and 2023 due to various problems. Clinical findings and treatment protocols were recorded following the detailed anamnesis obtained from the patient owners. Routine clinical examinations were performed by taking body temperature, pulse, and respiratory counts. Parenteral antibiotics (benzylpenicillin procaine 1ml/20kg IM, Reptopen®, Ceva, Türkiye) were administered conservatively for 1 week, especially in septic arthritis cases with increased body temperature.

After routine clinical examinations, those requiring surgical intervention were evaluated for suitability for anesthesia. In cases with dehydration, parenteral fluid was administered in the preoperative period. In cases of fracture and atresia, radiographic examination was also performed after clinical examination. Two-way radiographs, mediolateral (ML) and anterioposterior (AP), were taken at 80 kV, 0.2 mAs dose. An indirect radiographic examination was performed for the diagnosis of obstruction in cases of intestinal atresia. Radiopaque material (Sodium diatrizoate, Urografin® 76%, Bayer Pharma, Germany) diluted one-to-one with saline was administered through the rectum via Foley catheter or rectal catheter at a dose of 10 ml/kg. Immediately after administration of the contrast agent, the bowel was evaluated by radiography in the laterolateral (LL) position.

In all cases in which atresia recti and coli operations were decided, xylazine hydrochloride (0.2 mg/kg IM, SantaVet®, Türkiye) was administered and 0.5-1 ml 2% Lidocaine HCL (Vilcain®, Vilsan, Türkiye) was administered every 1-2 cm along the incision line in the right fossa paralumbalis (Figure 1). Epidural anesthesia was provided with lidocaine simplex in cases of atresia ani, prolapsus recti, and prolapsus coli. In cases of atresia ani, a “+” shaped incision was made in the area where the anus should be located, and the

rectum was brought to the anatomical position where it should be located and fixed by applying purse-string suture.

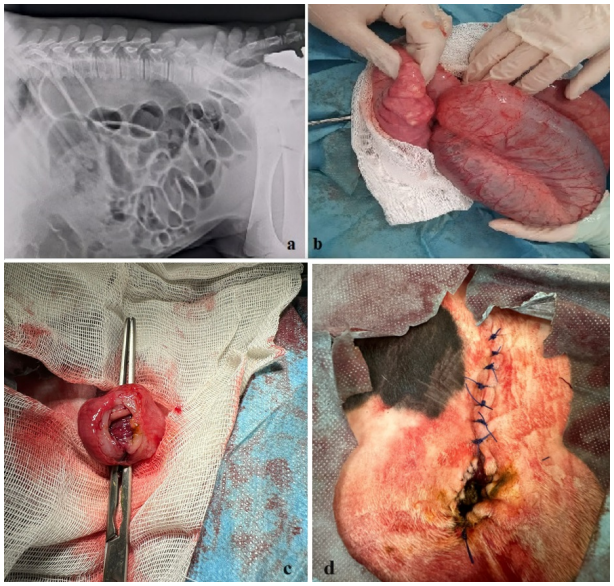

Figure 1. Congenital atresia coli. a. LL abdominal radiography, b. Operative view of the secum, c. Positioning the colon to the fossa paralumbalis region, d. Postoperative view.

Figure 2. a. Bilateral Arqure-Bouleture in a calf, b. Preoperative view, c. Image of accordion tenotomy operation, d. Postoperative view.

For orthopedic surgery, Xylazine hydrochloride (0.2 mg/kg IM, SantaVet®, Türkiye) was administered followed by Ketamine hydrochloride (0.3 ml IV, Keta-control®, Doğa İlaç, Türkiye), and general anesthesia was achieved. In cases with fractures, fracture reduction was achieved by retrograde intramedullary pin application in the related extremities. In cases where there was no indication of operation, a PVC bandage was applied. In one patient with arqure-bouleture, treatment was performed with the accordion tenotomy method. A supported bandage was applied in the other cases (Figure 2).

Local lidocaine HCl (Jetocaine Simplex®, Adeka, Türkiye) was applied to the conjunctival region, and Proparacaine HCl 0.5% (Alcaine®, Novartis, UK) was applied as corneal topical anesthesia in cyst dermoid cases. The cyst was removed with a dermoid corneal scalpel.

RESULTS

In terms of surgical diseases, 37.3% of 150 calves were soft tissue, 56.6% orthopedics, 2% ophthalmology, and 4% neurology cases (Figure 3). Congenital anomalies were found in 44.65% of 56 patients with soft tissue disease. Atresia coli (n=5), atresia ani (n=2), and atresia recti (n=3) were among the most common congenital anomalies. In addition to these, meconium ileus was detected in 6 cases. Congenital anomalies including hernia umbilicalis (n=3), anuria (n=2), agenesis of the soft palate (n=1), and eventration (n=1) were detected (Table 1) (Figure 4).

Table 1. Distribution of the soft tissue diseases.

Soft Tissue			
Acquired		**Congenital**	
Hernia abdominalis	4	Uracus fistula	2
Abomaso-umbilical fistula	1	Atresia coli	5
Prolapsus recti	3	Atresia ani	2
Prolapsus coli	1	Atresia recti	3
Omphalitis	4	Hernia umbilicalis	3
Phlegmon	1	Anuria	2
Wound	3	Soft palate agenesis	1
Soft tissue injury	12	Meconium ileus	6
Vesica urinaria rupture	1	Eventration	1
Peritonitis	1		
Total	**31**	**Total**	**25**

Orthopedic examination revealed, 57.1% of the cases had fractures, 23.8% had arthritis and 19% had tendon diseases. In the fracture cases, it was determined that the fractures were caused by the excessive application of force during the intervention of the patient owners to the birth and the incorrect application of the calf birth ropes. 19

of 47 fracture cases were metacarpal fractures (Table 2) (Figure 5).

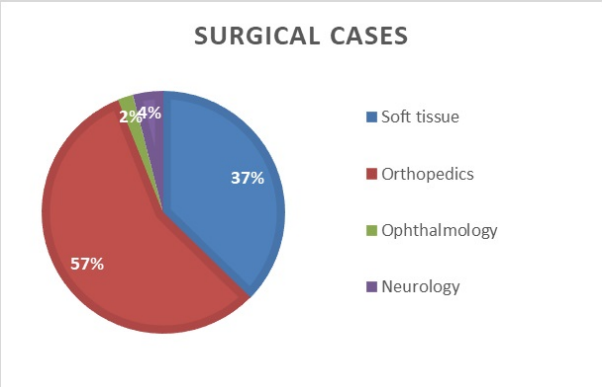

Figure 3. Schematic distribution of the diseases encountered in 150 newborn calves.

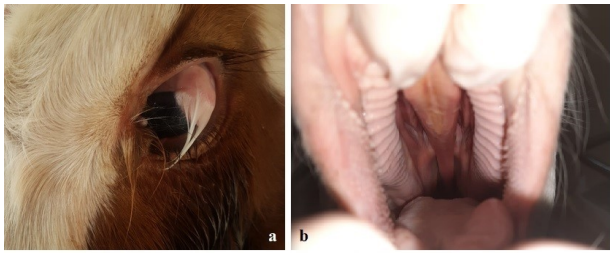

Figure 4. Image of congenital calf diseases. a. Cyst dermoid, b. Soft palate agenesis and cleft palate.

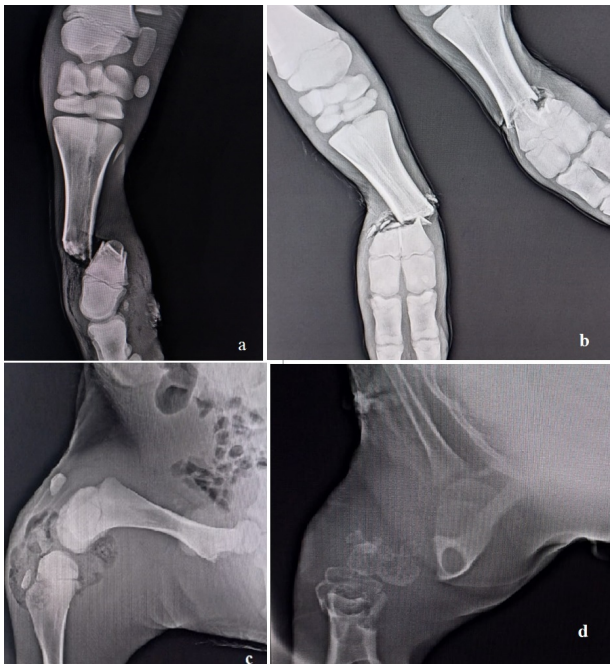

Figure 5. Radiographic appearance of fracture cases. a. ML appearance of metacarpal transversal fracture, b. Bilateral metacarpal 3 transversal fracture, c. ML appearance of septic arthritis, d. Congenital agenesis of the humerus.

In two cases with metacarpus fractures and one case with femur fracture, reduction was achieved with multiple intramedullary pins. However, during the postoperative care process, inadequate attention of the animal owner, and pin migration occurred as a complication. Improper antibiotic administration and lack of hygiene in the housing conditions caused infection in the operation area. Treatment of other fracture cases was provided with a supported bandage.

Table 2. Distribution of bone and joint diseases.

Fracture		Other	
Antebrachium	2	Arthritis	12
Metacarpus	19	Septic arthritis	6
Femur	8	Polyarthritis	2
Tibia	4	Tendinitis	1
Coxa	1	Actinomycosis	1
Metatarsus	4	Arqure	6
Madibula	1	Bouleture	5
Sacroiliac	2	Arthrogryposis	4
Humerus	3		
Other	4		
Total	**48**	**Total**	**37**

Mandibular, sacroiliac, and coxa fractures could not be treated because the owner could not afford the operation cost. In the musculoskeletal system diseases, arthritis (n=20) was the most common disease in neonatal calves. Septic arthritis (n=6) was among the most common arthritis cases. In septic arthritis cases, the pus was removed by puncture and the joint was lavaged. In addition to cases with omphalitis, polyarthritis was seen in two cases. Surgical cases due to tendon injuries were determined as arqure and bouleture (n=11). According to the anamnesis, it was learned that the cases with tendon damage were examined for pregnancy in the 6th month of the embryonic period and pregnancy was achieved by artificial insemination. It was determined that four cases having musculoskeletal diseases were due to arthrogryposis. In addition to these cases, actinomycosis was observed in one case. Among the neurologic cases, paraplegia in the hind limbs in four cases due to compression in the birth canal, and radial paralysis in two cases due to excessive force applied to the forelimb during intervention for difficult delivery were observed.

It was determined that 37.3% of the surgical cases were soft tissue-related. Among these cases, the most common cause was the inability of the animal to stand up due to soft tissue injury (n=9). This was followed by hernia abdominalis (n=4), omphalitis (n=4), wound (n=3), urinary bladder rupture (n=1), phlegmon (n=1), peritonitis (n=1) and abomaso-umbilical fistula (n=1). According to the anamnesis taken from the owner, it was concluded that the rupture and fistula were caused by the unconscious intervention of the owner. Prolapsus recti (n=3) and prolapsus coli (n=1) were determined due to neonatal calf diarrhea. In these cases, the rectum and colon were rejected, and the purse-string suture was applied.

In addition to soft tissue diseases, there were 5 ophthalmologic cases, including orbital mass (n=2) and dermoid cyst (n=3).

DISCUSSION

Umbilical lesions are among the most common diseases in newborn calves. In the studies conducted, 28.57% of diseases due to umbilical lesions were detected (Yurdakul et al., 2016; 2021). Omphalophlebitis and urachus fistula are among the most common surgical cases of umbilical infections. Kılıç et al. (2005) reported that they encountered 25.2% omphalophlebitis and 28.4% urachus fistula. Moscuzza et al. (2014) reported that omphalophlebitis was the most common case. Yurdakul et al. (2021) reported that omphalophlebitis was seen in 35% and hernia umbilicalis in 25% in their retrospective study. In our study, umbilical lesions constituted 10% of 150 cases; however, it was determined that soft tissue cases constituted 27.78% of the cases. Although umbilical lesions were determined as the most common disease in calves according to previous studies, the patients were mostly referred to our clinic for long extremity fractures. The reason for this is thought to be that the owners apply empirical treatment methods for umbilical infections and do not bring the patient for examination due to economic reasons or transportation difficulties.

The narrow pelvic canal of the mother, large litter size, or use of semen of different breeds during artificial insemination are some of the reasons that cause difficult delivery. During difficult parturition, fracture lesions are formed because of ignorant and incorrect intervention of animal owners (Nuss et al., 2011). Yurdakul et al. (2021) reported that fractures were the most common lesions after umbilical lesions and arthritis. In our study, musculoskeletal system diseases constituted 56% of all cases. Fractures occur due to incorrect tying of nylon-like ropes to the metacarpal region of the calf and pulling with disproportionate force, especially during delivery. In our study, metacarpal fractures were detected in 39% of cases and this supports our views.

Intestinal atresia cases have an important place in terms of calf losses. It has been reported that rectal examinations performed before day 42 for early diagnosis of pregnancy may cause intestinal atresia cases (Syed et al. 1992; Atiba et al. 2016; Yurdakul, 2019). In our study, early pregnancy examination was performed in all cases with intestinal atresia. Studies have reported that the most common anorectal congenital anomaly is atresia ani (Carraro et al., 1996; Belge et al., 2000; Aslan et al., 2009). In our study, 31.25% of intestinal atresia cases were atresia coli, while 12.5% were atresia ani. At the same time, in cases of atresia ani, the unconscious intervention of the patient owner with sharp objects caused calf losses and a decrease in the number of patients brought to the clinic. Aslan et al. (2009) reported that surgical treatment was successful in anorectal anomalies. In our study, it was reported that the animal continued to live until it reached slaughter weight, especially in cases of atresia coli.

CONCLUSION

Finally, while cattle breeding has great economic importance in our country, calf losses occur due to economic conditions such as artificial insemination errors, irregular registration systems, lack of standardization of care and housing, incorrect intervention of the patient owner, and treatment costs. It is envisaged that economic losses will be minimized with early diagnosis, early intervention, appropriate medical treatment, and surgical treatment options, and awareness will be raised by educating the patient owners about the diseases and thus animal welfare will be increased.

REFERENCES

Alkan F, Koç Y, Sulu K. Yeni doğan buzağılarda göbek lezyonları: Yeni doğan buzağılarda enfeksiyöz seyirli göbek lezyonları ve sağaltım seçenekleri. In: Baran V, ed. Ruminantlarda Neonatal Cerrahi. 1. Baskı. Ankara: Türkiye Klinikleri; 2019. pp.40-44.

Anonymous. Available at: https://data.tuik.gov.tr/Bulten/Index?p=Hayvansal-Uretim-Istatistikleri-Haziran-2023-49680. Accessed Nov 20, 2023.

Arıcan M, Erol H, Esin E, Parlak K. A retrospective study of fractures in neonatal calves: 181 cases (2002-2012). Pak Vet J. 2014; 34:247-250.

Aslan L, Karasu A, Gençcelep M, Bakır B, Alkan I. Evaluation of cases with congenital anorectal anomalies in ruminants. YYU Vet Fak Derg. 2009; 20(1):31-36

Atiba AS, Farrag FA. Comparison between colostomy and coloanal reconstruction technique for colonic atresia in calves. Alexandria J Vet Sci. 2016; 48:27-33.

Azizi S, Mohammadı R, Mohammadpour I. Surgical repair and management of congenital intestinal atresia in 68 calves. Vet Surg. 2010; 39:115-120.

Belge A, Gönenci R, Biricik HS, Ormancı S. The cases of congenital anomlies in calves. YYU Vet Fak Derg. 2000; 11:23-26.

Carraro DB, Dart AJ, Hudson NP, Dart CM, Hodgson DR. Surgical correction of anorectal atresia and rectovaginal fistula in an alpaca cria. Aust Vet J. 1996; 74(5):352-354

Goodarzi M, Khamesipour F, Mahallati SA, Karimi M, Azizi D, Azizi S. Study on prevalence of bacterıal causes ın calves arthrıtıs. JABS. 2015; 10:6.

Hosie BD. Prolapse and hernia. In: Aitken ID, ed. Diseases of Sheep. 4th ed. Oxford UK: Blackwell Publishing; 2007. p.98.

Jesse FFA, Bıtrus AA, Abba Y, *et al*. Clinical management of septic arthritis in a sheep: A case report. Adv Anim Vet Sci. 2017; 5(6):267-270.

Kılıç N, Derincegöz OÖ, Yaygıngül R. Surgical correction of umbilical disease in calves: A Retrospective study of 95 Cases. YYÜ Vet Fak Derg. 2005; 16:35-38.

Marchionatti E, Nichols S, Babkine M, *et al*. Surgical management of omphalophlebitis and long term outcome in calves: 39 Cases (2008-2013). Vet Surg. 2016; 45:194-200.

Mejer T. Bovine colostrum and factors impacting colostrum quality in conventional and organic dairy herds. [Master's thesis]. Denmark: Aarhus University, Department of Animal Science Animal Nutrition and Environmental Impact; 2015.

Moscuzza C, Milicich H, Álvarez G, Gutierrez B, Nahum M. Calving assistance influences the occurrence of umbilical cord pathologies treated surgically in calves. Turk J Vet Anim Sci. 2014; 38:405-408.

Nuss K, Spiess A, Feist M, Köstlin R. Treatment of long bone fractures in 125 newborn calves: A retrospective study. Tieraerztl Prax. 2011; 39:15-26.

Syed M, Shanks RD. Incidence of atresia coli and relationships among the affected calves born in one herd of holstein cattle. J Dairy Science. 1992; 75:1357-1364.

Wieland M, Mann S, Guard CL, Nydam DV. The influence of 3 different navel dips on calf health, growth performance, and umbilical infection assessed by clinical and ultrasonographic examination. J Dairy Sci. 2016; 100:513-524

Yudakul I, Kulualp K, Yalçın M. Evaluation of surgical diseases detected in calves: a retrospective study of 350 cases. Turk Vet J. 2021; 3(1):21-25

Yurdakul I. Clinical and treatment evaluation of congenital intestinal atresia cases in calves. Van Vet J. 2019; 30(1):31-36

Yurdakul I, Kuluap K, Yalçın M. Clinical and treatment evaluation of umbilical lesions in calves: a retrospective study of 100 cases. Dicle Üniv Vet Fak Derg. 2021; 14(1):67-72

Yurdakul I. Complications due to umblical cord infection infections in lamb and kids. Cumhuriyet Üniv Sağ Bil Enst Derg. 2016; 1:39-45.

ACKNOWLEDGMENTS

Author contributions: NA, FG, BÖ and MS designed the study. NA and FG performed surgeries. MS and BÖ participated in revising the manuscript. NA: Nihan Avcı, FG: Fehmiye Gümüş, BÖ: Başak Boztok Özgermen, MS: Mehmet Sağlam

Financial Disclosure: The authors declared that this study has received no financial support.

Conflict of Interests: The authors declared that there is no conflict of interests.

Additional information: All authors have read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to NA.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Original Article https://doi.org/10.47748/tjvr.1428357

TJVR 2024; 8 (2): 95-101

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Therapeutic effect of D-Carvone on inflammation, apoptosis, and cell damage in lithium-induced liver injury model in rats

Elif Erbaş[1] Volkan Gelen[2] Emre Öztürk[3]

[1] *Department of Histology and Embryology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Türkiye,*
[2] *Department of Physiology, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye,*
[3] *Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum, Türkiye.*

Correspondence: *Elif Erbaş (eliferbas4154@gmail.com)*

Received: 30.01.2024 Accepted: 07.04.2024

ABSTRACT

Objectives: Lithium is an element denoted by the symbol Li in the periodic table. Lithium salts are widely used worldwide as therapeutic agents in treating excitement. However, the use of lithium causes liver damage. Carvone is an unsaturated monoterpenoid ketone usually found in essential oil extracts of aromatic medicinal plants such as dill, mint, and cumin. Various studies have shown that D-Carvone has strong antioxidant and anti-inflammatory effects. This study aimed to investigate the therapeutic effect of D-carvone on apoptosis, inflammation, and cell damage in lithium-induced liver injury.

Materials and Methods: The rats in our study were divided into 4 groups control, D-Carvone, Lithium, and Lithium+D-Carvone. After the treatments, rats were decapitated and liver tissues were removed. Histopathological analyses were performed on liver tissue and Bcl-2, Bax, P2X7R, and NfκB-p65 expression levels were evaluated by the Western Blot method.

Results: We determined that lithium administration caused liver tissue damage and increased Bax, P2X7R, and Nfkβ-p65 expression and decreased Bcl-2 expression. D-Carvone administration ameliorated these changes.

Conclusions: As a result, it was observed that D-Carvone administration ameliorated lithium-induced liver tissue damage and showed this effect by suppressing the inflammatory and apoptotic process.

Keywords: *Lithium, D-Carvone, Bcl-2, Bax, P2X7R, Nfkβ-p65*

INTRODUCTION

Lithium is a soft metallic element, denoted by the symbol Li in the periodic table. Lithium-ion batteries and lithium carbonate tablets for mood stabilization are the two best-known applications of lithium. Clinically, lithium has been used as a classical mood stabilizer in the treatment of bipolar disorder for more than fifty years (Johnson and Gershon, 1999).

The very strong anti-suicide effect of lithium has also been recently demonstrated (Tondo and Baldessarini, 2009). Lithium salts are widely preferred worldwide as therapeutic agents for treating excitement and mania. Lithium salts are easily distributed in the body, easily absorbed from the intestine and almost all of them are excreted through the urinary system (Rosenthal and Goodwin, 1982). Long-term lithium use has been found to have adverse effects on various organs, especially the liver. Numerous reports reflecting the toxic effects of lithium on liver function and structure have been put forward (Hunt et al., 1983; Abbas and Kathem, 2021; Bouyahya et al., 2021). It

has been suggested that lithium is a xenobiotic and affects the hepatic drug-metabolizing enzyme system (Hunt et al., 1983).

Carvone is an unsaturated monoterpenoid ketone usually found as the main phytochemical component in essential oil extracts of aromatic medicinal plants such as dill, mint, and cumin, with a boiling point of 230°C (Abbas et al., 2020; Zhao and Du, 2020; Bouyahya et al., 2021). Carvone has two enantiomers that differ in their biological properties, Carvone (L-Carvone) found in mint leaves and Carvone (D-Carvone) found in cumin leaves (Alsanea and Liu, 2017).

Pharmacologically, D-Carvone has been found to have anti-hyperlipidemic, fungicidal, anti-cancer, immunomodulatory, antioxidant, antimicrobial, anti-hypertensive, and anti-inflammatory effects (Lv et al., 2021). It was reported that D-Carvone showed no toxic effects when taken at 0.6 mg/kg body weight/day (Mahboubi, 2019). In mice fed a fatty diet, D-Carvone was found to reduce lipid accumulation by regulating gene expression of proteins responsible for lipid synthesis and transport in the liver. In another study, it was found that Carvone decreased hepatic cholesterol and triglyceride levels and decreased hepatic steatosis in rats with non-alcoholic fatty liver disease (Günther et al. 2019). The present study aimed to investigate the ameliorative effects of D-Carvone against lithium-induced hepatocyte damage.

MATERIALS and METHODS

Animal and experimental procedure

All studies were approved by Bingöl University Animal Experiments Local Ethics Committee (Decision no: BÜHADYEK-01/09). Adult 12-week-old male rats (n=32) obtained from BÜDAM were randomly divided into 4 groups of 8 rats per group based on body weight and housed in separate cages at an ambient temperature of 22 (±2)°C. The light regime was set to 12 hours light and 12 hours dark cycle. Rats in all groups were allowed free access to food and water. The lithium chloride (LiCl) supplemented feeds used in the experimental study were prepared by a commercial company that prepares certified experimental animal feed at a rate of 40 mmol LiCl per 1 kg dry feed (40 mmol/kg feed). From the beginning to the end of the experimental study, housing, feeding and care of all rats and all experimental procedures were carried out at BÜDAM.

Table 1. Groups and applications.

Group Name	Day 0-28. (n=8)	Day 28-42. (n=8)
Group 1: Control (CNT, n=8)	For 28 days, rats were fed with unadulterated standard commercial feed.	Rats were fed with standard commercial feed without additives.
Group 2: D-Carvone (D-CAR, n=8);	For 28 days, rats were fed with unadulterated standard commercial feed.	Rats were fed with unadulterated standard commercial feed and at the same time, each rat in the group was given 20 mg/kg D-carvone intraperitoneally daily for 14 days.
Group 3: Lithium (LIT, n=8);	Rats were fed 1 kg of standard commercial feed supplemented with 40 mmol Lithium chloride (LiCl) for 28 days.	Rats were fed with 1 kg standard commercial feed supplemented with 40 mmol Lithium chloride (LiCl).
Group: Lithium+D-Carvone (LIT+D-CAR, n=8)	Rats were fed 1 kg of standard commercial feed supplemented with 40 mmol Lithium chloride (LiCl) for 28 days.	Rats were fed with 1 kg of standard commercial feed supplemented with 40 mmol LiCl and 20 mg/kg D-carvone were administered intraperitoneally daily to each rat in the group for 14 days.

From the beginning of the study, except for the rats in the Control and D-Carvone groups, the other groups were fed with rat pellet feed supplemented with 40 mmol LiCl for 42 days. Rats in the D-Carvone group were fed with standard rat chow for 42 days and 20 mg/kg D-Carvone was administered intraperitoneally for 14 days after the 28th day. Rats in the LiCl+D-Carvone group were

fed with rat pellet feed supplemented with LiCl for 28 days and then 20 mg/kg D-carvone was administered intraperitoneally every day for 14 days. The groups formed within the scope of the study and the experimental procedure applied to the rats in each group are presented in Table 1.

Completion of the study

At the end of the 42-day experiment, the rats in all groups were weighed and their body weights were determined. Following general anesthesia, blood samples were collected from the intracardiac, and decapitation was performed. Blood samples were collected into serum tubes with yellow caps and centrifuged at 4000 rpm for 5 minutes and the sera obtained were stored in a deep freezer at -80°C.

After blood samples were taken, liver tissues were taken and their weights were determined. Some of the liver tissue samples were immersion fixed in 10% buffered formaldehyde solution for histopathological analysis and some were stored in a -20°C deep freezer for the first 24 hours and then in a -80°C deep freezer for Western-Blot analysis.

Histopathologic analysis and evaluation

Liver tissue samples were fixed in 10% buffered neutral formalin and after routine histological technique procedure embedded in paraffin. Paraffin blocks were sectioned at 5 µm thickness and stained with Mallory's triple stain modified by Crossman. They were then evaluated using a light microscope (Zeiss AXIO Scope.A1, German) with 200x magnification. Histologic photomicrographs were converted to quantitative analysis using a scoring system (Niazvand et al., 2023). Depending on the extent of sinusoidal dilatation, degeneration, congestion, and hemorrhage, the results were scored as 0, 1, 2, and 3, representing normal, mild, moderate, and severe damage, respectively.

Western blot analysis

Before western blot analysis, the acquired hepatic tissue samples were kept at -80 °C in a deep freezer. The hepatic tissue samples were weighted and crushed in nitrogen gas, treated with radioimmunoprecipitation (RIPA buffer, Ecotech Bio, Turkey) supplemented with protease and phosphatase inhibitors, and homogenized using a tissue lyser device (Qiagen, USA) at 30 Hz for 20 sec to determine the relative protein expressions of Bcl-2 (sc-7382, Santa Cruz), Bax (Sc-7480, Santa Cruz), P2X7R (11144-1-AP, Proteintech), and NfκB-p65 (sc-109, Santa Cruz). A protein assay kit was used to quantify hepatic tissue's total protein (Pierce BCA, Thermo Sci., USA). 30 ug of protein were then put into the PVDF membrane after being separated by 10% SDS-PAGE. First, at room temperature, 5% bovine serum albumin was used to block the membranes for 90 minutes. Then, the membranes were incubated at 4°C overnight with the appropriate primary antibodies. After primary antibody incubation, the PVDF membranes were washed with TBST and then incubated for an additional 90 minutes at room temperature with the second antibody (Santa Cruz, sc-2004/sc-2005) coupled to horseradish peroxidase. Then, the protein bands were captured using the enhanced chemiluminescence reagent Western ECL substrate (Thermo, 3405), visualized, and analyzed by Image Lab™ Software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Statistical analysis was performed using the SPSS (version 25.0; IBM SPSS Inc, Chicago, IL, USA) package program. Descriptive statistical analyses (mean ± standard deviation) were used. One-way ANOVA test and post hoc Tukey test were performed to compare groups. P values less than 0.05 at the 95% confidence interval were considered statistically significant.

RESULTS

Histopathologic evaluation

Photomicrographs of liver tissue in different groups are presented in Figure 1.

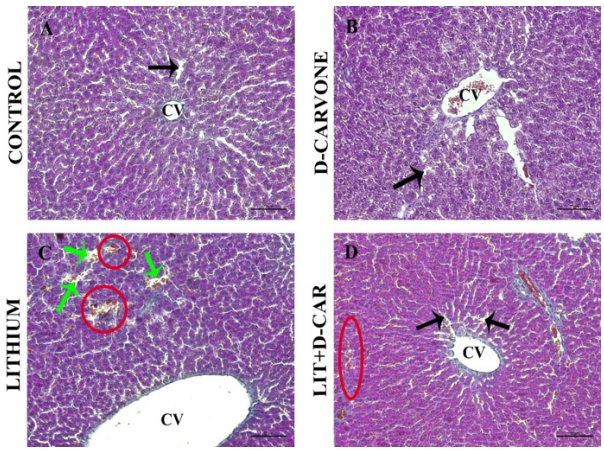

Figure 1. Photomicrographs of liver tissue in different groups. A: Control, B: D-Carvone, C: Lithium, D: Lithium+D-Carvone. Central vein (CV), sinusoid (black arrow), sinusoidal congestion and bleeding (red circle), and sinusoidal dilatation (green arrow). Staining: Mallory's triple stain modified by Crossman, magnification: 200X.

The control group (Figure 1-A) showed normal histologic architecture of the lobules (sinusoids, hepatocytes, and Kupffer cells) in the liver. No

changes were observed in the liver architecture in the D-Carvone (D-CAR) group (Figure 1-B). Figure 1-C shows the liver tissue in the Lithium (LIT) group. Lithium administration resulted in irregularities in the sinusoids such as dilatation, degeneration, congestion, and hemorrhage. On the other hand, the group treated with Lithium and D-Carvone showed reduced liver damage compared to the group treated with only Lithium (Figure 1-D). The damage in these groups was characterized by mild dilatation and congestion of some blood sinusoids. The severity of damage in all groups is listed in Figure 2 according to the defined scoring.

Western blot analysis results

When the relative protein expression levels of liver tissue were evaluated, Bax, NfκB-p65, and P2X7R expression levels were low in the control and D-Carvone groups and there was no significant difference between them ($p>0.05$). However, Bax, NfκB-p65, and P2X7R expression levels were significantly increased in the lithium-treated group compared to the other groups ($p<0.05$). In the lithium+D-Carvone group, these protein expressions were significantly decreased compared to the lithium group ($p<0.05$). On the other hand, the Bcl-2 expression level was higher in the control and D-Carvone groups compared to the other groups and there was no significant difference between them ($p>0.05$). We determined that the Bcl-2 expression level was significantly decreased in the lithium-treated group compared to the other groups ($p<0.05$). In the lithium+D-Carvone group, the expression of this protein was significantly increased compared to the lithium group ($p<0.05$), (Figure 3).

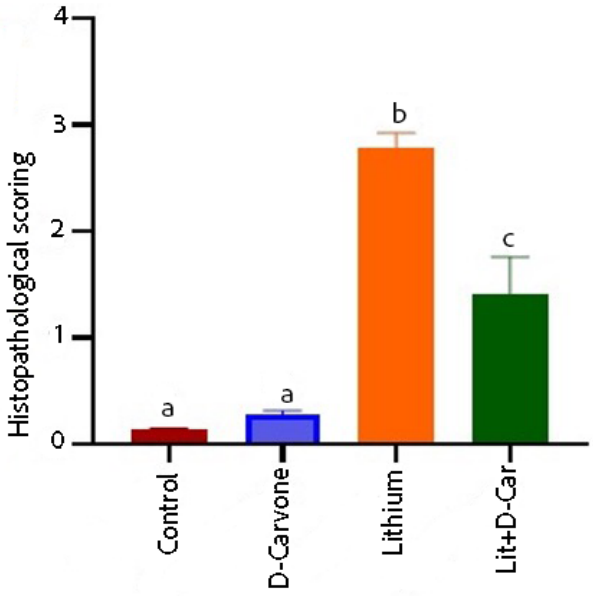

Figure 2. Assessment of liver histology. The values are given as mean ± SD (n=6) and analyzed by one-way ANOVA followed by the Tukey test. The letters (a, b, c) indicate statistically significant difference between the groups, $p<0.05$

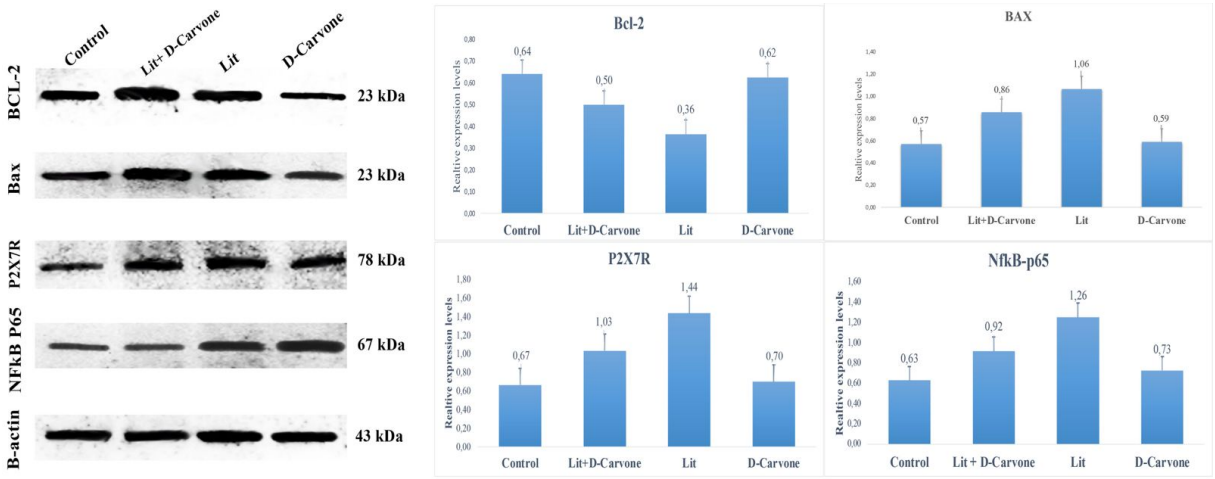

Figure 3. Relative expression of proteins for Bcl-2, Bax, P2X7R, and NfκB-p65. The values are given as mean ± SEM (n=7) and analyzed by one-way ANOVA followed by the Tukey test, $P<0.05$.

DISCUSSION

Lithium salts are a widely used agent in excitement control worldwide. When taken by mouth, lithium is easily absorbed from the intestines, easily distributed throughout the body, and excreted through the kidneys (Pert et al., 1978; Rosenthal and Goodwin, 1982). Long-term treatment with

lithium has been found to cause adverse effects on various organs including the liver (Laakso and Oja, 1979; Casado et al., 1989). There are various reports reflecting the toxic effects of lithium on liver structure and function (Ghoshdastidar, 1990; Lydiard and Gelenberg, 1982). Lithium, a xenobiotic, has been shown to affect the hepatic drug-metabolizing enzyme system (Schou et al., 1968; Aniya and Matsusaki, 1983; Hunt et al., 1983). As a result of various studies, it has been reported that lithium causes cellular damage in liver tissue and stimulates apoptosis and inflation (Tandon et al., 1997). This study aimed to determine the therapeutic effect of D-carvone, which is reported to have antiapoptotic and anti-inflammatory effects (Zhu et al., 2020; Engin et al., 2023), on cellular damage, apoptosis, and inflammation caused by lithium.

In the study, dilatation, degeneration, congestion, and hemorrhage in the sinusoids of hepatocytes in lithium-treated animals were significantly damaged compared to normal rats. These histopathologic changes may be explained by the fact that lithium exerts its toxic effects primarily through the formation of reactive oxygen species, resulting in damage. The necrotic conditions observed in the liver of lithium-treated animals are in agreement with the molecular changes observed. Previous studies are consistent with our findings (Ben Saad et al., 2017; Dai et al., 2020). Co-administration of D-Carvone with lithium-treated animals resulted in a remarkable normalization of hepatic changes. Such hepatoprotective effects of D-Carvone have been observed in several previous studies (Mezni et al., 2022). The data obtained in the present study are similar to previous studies.

The transcription factor NF-κB regulates many aspects of innate and adaptive immune functions and serves as an important mediator of inflammatory responses. Nf-κB induces the expression of several pro-inflammatory genes, including those encoding cytokines and chemokines, and also participates in inflammatory regulation (Erbaş et al., 2024). In addition, NfκB plays a critical role in regulating the survival, activation, and differentiation of innate immune cells and inflammatory T cells (Mohamed and Younis, 2022). In a study, it was reported that there was a significant increase in NfκB levels due to lithium administration. As seen in the present study, NfκB levels analyzed in liver tissues showed a significant increase in the lithium-treated group compared to the control group (Liu et al., 2017). In another study, it was shown that D-Carvone administration maintained NfκB levels at physiologic levels (Makola et al., 2021; Sousa et al., 2023). In the present study, NfκB levels, which increased due to lithium administration, remained at normal physiologic values in rats administered lithium and D-Carvone as in the control groups.

Anti-apoptotic Bcl-2 and pro-apoptotic Bax give us information about whether apoptosis occurs in cells (Ogaly et al., 2022; Gelen et al., 2024). In the study, it was observed that the Bax level was significantly higher than the control due to lithium administration. It was observed that Bcl-2 level, which has anti-apoptotic properties, decreased due to lithium administration. In a study, it was observed that tissue damage occurred in rats given lithium and accordingly, the anti-apoptotic Bcl-2 level decreased and the proapoptotic Bax level increased (Neamatallah et al., 2018). Previous studies reported that D-Carvone administration was effective on Bax and Bcl-2 (Alural et al., 2015). In the present study, it was determined that Bax and Bcl-2 levels in rats administered D-Carvone together with lithium remained close to the control and there was no statistical difference between them and the control.

The P2X7 receptor mediates oxidative stress, inflammatory mechanisms (Gopalakrishnan et al., 2019; Kara and Özkanlar, 2023), and fibrogenesis (Das et al., 2013) in the liver. This receptor triggers Kupffer cell inflammatory cellular responses and hepatocyte damage in liver diseases. In some studies, P2X7 receptor expression was found to be increased in the liver of septic mice. Furthermore, AST and ALT enzymes released by hepatic cell disruption are decreased in the serum of septic P2X7-deficient mice, providing evidence for the importance of this receptor in sepsis-related liver injury. Accordingly, P2X7-deficient mice showed a significant reduction in serum ALT levels in experimental models of acetaminophen-induced acute liver injury (Huang et al., 2014) and nonalcoholic steatohepatitis (Gopalakrishnan et al., 2019). It is also in agreement with a previous study showing impaired glucose metabolism in P2X7-deficient mice resulting in hepatic injury with higher glycemia, dyslipidemia, increased susceptibility to glucose intolerance, and insulin resistance (Hoque et al., 2012; Arguin et al., 2017). In our study, we found that lithium administration increased P2X7 receptor expression in liver tissue, which is an indication that lithium causes damage in liver tissue. We also showed that D-Carvone

administration suppressed lithium-induced P2X7 receptor expression.

CONCLUSION

In conclusion, our data show that lithium administration induces cellular damage, apoptosis, and inflammation in liver tissue. On the other hand, D-carvone administration inhibits lithium-induced tissue damage, inflammation, and apoptosis. This effect of D-carvone was thought to be due to its antioxidant and anti-inflammatory activity.

REFERENCES

Abbas BW, Kathem SH. Carvone attenuates irinotecan-induced intestinal mucositis and diarrhea in mice. Iraqi J Pharm Sci. 2021; 30(2):58-63.

Abbas MM, Kandil Yİ, Abbas MA. R-(-)-carvone attenuated doxorubicin induced cardiotoxicity in vivo and potentiated its anticancer toxicity in vitro. Balkan Med J. 2020; 37(2):98-103.

Alsanea S, Liu D. BITC and S-carvone restrain high-fat diet-induced obesity and ameliorate hepatic steatosis and insulin resistance. Pharm Res. 2017; 34(11):2241-2249.

Alural B, Ozerdem A, Allmer J, Genc K, Genc S. Lithium protects against paraquat neurotoxicity by NRF2 activation and miR-34a inhibition in SH-SY5Y cells. Front Cell Neurosci. 2015; 28(9):209.

Aniya Y, Matsusaki K. Effects of neutral salts on hepatic microsomal drug-metabolizing enzyme system in rats. Jpn J Pharmacol. 1983; 33(3):647-653.

Arguin G, Bourzac JF, Placet M, *et al.* The loss of P2X7 receptor expression leads to increase intestinal glucose transit and hepatic steatosis. Sci Rep. 2017; 7:1-16.

Ben Saad A, Dalel B, Rjeibi I, *et al.* Phytochemical, antioxidant and protective effect of cactus cladodes extract against lithium-induced liver injury in rats. Pharm Biol. 2017; Dec;55(1):516-525.

Bouyahya A, Mechchate H, Benali T, Ghchime R, Charfi S, Balahbib A. Health benefits and pharmacological properties of carvone. Biomolecules. 2021; 11(12):1-26.

Casado M, Aragon MC, Gimémez C. Determination of monoamines in rat brain regions after chronic administration of lithium. Neurochem Res, 1989; 14: 905-908.

Dai M, Wu L, Yu K, *et al.* D-Carvone inhibit cerebral ischemia/reperfusion induced inflammatory response TLR4/NLRP3 signaling pathway. Biomed Pharmacother. 2020; 132:110870.

Das S, Seth RK, Kumar A, *et al.* Purinergic receptor X7 is a key modulator of metabolic oxidative stress-mediated autophagy and inflammation in experimental nonalcoholic steatohepatitis. Am J Physiol Liver Physiol. 2013; 305:950-963.

Engin AB, Engin A, Engin ED, Memis L. Does lithium attenuate the liver damage due to oxidative stress and liver glycogen depletion in experimental common bile duct obstruction? Toxicol Appl Pharmacol. 2023; 466:116489.

Erbaş E, Gelen V, Kara H, *et al.* Silver nanoparticles loaded with oleuropein reduce doxorubicin-induced testicular damage by regulating endoplasmic reticulum stress, and apoptosis. Biol Trace Elem Res. 2024; 10:1-12.

Gelen V, Özkanlar S, Kara A, Yeşildağ A. Citrate-coated silver nanoparticles loaded with agomelatine provide neuronal therapy in acute cerebral ischemia/reperfusion of rats by inhibiting the oxidative stress, endoplasmic reticulum stress, and P2X7 receptor-mediated inflammasome. Environ Toxicol. 2024; 39(3):1531-1543.

Ghoshdastidar D, Poddar MK. Long term effect of lithium on brain regional catecholamine metabolism. Indian J Exp Biol, 1990; 28(5): 444-450.

Gopalakrishnan T, Ganapathy S, Veeran V, Namasivayam N. Preventive effect of D-carvone during DMBA induced mouse skin tumorigenesis by modulating xenobiotic metabolism and induction of apoptotic events. Biomed Pharmacother. 2019; 111:178-187.

Günther J, Schneider I, Krämer A. Carvone prevents and alleviates hepatic steatosis in rat model with nonalcoholic fatty liver disease. GMJ Med. 2019; 3(1):118–24.

Hoque R, Sohail MA, Salhanick S. P2X7 receptormediated purinergic signaling promotes liver injury in acetaminophen hepatotoxicity in mice. Am J Physiol-Gastrointest Liver Physiol. 2012; 302(10):1171-1179.

Huang C, Yu W, Cui H. P2X7 blockade attenuates mouse liver fibrosis. Mol Med Rep. 2014; 9:57-62.

Hunt GE, Beilharz GR, Storlien LH, Kuchel PW, Johnson GF. The effect of lithium on rat erythrocyte choline, glycine and glutathione levels. Biochemical Pharmacol. 1983; 32(19):2981-2983.

Johnson G, Gershon S. Early North American Research on Lithium. Aust N Z J Psychiatry. 1999; 33:48-53.

Kara A, Ozkanlar S. Blockade of P2X7 receptor-mediated purinergic signaling with A438079 protects against LPS-induced liver injury in rats. J Biochem Mol Toxicol. 2023; 37(10):23443.

Laakso ML, Oja SS. Transport of tryptophan and tyrosine in rat brain slices in the presence of lithium. Neurochem Res, 1979; 4, 411-423.

Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017; 2:1-9.

Lv L, Yang N, Cao Y, Dang J, Cheng L, El-Sheikh MA. D-carvone inhibits the JAK/STAT3 signaling pathway and induced the apoptotic cell death in the human gastric cancer AGS cells. J Biochem Mol Toxicol. 2021; 35(5):1-7.

Lydiard RB, Gelenberg A. Hazards and adverse effects oflithium. Ann Rev Med. 1982; 33:327-344.

Mahboubi M. Caraway as important medicinal plants in management of diseases. Nat Products Bioprospect 2019; 9(1):1-11.

Makola RT, Kgaladi J, More GK, Jansen van Vuren P, Paweska JT, Matsebatlela TM. Lithium inhibits NF-κB nuclear translocation and modulate inflammation profiles in Rift valley fever virus-infected Raw 264.7 macrophages. Virol J. 2021; 18(1):116.

Mezni A, Khazri O, Jarnier F, *et al.* Hepatoprotective effect of grape seed and skin extract against lithium exposure examined by the window of proteomics. Dose Response. 2022; 20(4):15593258221141585.

Mohamed ME, Younis NS. Ameliorative effect of D-Carvone against hepatic ischemia-reperfusion-induced injury in rats. Life (Basel). 2022; 12(10):1502

Neamatallah T, El-Shitany NA, Abbas AT, Ali SS, Eid BG. Honey protects against cisplatin-induced hepatic and renal toxicity through inhibition of NF-κB-mediated COX-2 expression and the oxidative stress dependent BAX/Bcl-2/caspase-3 apoptotic pathway. Food & Function. 2018; 9:3743-3754.

Niazvand F, Ashtari A, Chamkouri N, Azari M. Hepatoprotective effects of Alpinia officinarum rhizome extract on cisplatin-induced hepatotoxicity in rats: A biochemical, histopathological and immunohistochemical study. J Trace Elem Med Biol. 2023; 80:127306.

Ogaly HA, Aldulmani SAA, Al-Zahrani FAM, Abd-Elsalam RM. D-Carvone attenuates CCl_4-induced liver fibrosis in rats by inhibiting oxidative stress and TGF-ß 1/SMAD3 signaling pathway. Biology. 2022; 11:739.

Pert A, Rosenblatt JE, Sivit C, Pert CB, Bunney Jr, WE. Long-term treatment with lithium prevents the development of dopamine receptor supersensitivity. Science. 1978; 201(4351): 171-173.

Rhee C, Dantes R, Epstein L. Incidence and trends of sepsis in US hospitals using clinical vs claims data, 2009-2014. JAMA. 2017; 318:1241-1249.

Rosenthal NE, Goodwin FK. The role of the lithium ion in medicine. Ann Rev Med. 1982; 33(1):555-568.

Schou M, Amdiesen A, Eskajaer-Olsen T, Jemson SQ. Occurrence of goiter during lithium treatment. Br J Med. 1968; 3:710-703.

Sousa C, Neves BM, Leitão AJ, Mendes AF. Molecular mechanisms underlying the anti-inflammatory properties of (R)-(-)-Carvone: Potential Roles of JNK1, Nrf2 and NF-κB. Pharmaceutics. 2023; 5(1):249.

Tandon A, Nagpaul JP, Dhawan D. Effect of lithium on thehepatic drug metabolizing enzymes of protein deficient rats. Biol Trace Elem Res. 1997; 59:1-7.

Tondo L, Baldessarini RJ. Long-term Lithium Treatment in the Prevention of Suicidal Behavior in Bipolar Disorder Patients. Epidemiol Psichiatr Soc. 2009; 18:179-183.

Zhao M, Du J. Anti-inflammatory and protective effects of d-carvone on lipopolysaccharide (LPS)-induced acute lung injury in mice. J King Saud Univ Sci. 2020; 32(2):1592-1596.

Zhu X, Wang G, Wu S, Li C. Protective effect of D-Carvone against dextran sulfate sodium induced ulcerative colitis in Balb/c mice and LPS induced RAW cells via the inhibition of COX-2 and TNF-α. J Environ Pathol Toxicol Oncol. 2020; 39(3):235-245.

ACKNOWLEDGMENTS

Author contributions: EE, VG and EÖ designed the study. EE and VG performed surgeries. EÖ performed statistical analysis. EE and VG participated in drafting and revising the manuscript. EE: Elif Erbaş, VG: Volkan Gelen, EÖ: Emre Öztürk

Financial Disclosure: The authors declared that this study has received no financial support.

Conflict of Interests: The authors declared that there is no conflict of interests.

Additional information: All authors have read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to EE.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Original Article https://doi.org/10.47748/tjvr.1430913

TJVR 2024; 8 (2): 103-111

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Prediction of live weight from body measurements using stepwise regression models in Karacabey Merino lambs

Adem Kabasakal

Susurluk Agriculture and Forest Vocational School, Bandırma Onyedi Eylül University, Balıkesir, Türkiye.

Correspondence: *Adem Kabasakal (akabasakal@bandirma.edu.tr)*

Received: 02.02.2024 Accepted: 22.04.2024

ABSTRACT

Objectives: The aim of this study was to determine regression models that can be used to estimate live weight from body measurements in Karacabey Merino lambs of different ages (6th, 8th and 12th months).

Material and Methods: The animal material for the study consisted of 200 Karacabey Merino lambs. Some body measurements were taken from all lambs and live weights were also recorded. For live weight prediction equations with multiple linear regression analysis using some measurements according to age groups. stepwise multiple regression procedure was used in SAS (1999). DUNCAN test. one of the tests for multiple comparisons, was used to detect the differences between groups.

Results: The least squares mean for body length (BL), withers height (HW), back height (BH), rump height (RH), breast depth (CD), breast width (CW), rump width (RW), and live weight (LW) were 71.28 cm, 69.91 cm, 70.50 cm, 71.57 cm, 30.05 cm, 87.25 cm, 21.50 cm, 23.32 cm, and 51.05 kg, as the average of all lambs, respectively. High positive phenotypic correlation coefficients were found between body measurements and live weight in the different age groups. It is noteworthy that the live weight estimation models for three different age groups using stepwise regression analysis (second, third, and fourth models) can be recommended for the 6-month ages (R^2:0.82), 8-month ages (R^2:0.71), and 12th (R^2:0.79) months of life. The variables that can be used in the equations to estimate body weight for this breed. at these ages are HW, CW, RW, CG, CD, and BL.

Conclusion: Finally. it has been demonstrated that the live weights of Karacabey Merino lambs can be estimated with high accuracy using the stepwise regression method based on body measurements.

Keywords: *Body measurements, Extensive, Karacabey Merino sheep, Regression models*

INTRODUCTION

It can be said that studies on sheep breeding in Turkey began with the establishment of the Republic. Within the framework of these studies. the initial focus was on "merinoization" studies to improve the wool quality of Turkish sheep breeds (Kaymakçı and Taskin, 2008; Sezenler and Özder, 2009). Later. efforts were made to improve the meat and milk yield of indigenous breeds in Turkey (Kaymakçı, 2006; Sönmez et al., 2009). For this purpose. crosses of German Meat Fleece Merino x Kıvırcık were performed on Karacabey farm. As a result of this study. Karacabey Merino was developed for wool and meat performance (Özcan, 1990). Karacabey Merino bred mainly in the Marmara region of Turkey, has become an important breed with medium size, high quality fleece and carcass. (Sezenler et al., 2013; Yilmaz et al., 2014; Yilmaz et al., 2015; Ambarcioglu et al. 2017).

Breeding objectives in livestock include the determination of traits of economic importance. In this context. body measurements and live weight control. which can be considered the most important of these measures. are important criteria that are widely used for both scientific research and selection procedures (Yilmaz et al., 2013; Siddiqui et al., 2015; Kumar et al., 2018; Jawasreh et al., 2018; Hamadani et al., 2019; Sabbioni et al., 2020). Growth characteristics and live weights of sheep are economically important characteristics for breeding farms. Therefore. the accuracy of the measurements required to monitor the development of sheep is extremely significant. (Hossein-Zadeh, 2012; Abdel-Mageed and Ghanem, 2013; Eteqadi et al., 2014; Jafari and Hashemi, 2014; Mulyono et al., 2018) Livestock has a balance between body measurements and live weight in livestock. Studies on this topic have found significant phenotypic correlation values between live weight and body measurements. Many studies on this subject show a phenotypic correlation (Yilmaz et al., 2013; Ghotbaldini et al., 2019; Ibrahim et al., 2021; Panda et al., 2021). Research on body measurements is very important for describing breeds.

Since sheep breeding in Turkey is mainly carried out under extensive conditions. there is often not enough infrastructure available to perform live weight checks. Which is an important selection criterion (Yilmaz et al., 2013; Inan and Aygun. 2019). This important selection criterion cannot be adequately be used on some sheep farms. Estimation of liveweight of animals with different statistical approaches using some body measures is particularly important to more effectively include farms that do not have sufficient infrastructure in breeding programs. Several studies have found high and positive phenotypic correlation coefficients between body weight and body measurements. Scientific studies have shown that live weight can be estimated from various body measurements with a high degree of accuracy (Yilmaz et al., 2013; Sun et al., 2020; Esen and Elmaci, 2021; Kumar et al., 2021). Due to inadequate infrastructure on some sheep farms, estimation of live weight using body measurements will provide more practical and rapid results and will also make an important contribute to breeding programs. In the present study, the objective was to predict the live weight of Karacabey Merino lambs of different age groups from body measurements using stepwise regression models.

MATERIALS and METHODS

The animal material for the study consisted of 200 Karacabey Merino lambs. The Karacabey Merino lambs were divided into three groups according to age. The birth dates of the lambs are registered. There are male and female lambs in every age group. At the sheep farm where the study was conducted. controlled mating of sheep is practiced. leading to synchronized births. While there may be differences in individual birth dates in days. there is no age difference among the lambs within each group. All lambs were measured on the same day. The number of animals in each age group is shown in Table 1.

Table 1. Distribution of animal material by age groups.

Age Group (Months)	n
6th	54
8th	35
12th	111
Total	**200**

The live weights of the animals were determined using an electronic balance with a sensitivity of 50 g. Of the body measurements defined in the study. body length (BL). withers height (HW). back height (BH). rump height (RH). chest depth (CD). chest width (CW). and rump width (RW) were measured with a measuring stick. and chest girth (CG) was measured with a tape measure (Figure 1).

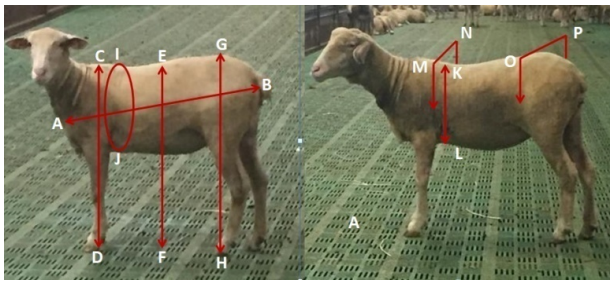

Figure 1. Karacabey Merino ewe showing the exact points at which the body measurements were taken.

To check the normality of the data, the procedure UNIVARIATE of the statistical programme SAS (1999) was used. The result of this analysis showed that the data were normally distributed for all measured characteristics. Subsequently, the general linear model (GLM) procedure of the same software was used to perform an analysis of

variance and obtain least squares means for the studied traits. Phenotypic correlations between variables were also obtained using the procedures PROC CORR in SAS (1999). DUNCAN test, one of the tests for multiple comparisons, was used to show the differences between groups.

The mathematical models used for the analysis of variance are presented below:

Model used for live weight:

$\gamma_{ijk} = \mu + a_i + b_j + e_{ij}$

Model used for body measurement

$\gamma_{ijk} = \mu + a_i + b_j + \beta_k(X_i - \bar{X}) + e_{ijk}$

where:

$\mathbf{Y}_{ijk}$= Observations for body measurement and weight

μ= Overall mean of the trait

a_i= Fixed effect of age group (i=6th. 8th and 12th months)

b_j= Fixed effect of gender (j= male and female)

β_1= Coefficient of regression of live weight

$\bar{X}$= Mean live weight

X_i= Live weight

$\mathbf{e}_{ijk}$**and**$\mathbf{e}_{ijk}$ =Random errors with the assumption of N (0. σ^2)

Estimation equations of live weights with multiple linear regression analysis using some measurements according to age groups were obtained by using stepwise multiple regression procedure in SAS (1999).

Multiple linear regression model given below was used for estimation equations.

$\hat{\boldsymbol{\gamma}}_{\boldsymbol{i}} = \hat{\beta}_0 + \hat{\beta}_1 x_{1+} \hat{\beta}_2 x_{2+} \hat{\beta}_3 x_{3+} \hat{\beta}_4 x_{4+} \hat{\beta}_5 x_{5+} \hat{\beta}_6 x_{6+}$ei

$\widehat{\boldsymbol{\beta}}_{\mathbf{0}}$=Constant

$\widehat{\boldsymbol{\beta}}_{\boldsymbol{i}}$=Regression coefficient

$\boldsymbol{x}_{\boldsymbol{i}}$=Body measurements

$\boldsymbol{x}_1$= Height at withers (HW)

$\boldsymbol{x}_2$=Chest width (CW)

$\boldsymbol{x}_3$= Rump width (RW)

$\boldsymbol{x}_4$=Chest Girth (CG)

$\boldsymbol{x}_5$= Chest depth (CD)

$\boldsymbol{x}_6$=Body length (BL)

Afterwards. the obtained estimates were compared with the actual live weights.

RESULTS

Descriptive statistics obtained for the characteristics addressed in the study are shown in Table 2. When examining the descriptive statistics. it was noticeable that the coefficients of variation for CW, RW, and LW were relatively high compared to other traits.

The least squares means and standard errors are shown in Table 3. The general mean values for BL, HW, BH, RH, CD, CG, CW, RW and LW were 71.28 cm, 69.91 cm, 70.50 cm, 71.57 cm, 30.05 cm, 87.25 cm, 21.50 cm, 23.32 cm and 51.05 kg as the average of all lambs, respectively. The analysis results showed that there was a statistically highly significant difference between the age groups in the present study in terms of HW, BH, RH RW and LW ($p<0.01$).

To estimate body weight considering body measurements, separate models were developed for different age groups using the stepwise regression model. The developed models and standard error values of coefficients of determination (R^2) are shown in Table 4. The independent variables of the final models selected for each age group analyzed were as follows: HW, CW, RW and CG for 6 months; HW, CW, RW and CG for 8 months; HW, CW, RW, CG and CD for 12 months. In the 8-month group, the independent variable CG (2.04) and in the 12-month group, CD (2.03) are observed to have a higher impact on the dependent variable.

When the determination coefficients (R^2) obtained for the developed models were evaluated, the lowest value was achieved in the first model developed for the 8th-month age group, while the highest value was obtained in the second model developed for the 6th-month age group. Additionally, all obtained regression models were found to be statistically significant.

The ANOVA significance tests for models II, III, and IV for these age groups and the t-test for regression coefficients were conducted according to the results presented in Table 4 in order to determine the best model with the stepwise regression method. The root means square error values (S) obtained for these models are lower than the other models in their age group, and the R^2 values are higher. In addition, since the Durbin-Watson (DW) test statistic values obtained are close to 2, there is no autocorrelation problem in these models. The fact that the Variance Inflation Factor (VIF) values obtained for each coefficient are less than 10 indicates that there is no multicollinearity problem.

Phenotypic correlation coefficients between body measurements and live weights for the age groups included in the study are shown in Table 5.

In general, high positive phenotypic correlation coefficients between body measurements and live weight were obtained for all age groups. Among the age groups, the highest phenotypic correlation coefficients between body measurements and live weight were obtained in the 6-month-old age group, with the exception of CD and CG.

Table 2. Basic statistics on body measurements and live weight.

Variable	Age	n	Mean	SD	CV	Min	Max
LW	6	54	39.90	7.59	19.03	26.50	59.00
	8	35	48.29	8.67	17.96	33.50	63.50
	12	111	56.07	9.75	17.38	35.00	89.00
BL	6	54	67.07	4.24	6.32	59.00	77.00
	8	35	73.13	3.24	4.44	65.00	79.00
	12	111	73.26	4.38	5.98	63.50	85.00
HW	6	54	65.93	3.52	5.33	60.00	75.00
	8	35	70.81	2.12	3.00	68.00	**76.50**
	12	111	71.22	3.37	4.73	64.00	81.00
BH	6	54	66.25	3.32	5.01	60.00	74.00
	8	35	71.29	1.82	2.56	68.50	77.00
	12	111	72.15	3.46	4.80	66.00	82.00
RH	6	54	67.03	3.33	4.96	60.00	75.00
	8	35	72.20	1.87	2.59	69.00	78.00
	12	111	73.59	3.32	4.50	66.50	83.00
CD	6	54	26.07	1.81	6.94	21.00	30.00
	8	35	27.69	1.62	5.85	24.00	30.00
	12	111	29.19	2.04	7.00	21.00	35.00
CG	6	54	80.58	5.30	6.57	72.00	94.00
	8	35	87.16	5.79	6.64	75.00	102.00
	12	111	91.51	6.70	7.32	78.00	109.00
CW	6	54	19.35	1.80	9.30	15.00	23.00
	8	35	21.20	2.43	11.44	16.00	27.00
	12	111	22.99	2.41	10.47	19.00	31.00
RW	6	54	21.67	1.97	9.09	18.00	28.00
	8	35	22.44	2.26	10.08	17.00	28.00
	12	111	24.62	2.41	9.78	18.00	31.00

BL:body length. HW:withers height. BH:back height. RH:rump height .CD: chest depth. CG: chest girth. CW: chest width. RW: rump width. LW: live weight. CV: coefficient of variation

Table 3. Least squares means and standard errors for body measurements and live weight belonging to Karacabey Merino lambs

Factors	n	BL	HW	BH	RH	CD	CG	CW	RW	LW
Age Group		p=0.546	p=0.000	p=0.000	p=0.000	p=0.498	p=0.419	p=0.843	p=0.043	p=0.000
6th months	54	70.84±0.962	67.81±0.461^{a}	68.04±0.456^{a}	68.90±0.424^{a}	29.75±2.495	85.88±1.284	21.57±0.269	23.36±0.299^{a}	39.90±0.817^{a}
8th months	35	72.04±0.921	71.25±0.441^{b}	71.72±0.436^{b}	72.65±0.406^{b}	28.12±2.389	88.18±1.229	21.58±0.258	22.79±0.287^{b}	48.29±1.016^{b}
12th months	111	70.95±1.032	70.68±0.494^{b}	71.76±0.489^{b}	73.17±0.455^{b}	32.29±2.677	87.69±1.377	21.35±0.289	23.81±0.321^{c}	56.07±0.710^{c}
Gender		p=0.709	p=0.103	p=0.051	p=0.029	p=0.059	p=0.869	p=0.017	p=0.552	p=0.000
Male	50	71.01±0.977	70.48±0.468	71.18±0.463	72.28±0.431	33.64±2.534	87.09±1.304	21.01±0.273	23.45±0.304	59.47±0.860
Female	150	71.55±0.728	69.34±0.348	69.83±0.344	70.87±0.321	26.47±1.887	87.41±0.971	21.99±0.204	23.18±0.226	42.63±0.585
Reg (Linear)		p=0.000	p=0.000	p=0.000	p=0.000	p=0.812	p=0.000	p=0.000	p=0.000	
Live weight		0.365±0.065	0.174±0.031	0.164±0.031	0.172±0.029	-0.04±0.168	0.509±0.086	0.217±0.018	0.160±0.020	
Overall	**200**	71.28±0.463	69.91±0.222	70.50±0.219	71.57±0.204	30.05±1.2	87.25±0.618	21.50±0.129	23.32±0.144	51.05±0.509

BL:body length. **HW:**withers height. **BH:**back height. **RH:**rump height. **CD:** chest depth. **CG:** chest girth. **CW:** chest width.**RW:** rump width. **LW:** live weight

Table 4. Live weight estimation models for three different age groups using stepwise regression analysis

Age Groups	Models		$\widehat{\beta}_0$	β_1	β_2	β_3	β_4	DW	S	R^2	P
6th months	I	βi	86.51	1.92				2.21	3.53	0.78	<0.001*
		S.E. Coeff.	9.11	0.14							
		t-value	-9.49	13.89							
		VIF		1							
		P	<0.001*	<0.001*							
	II	βi	-82.86	1.55	1.06			2.35	3.26	0.82	<0.001*
		S.E. Coeff.	8.5	0.17	0.34						
		t-value	-9.74	8.97	3.14						
		VIF		1.84	1.84						
		P	<0.001*	<0.001*	0.003*						
8th months	I	βi	-17.64	2.94				1.86	5.65	0.57	<0.001*
		S.E. Coeff.	9.68	0.43							
		t-value	-1.82	6.85							
		VIF		1							
		P	0.077	<0.001*							
	II	βi	-49.8	2.13	0.58			1.52	4.97	0.67	<0.001*
		S.E. Coeff.	13	0.45	0.18						
		t-value	-3.84	4.72	3.28						
		VIF		1.43	1.43						
		P	0.001*	<0.001*	0.002*						
	III	βi	-69.2	1.57	0.43	1.6		1.73	4.67	0.71	<0.001*
		S.E. Coeff.	14.9	0.49	0.18	0.7					
		t-value	-4.65	3.22	2.44	2.28					
		VIF		1.9	1.64	2.02					
		P	<0.001*	0.003*	0.02*	0.03*					
12th months	I	βi	16.8	3.17				1.73	6.09	0.61	<0.001*
		S.E. Coeff.	5.58	0.24							
		t-value	-3.01	13.13							
		VIF		1							
		P	0.003*	<0.001*							
	II	βi	-62.46	1.88	1.03			2.05	5.16	0.72	<0.001*
		S.E. Coeff.	8.34	0.28	0.15						
		t-value	-7.49	6.69	6.64						
		VIF		1.9	1.9						
		P	<0.001*	<0.001*	<0.001*						
	III	βi	-76.14	1.61	1.3	0.78		2.04	4.72	0.77	<0.001*
		S.E. Coeff.	8.16	0.26	0.28	0.15					
		t-value	-9.33	6.1	4.7	5.2					
		VIF		2	1.57	2.15					
		P	<0.001*	<0.001*	<0.001*	<0.001*					
	IV	βi	-80.52	1.43	0.16	1.22	0.73	2.03	4.5	0.79	<0.001*
		S.E. Coeff.	7.9	0.26	0.05	0.26	0.14				
		t-value	-10.19	5.55	3.38	4.64	5.08				
		VIF		2.08	1.23	1.58	2.17				
		P	<0.001*	<0.001*	0.001*	<0.001*	<0.001*				

* The test is important at the 0.05 significance level. $\widehat{\beta}_0$=constant. β_i=regression coefficient. R^2=adjusted estimation power **S.E. Coeff.:** Standard error values of coefficients **VIF:** Variance Inflation Factor **DW:** Durbin-Watson test **S:** Root Mean Square Error value of model

Table 5. Phenotypic correlation coefficients between weight and body measurements according to age group.

	Age Group	LW	BL	HW	BH	RH	CD	CG	CW
	6th months	0.812***							
BL	8thmonths	0.148ns							
	12th months	0.782***							
	6th months	0.888***	0.817***						
HW	8thmonths	0.341*	-0.109ns						
	12th months	0.537***	0.603***						
	6th months	0.862***	0.823***	0.968***					
BH	8thmonths	0.332ns	-0.120ns	0.934***					
	12th months	0.548***	0.560***	0.888***					
	6th months	0.859***	0.818***	0.958***	0.983***				
RH	8thmonths	0.434**	-0.002ns	0.913***	0.941***				
	12th months	0.598***	0.595***	0.878***	0.924***				
	6th months	0.123ns	0.194ns	0.136ns	0.126ns	0.134ns			
CD	8thmonths	0.739***	0.145ns	0.257ns	0.295ns	0.377ns			
	12th months	0.683***	0.575***	0.510***	0.494***	0.530***			
	6th months	0.559***	0.567***	0.477***	0.443**	0.393**	0.076ns		
CG	8thmonths	0.690***	-0.037ns	0.243ns	0.231ns	0.329ns	0.586***		
	12th months	0.512***	0.372***	0.222*	0.217*	0.252**	0.310**		
	6th months	0.736***	0.680***	0.675***	0.668***	0.667***	0.017ns	0.690***	
CW	8thmonths	0.573***	0.094ns	0.207ns	0.208ns	0.173ns	0.458**	0.401*	
	12thmonths	0.783***	0.688***	0.445***	0.430***	0.490***	0.527***	0.410***	
	6th months	0.770***	0.692***	0.738***	0.745***	0.707***	0.071ns	0.548***	0.749***
RW	8thmonths	0.766***	0.056ns	**0.298ns**	0.316ns	0.327ns	0.658***	0.548**	0.572***
	12th months	0.619***	0.493***	0.391***	0.427***	0.450***	0.526***	0.187*	0.668***

BL:body length. HW:wither height. BH:back height. RH:rump height. CD: chest depth.CG: chest girth. CW: chest width. RW: rump width. LW: live weight. ns:non-significant.*: p<0.05.**: p<0.01.***: p<0.001

Table 6. Average live weight for age groups. live weight estimations and hit rates (%) according to models

Age Groups	LW	Models							
		I		II		III		IV	
			%		%		%		%
6th months	39.90	40.07	99.996	39.84	99.998				
8th months	48.29	48.34	99.999	48.54	99.995	47.79	99.990		
12th months	56.07	56.07	100	56.21	99.998	55.96	99.998	55.96	99.998

Average live weights for age groups. live weight estimates and hit rates according to models are given in Table 6. The lowest hit rate was found to be 99.990% in model III in the 8th-month age group. The highest hit rate was found to be 100% in model I in the 12th-month age group. In this case, it has been revealed that live weight estimation can be made with high accuracy from the body measurements subject to the models.

DISCUSSION

The average body weight values obtained in the present study were lower than those obtained in studies of native Turkish and foreign breeds in different countries (Yilmaz et al., 2004; Sezenleret al., 2011; Yilmaz et al. 2013; Jafari and Hashemi, 2014; Zishiriet al., 2014). These differences, which have emerged in the literature, are due to the differences in breeds and breeding systems. The study found a high positive phenotypic correlation

coefficient between body measurements and live weight. This result is also consistent with the studies conducted in different breeds on this subject (Yilmaz et al., 2013; Lakew et al., 2018; Gul et al., 2019; Sabbioni et al., 2020; Canul-Solis et al., 2020; Panda et al., 2021).

In addition to BL, CG, and CW, other body measurements and live weight values were higher in males. The regression between live weights proved statistically highly significant ($p<0.01$) when all body characteristics except CD were measured. The effect of gender on RH and CW and live weight values was statistically significant. Regression between live weights in the measurement of all body characteristics was found to be statistically significant. The difference between the genders is an expected finding, and numerous literatures supports this (Sabbioni et al., 2020; Esen and Elmaci, 2021). The genders of the lambs used in the study are not equal. Therefore, the main focus of the study is to develop models for different age groups.

As can be seen from the models presented for estimating live weight by age, the addition of more than one body feature to the model results in an increase in the R^2 value. However, it is well known that taking a minimal number of measurements in field studies not only saves time, but also allows for practical application. For this reason, it is useful to make sure that the models to be created contain, few features and have a high R^2 value. Consistent with this information, it is noteworthy that the second, third, and fourth models can be recommended for the 6^{th}, 8^{th}, and 12^{th} months of age, respectively. The R^2 values of these models were relatively high compared with the other models. The body measures that can be used in the equations to estimate body weight for this breed and these age groups are HW, CW, RW, CG, CD and BL. Sun et al. (2000) determined by stepwise multiple regression analysis according to age that the most appropriate equation with the highest R2 value was HW, RH, BL, CD, RW, and the parameter that best predicted body weight and BW. Yilmaz et al. (2013) in their study on Karya sheep, CG and BL showed the most significant effects on BW according to multiple linear regression models. In their study on 4 sheep, Esen and Elmacı (2021) found a high correlation between LW and such body measurements (e.g. HW and CG). They stated that these two traits could be used instead of BL and BH in Karacabey Merino and Ramlıç lambs. Ambarcıoğlu et al. (2017) stated that the body size with the highest direct effect on live weight was determined to be CG. and CD and RW had an indirect effect.

CONCLUSION

Live weight measurement in animal husbandry is an important application for both breeding and commercial sheep farms. However, due to the difficulty and time involved in weighing. especially in commercial sheep farms operating under intense conditions, it cannot always be done exactly. Due to both physical and financial difficulties. weighing is not possible at some sheep farms.

The study revealed that live weight determination from body measurements can be achieved successfully. Particularly in extensive conditions and in enterprises that do not have sufficient infrastructure, it is absolutely necessary to know the live weight data in order to be integrated into animal breeding programs. In this case, live weight estimation models can be used by using some measurements to determine the live weights in sheep farms that do not have sufficient infrastructure. In this way, even if there is a deviation in the weight. the condition of the flock can be followed with the estimated weights according to the body measurements, and it can guide the breeding programs.

Consequently, the live weights of Karacabey Merino lambs can be successfully predicted from body measurements using stepwise regression models. Live weight and body measurements are directly related. However, it is well known that it varies according to species, breed, nutritional status, age and body size. For this reason, the models created for live weight estimation may differ for each breed. Considering this situation, live weight estimation from body measurements can be successfully performed in other sheep breeds.

REFERENCES

Abdel-Mageed I, Ghanem N. Predicting body weight and longissimus muscle area using body measurements in subtropical goat kids. Egyptian J Sheep and Goat Sci. 2013; 8:95-100

Ambarcıoğlu P, Kaya U, Özen D, Gürcan İS. An examination of the relationships between live weight and body measurements in Karacabey Merino sheep through the path analysis approach. Kafkas Üniv Vet Fak Derg. 2017; 23(6):857-863.

Canul-Solis J, Angeles-Hernandez JC, García-Herrera RA, *et al.* Estimation of body weight in hair ewes using an indirect measurement method. Trop Anim Health Produc. 2020; 52(5):2341-2347.

Esen VK, Elmacı C. The estimation of live weight from body measurements in different meat-type lambs. J Agr Sci. 2021; 27(4):469-475.

Eteqadi B, Hossein-Zadeh NG, Shadparvar AA. Population structure and inbreeding effects on body weight traits of Guilan sheep in Iran. Small Rumin Res. 2014; 119:45-51.

Ghotbaldini H, Mohammadabadi M, Nezamabadi-pour H, Babenko OI, Bushtruk MV, Tkachenko SV. Predicting breeding value of body weight at 6-month age using Artificial Neural Networks in Kermani sheep breed. Acta Sci. Anim Sci. 2019; 41:4582.

Gul S, Keskin M, Guzey YZ, Behrem S, Gunduz Z. Path analysis of the relationship between weaning weight and some morphological traits in Awassi lamb. Kahramanmaraş Sütçü İmam Üniv Tar ve DoğaDerg. 2019; 22:431-435.

Hamadani A, Ganai NA, Khan NN, Shanaz S, Ahmad T. Estimation of genetic. heritability. and phenotypic trends for weight and wool traits in Rambouillet sheep. Small Rumint Res. 2019; 177:133-140.

Hossein-Zadeh NG. Inbreeding effects on body weight traits of Iranian Moghani sheep. Archives Anim Breed. 2012; 55:171-178.

Ibrahim A, Artama WT, Budisatria IGS, Yuniawan R, Atmoko BA, Widayanti R. Regression model analysis for prediction of body weight from body measurements in female Batur sheep of Banjarnegara District, Indonesia. Biodiversitas J Biol Diver. 2021; 22(7):2723-2730.

Inan I, Aygün T. The body weight after shearing and the greasy wool yield of Red Karaman ewes at different raising conditions in Turkey. J Advd Agri Tech. 2019; 6(2):139-143.

Jafari S, Hashemi A. Estimation of genetic parameters for body measurements and their association with yearling liveweight in the Makuie sheep breed. South African J Anim Sci. 2014; 44(2):140-147.

Jawasreh K, Ismail ZB, Iya F, Castañeda-Bustos VJ, Valencia-Posadas. M. Genetic parameter estimation for pre-weaning growth traits in Jordan Awassi sheep. Vet World. 2018; 11(2):254-258.

Kaymakçı M. İleri koyun yetiştiriciliği. Bornova/İzmir: İzmir İli Damızlık Koyun ve Keçi Yetiştiricileri Birliği Yayınları; 2006.

Kaymakçı M, Taşkın T. Türkiye koyunculuğunda melezleme çalışmaları. Hayv Üret. 2008; 49(2):43-51.

Kumar S, Dahiya SP, Malik ZS, Patil CS. Prediction of body weight from linear body measurements in sheep. Indian J Anim Res. 2018; 52(9):1263-1266.

Kumar A, Mallick PK, Misra SS, Sharma RC, Gowane GR. The morphometric scale to predict the live weight of Malpura Sheep in Semi-arid region of Rajasthan. Indian J Anim Res. 2021; 55(9):1105-1110.

Lakew M, Tesema Z, Zegeye A. Body weight prediction from linear body measurements in Awassi crossbred sheep of North Eastern Ethiopia. J App Agri and Biotec. 2018; 2(2):28-36.

Özcan L. Koyunculuk. Ankara: Tarım Orman ve Köyişleri Bakanlığı; 1990.

Panda S, Gaur GK, Chauhan A, Kar J, Mehrotra. A. Accurate assessment of body weights using morphometric measurements in Landlly pigs. Trop Anim Health Produc. 2021; 53(3):1-6.

Sabbioni A, Beretti V, Superchi P, Ablondi M. Body weight estimation from body measures in Cornigliese sheep breed. Italian J Anim Sci. 2020; 19(1):25-30.

SAS. The SAS System. Version 8. NC, USA: SAS Institute Inc Cary; 1999.

Sezenler T, Özder M. Türkiye'de merinoslaştırma çalışmaları. Hasad Hay Derg. 2009; 25:34-41.

Sezenler T, Soysal D, Yildirir M, *et al.* Karacabey Merinos koyunların kuzu verimi ve kuzularda büyüme performansı üzerine bazı çevre faktörlerinin etkisi. Tekirdağ Zir Fak Derg. 2013; 10(1):40-47.

Sezenler T, Özder M, Yildirir M, Ceyhan A, Yüksel MA. The relationship between body weight and body condition score in some indigenous sheep breeds in Turkey. The J Anim and Plant Sci. 2011; 21(3):443-447.

Siddiqui MU, Lateef M, Bashir MK, Bilal MQ, Muhammad G, Mustafa MI. Estimation of live weight using different body measurements in Sahiwal Cattle. Pakistan J Life and Soc Sci. 2015; 13(1):12-15.

Sönmez R, Kaymakçı M, Eliçin A, Tuncel E, Wassmuth R, Taşkın T. Türkiye koyun islahı çalışmaları. Uludağ Üni Zir Fak Derg. 2009; 23(2):43-65.

Sun MA, Hossain MA, Islam T, Rahman MM, Hossain MM, Hashem MA. Different body measurement and body weight prediction of Jamuna basin sheep in Bangladesh. SAARC J Agri. 2020; 18(1):183-196.

Yilmaz A, Ozcan M, Ekiz B, Ceyhan A, Altine A. The production characteristics of the indigenous Imroz and Kivircik sheep breeds in Turkey. Anim Gen Resources. 2004; 34:57-66.

Yilmaz O, Cemal I, Karaca. O. Estimation of mature live weight using some body measurements in Karya sheep. Trop Anim Health Prod. 2013; 45(2):397-403.

Yilmaz O, Sezenler T, Ata N, Yaman Y, Cemal I, Karaca O. Polymorphism of the ovine calpastatin gene in some Turkish sheep breeds. Turkish J Vet and Anim Sci. 2014; 38(4):354-357.

Yilmaz O, Sezenler T, Sevim S, *et al.* Genetic relationships among four Turkish sheep breeds using microsatellites. Turkish J Vet Animl Sci. 2015; 39(5):576-582.

Zishiri OT, Cloete SWP, Olivier JJ, Dzama K. Genetic parameters for live weight traits in South African terminal sire sheep breeds. Small Rum Res. 2014; 116(2-3):118-125.

ACKNOWLEDGMENTS

Author contribution: The conception and design of this study. data collection and analysis. review writing and editing of the last draft of the manuscript were performed by Adem Kabasakal.

Financial Disclosure: The author declared that this study has received no financial support.

Conflict of Interests: The author declared that there is no conflict of interests.

Additional information: The author has read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to Adem Kabasakal.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Original Article https://doi.org/10.47748/tjvr.1466125

TJVR 2024; 8 (2): 113-119

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Ultrastructural changes in the adrenal gland in terms of age and gender-related

Nejat Ünlükal[1] Abdullah Şentürk[1]

[1] *Department of Histology and Embryology, Faculty of Medicine, Selcuk University, Konya, Türkiye.*

Correspondence: *Nejat Ünlükal (nunlukal@selcuk.edu.tr)*

Received: 04.04.2024 Accepted: 03.07.2024

ABSTRACT

Objective: The adrenal glands are endocrine organs that synthesize hormones with crucial functions in the body. This irreplaceable structure performs multiple functions, from the metabolism of carbohydrates, fats, and proteins to sex development. Any disorder concerning the adrenal glands can give rise to life-threatening conditions. The aging process inevitably affects the adrenal glands. The effects of aging are marked by a rise in cortisol secretion in the zona fasciculata and a decrease in androgen secretion in the zona reticularis. Elevated cortisol levels in the blood disturb most bodily systems in favour of catabolism, amplifying the cellular decline and destruction that accompanies aging. The deterioration of the adrenal glands relates not just to spongiocytes and chromaffin cells, but also to the endothelium, which enables circulation. It is clear that a decline in vascular function will have a negative impact on endocrine activities. Our study aims to explore the influence of aging on the adrenal glands in rats, analyzing sex-specific ultrastructural scales.

Materials and Methods: A total of 28 Sprague-Dawley rats, 14 males, and 14 females, were planned to be used in the study. Of these 28 rats, 4 females and 4 males constitute the control group. The rats in the control group at approximately 10 weeks old and the rats in the experimental group at approximately 19 weeks old were sacrificed. All animals in the study have been anaesthetized and then sacrificed by removal of the heart. The right and left and adrenal glands were removed from the sacrificed animals. Removed and adrenal glands prepared for TEM examination. For each animal, at least four TEM images were taken from four different sections of the same block.

Results: Our findings demonstrate that the aging process not only affects spongiocytes within the adrenal gland but also contributes to the deterioration of endothelial cells. As anticipated, our results indicate the presence of senescence and apoptosis in endothelial cells. The observed vascular separations and ruptures are due to endothelial deterioration. Hypertrophy has been observed in the spongiocytes to compensate for the functional deficiencies following a decrease in their number due to aging. Elevated levels of lipofuscin, lipid droplets, and lysosomes were discovered in spongiocytes. Impaired endothelium potentially contributes to certain changes in spongiocytes.

Conclusions: While aging-related changes appear similar in both genders, males tend to be more impacted.

Keywords: *Adrenal gland, Endothelium, Ultrastructural, Endocrine, Gender-dependent difference*

INTRODUCTION

Adrenal (suprarenal) glands are cap-like endocrine glands located above the kidneys. Adrenal glands are retroperitoneal organs embedded in adipose tissue. Adrenal glands have a rich vascular support such as endocrine organs. The inferior phrenic artery, the abdominal aorta, and the renal artery supply the adrenal glands by giving rise to the superior, middle, and inferior suprarenal arteries respectively. The adrenal glands consist of two parts, the cortex (80-90%), which synthesizes

steroid hormones, and the medulla (10-20%), which synthesizes catecholamines (Hansen, 2021).

Adrenal cortex consists of zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR). These cell layers have different properties from each other. The zona glomerulosa makes up about 15% of the cortex and is responsible for the synthesis of mineralocorticoid aldosterone. The zona fasciculata makes up 80% of the cortex and is responsible for the synthesis of glucocorticoids, especially cortisol. The zona reticularis is the smallest part of the cortex (5-6%) and is responsible for the synthesis of androgens called dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEA-S). Unlike the cortex, the adrenal medulla is composed of chromaffin cells of neural crest origin. The adrenal medulla is not stimulated by hormones from the hypothalamic-pituitary-adrenal axis like the cortex but by nerves from the sympathetic ganglia. There are two types of chromaffin cells, norepinephrine-secreting and epinephrine-secreting. About 80% of the secretion from the adrenal medulla is epinephrine, 20% norepinephrine and a small amount of dopamine. The blood supply to the adrenal glands is provided by a system of the three vessels mentioned above, which enter the capsule. Capsular capillaries supply the capsule, vessels enter the cortex and form the fenestrated cortical sinusoidal capillaries, medullary arterioles pass through the trabeculae in the cortex, reach the medulla and bring arterial blood to the medullary capillary sinusoids. The fenestrated cortical sinusoidal capillaries supplying the cortex bring venous blood to the medullary capillary sinusoids. The medulla is supplied by two bloodstreams. Venous blood from venules originating in the cortical and medullary sinusoids empties into the adrenomedullary collecting veins and finally leaves the adrenal gland via the central adrenomedullary vein (Kierszenbaum and Tres, 2019; Mescher, 2013).

Since endocrine organs release their secretions into the blood, any disruption in their circulation will have undesirable effects on the body. So much so that endocrine organs are the places in the body where the most blood per gram is seen. The adrenal gland is one of the most blood-rich tissues and, as mentioned above, it has sinusoidal capillaries. Sinusoids are densely located in organs and tissues where the transport of large molecules is intense and where substance exchange is high (Augustin and Koh, 2017; Schaeffer et al., 2011). Disruption of the sinusoids prevents the adrenal gland from performing its full function.

The secretions of the adrenal glands play an important role in maintaining homeostasis. Corticotropin-releasing hormone (CRH) secreted by the hypothalamus stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH stimulates the adrenal cortex to produce steroid hormones, particularly cortisol. The rising levels of cortisol and other glucocorticoids in the circulation control hormone secretion through negative feedback on the hypothalamus and pituitary. The main stimulus for aldosterone synthesis from the cortex is the renin-angiotensin system. Synthesis and secretion of androgens are controlled by CRH-ACTH, as are glucocorticoids. Glucocorticoids secreted from the adrenal cortex mainly affect carbohydrate, protein, and lipid metabolism, but also the immune system, hematopoiesis, vascular action of catecholamines, bone reabsorption, glomerular filtration, gastrointestinal system, and central nervous system. The main action of aldosterone is to ensure the retention of sodium ions and associated water from the kidney. Aldosterone increases potassium and hydrogen ion excretion while increasing sodium retention. Androgens have effects on protein anabolism and growth promotion as well as the development of secondary sex characteristics during puberty (Jameson et al., 2016; Hall and Hall, 2020; Costanzo, 2021).

The functions of the adrenal glands change with age. The zona reticularis decreases in activity with age, while hypertrophy of the zona fasciculata and zona glomerulosa has been reported. There are several theories as to why adrenal function may deviate from physiological limits, one of which is that the blood supply to the adrenal glands decreases with age. In addition, the effect of aging on the adrenal glands differs between men and women. Men are said to be more affected by adrenal aging (Parker et al., 1997; Warde et al., 2023). In our study, we tried to understand the effects of aging on the adrenal glands in men and women by examining the adrenal glands ultrastructurally.

MATERIALS and METHODS

A total of 28 Sprague-Dawley rats, 14 males, and 14 females, were used in the study. Of these 28 rats, 4 females and 4 males constituted the control group. The rats in the control group were

approximately 10 weeks old, and the rats in the experimental group, which represented the aged group, were 19 weeks old. All animals in the study were anaesthetized with 60 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride and then sacrificed by removing the hearts and the right and left suprarenal glands were removed from the sacrificed animals.

The removed glands were immediately placed in a 0.1 M phosphate buffered container containing 2.5% glutaraldehyde for 24 hours. The next morning the tissues were postfixed in 1% osmium tetraoxide (OsO4) in 0.1 M phosphate buffer for 1 hour and dehydrated by graded alcohols (25-100%). After passing through propylene oxide, the samples were embedded in Araldyte CY 212, DDSA (2-dodecenylsuccinic anhydride), BDMA (benzyldimethylamine), and dibutylphthalate. Semi-thin sections (1µm) of suprarenal glands were stained with toluidine blue and examined by light microscopy. Ultrathin sections of adrenal glands were stained with uranyl acetate and lead citrate and examined by LEO 906E transmission electron microscopy (TEM). For each animal, at least four TEM images were obtained from four different sections obtained from the same block.

Ethical approval

The study was approved by the Ethical Committee of the Faculty of Medicine of Baskent University. The ethics approval number: is DA09/16.

RESULTS

In the control group, male rats of vessels lumen surrounded by endothelial cytoplasm, and the nucleus was characterized by a rich euchromatin content. The vessel was surrounded by actively secreting cells that were rich in mitochondria (Figure 1).

On the other hand, in the control female rat vessels, the endothelium is seamlessly connected to the basal lamina. Notably, vesicles located in both the apical and basolateral regions of the endothelium play a crucial role in the transit and transport of necessary substances to the adjacent secretory cell (Figure 1).

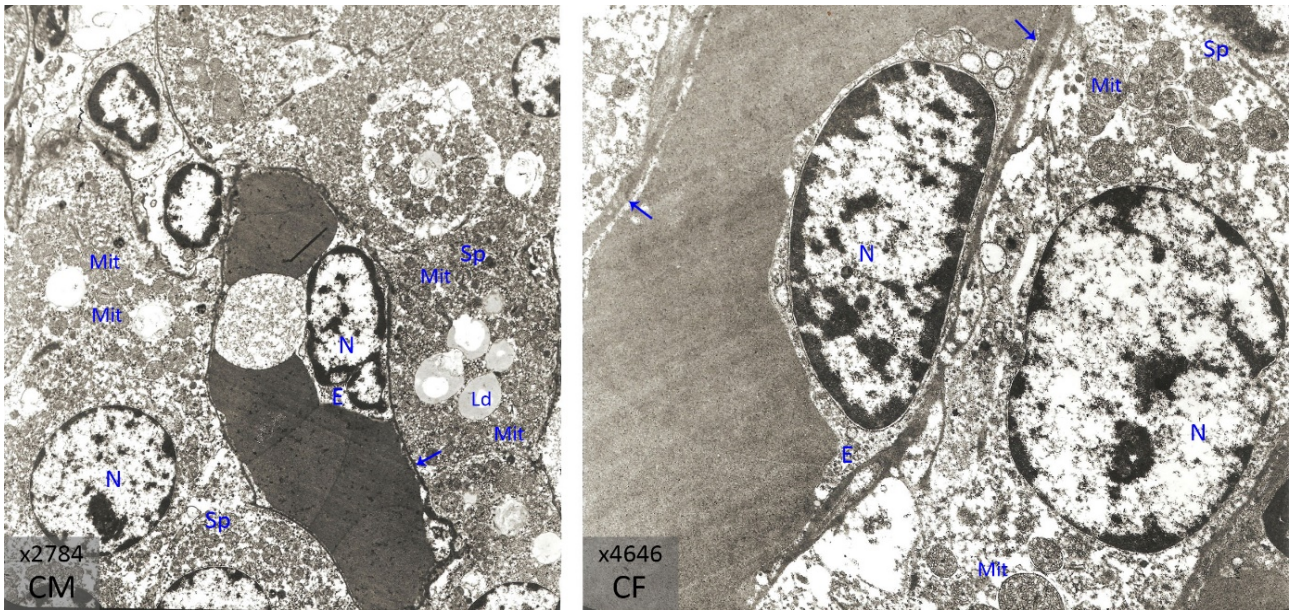

Figure 1. Control group electromicrographs (male on the left [CM], female on the right [CF]). E: endothelial cell, Sp: spongiocyte, Ld: lipid droplets, N: nucleus, Mit: mitochondrion, →: refers to basement membrane

The observations revealed various structural alterations within the endothelial cells and their microenvironment. Signs of degeneration were evident in specific regions of the endothelial cells and some spongiocyte mitochondria (Figure 2 OF-1). Vacuolization was noted in the endothelial part of the basal lamina and there were folds on its surface facing the spongiocytes. White spaces within spongiocytes were recognized as residual lipid droplet remnants following lipid removal, while an observed thickening occurred in the basal lamina. Mitochondrial degeneration in both endothelial and secretory cells was notable, alongside increased heterochromatin regions within cell nuclei. In addition, an increase in lipofuscin was also observed due to degenerated mitochondria, lysosomes, and lipid droplets increasing in spongiocytes (Figure 2 OF-2, OF-3).

Membrane folding was observed in the basal lamina and at regions of cell contact. Vacuolization at the border of the basal lamina and bubble-like separations within it were also reported. Details included dilated cristae in endothelial cell mitochondria, heterochromatin-rich regions in cell nuclei, and thickening of the basal lamina (Figure 2 OF-4, OF-5). These comprehensive observations highlight a range of structural changes and cellular variations within the endothelial cells and their immediate surroundings (Figure 2 OF-6).

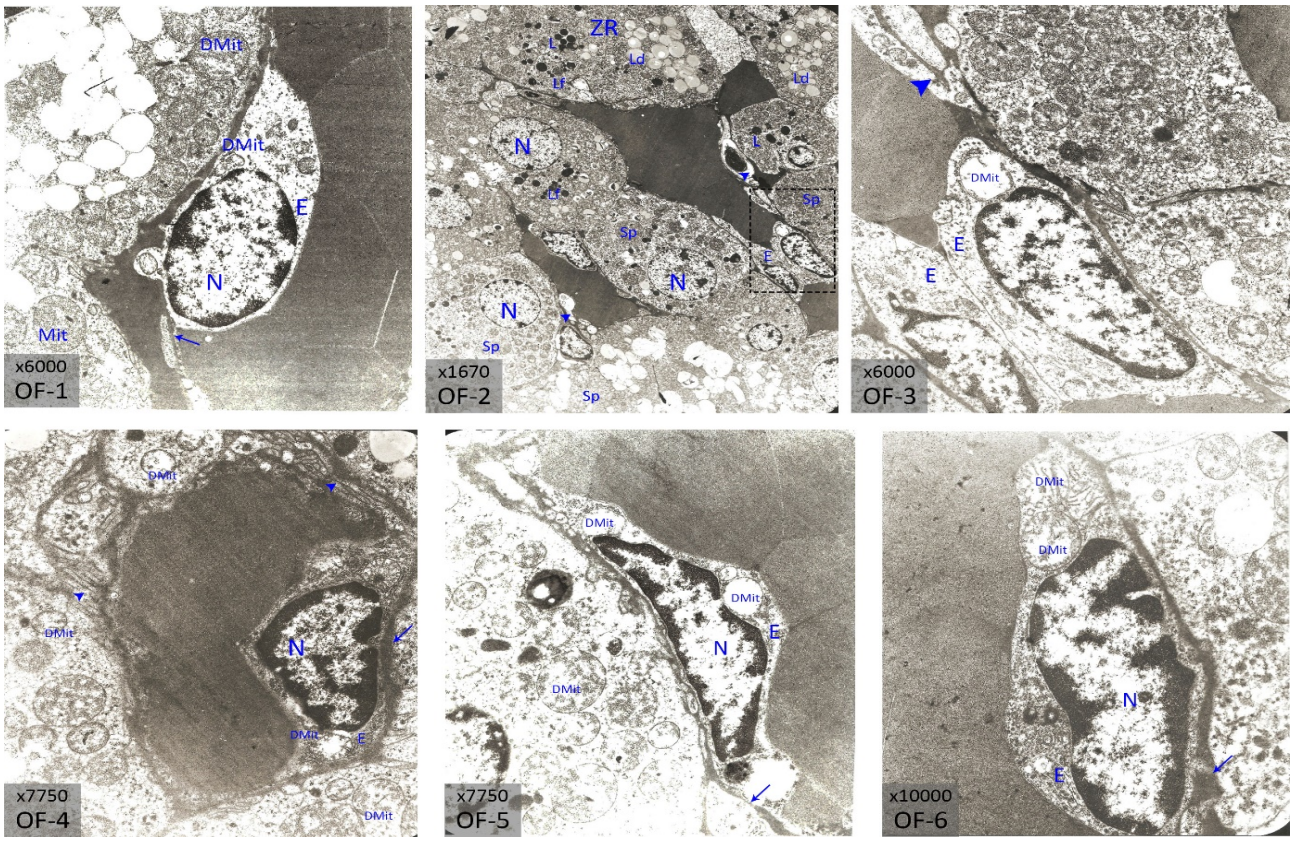

Figure 2. Electromicrographs of the old female group E: endothelial cell, Sp: spongiocyte, ZR: zona reticularis, L: lysosome, Lf: lipofuscin, Ld: lipid droplets, N: nucleus, Mit: mitochondrion, DMit: degenerated mitochondrion, →: refers to basement membrane

Darkening of the endothelial cell nucleus, mitochondrial degeneration, cytoplasmic vacuolization, lipofuscin, cell growth, separation, thickening of the basal lamina, and mitochondrial degeneration in the spongiocyte were observed (Figure 3 OM-1). Vacuolization of endothelial cells increased, and the lumen was covered with endothelial cells. It was observed that the integrity of the basal lamina was disrupted and some mitochondria in the spongiocytes were disrupted. Extravasation and stasis formation were observed due to deterioration of the vascular wall (Figure 3 OM-2, OM-3). There was GER dilatation in the endothelium and dilatation in the perinuclear space (Figure 3 OM-4). The endothelial nucleus was completely filled with heterochromatin, and there was dilation of the perinuclear space and thickening of the basal lamina between the endothelial cell and spongiocyte. Numerous detachments, degenerated mitochondria, and lipofuscin accumulations were observed in spongiocytes (Figure 3 OM-5, OM-6). There were many spaces within and between cells, and debris in some of the larger spaces has been thought to remain from degenerated cells (Figure 3 OM-7). An excessive increase in endothelial cytoplasm and detachment around the basal lamina were observed. There was a rupture between the spongiocyte and the vessel wall. In the sample, the erythrocyte extravasated abnormally into the endothelial cell line (Figure 3 OM-8.). Overgrowth of endothelial cells caused the artery to narrow, leading to blockage. Increased mitochondria were observed around the heterochromatin-rich nucleus in the endothelium, and lipofuscin formation was observed in the endothelium and spongiocytes. Additionally, cell contents were seen to leak into the arterial lumen (Figure 3 OM-9).

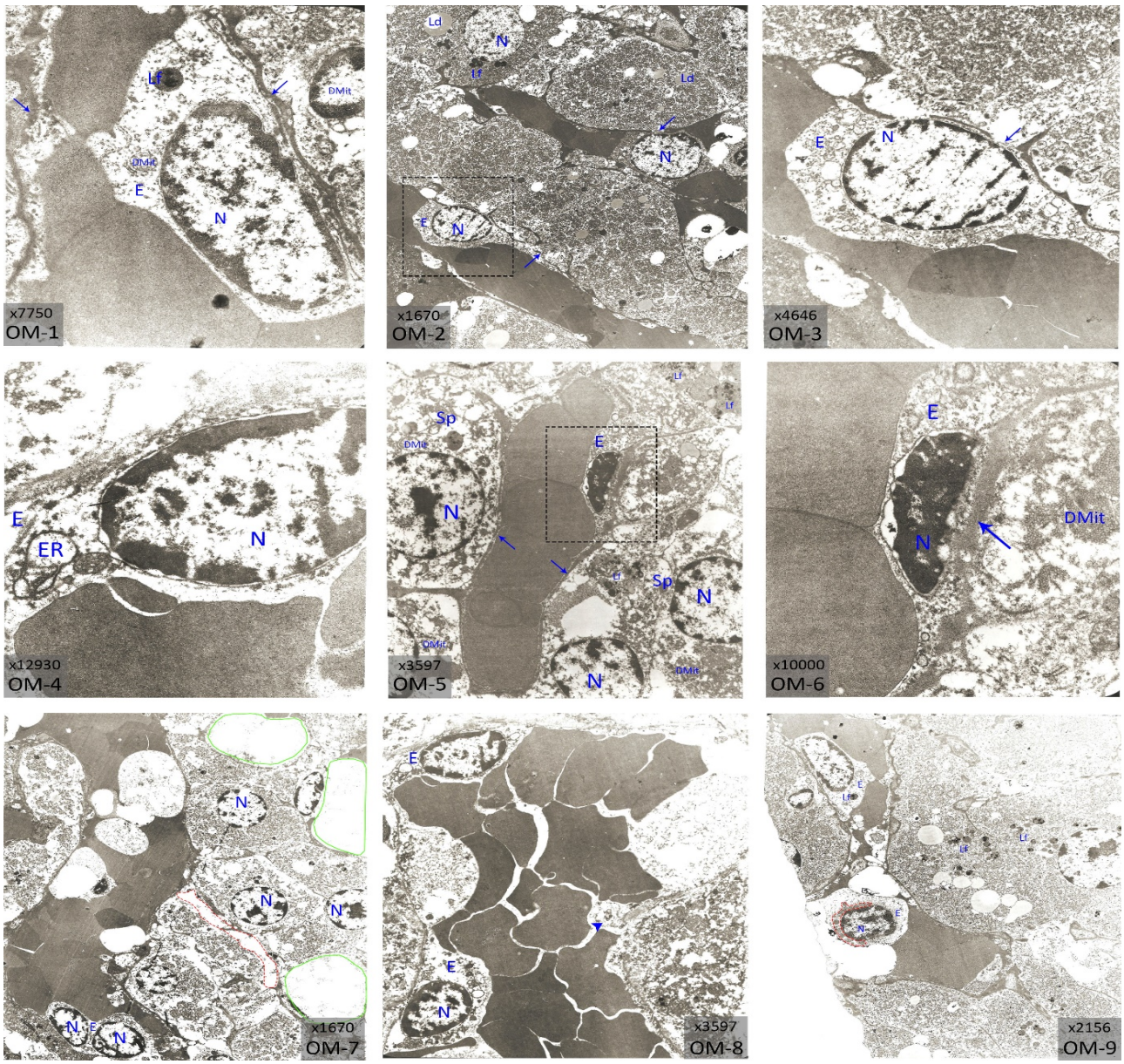

Figure 3. Electromicrographs of the elderly male group E: endothelial cell, Sp: spongiocyte, ZR: zona reticularis, L: lysosome, Lf: lipofuscin, Ld: lipid droplets, N: nucleus, Mit: mitochondrion, ER: endoplasmic reticulum, DMit: degenerated mitochondrion, →: basement membrane

DISCUSSION

Age-related changes in the endothelial cells of the adrenal gland have been suggested to play an important role in transcapillary transport under extreme conditions. These changes in the endothelium reflect the deterioration in energy production and protein synthesis in old animals. The effect of these changes has been the facilitation of the progression of pathological formations in the glands in old animals (Stupina and Shaposhnikov, 1981). One study suggested that the loss of function of the adrenal cortex in humans has been associated with senescence and apoptosis. The causes that may be responsible for the development of senescence and apoptosis are the development of ischaemia-reperfusion injury and its consequences as a result of impaired circulation. As a result of ischaemia-reperfusion injury, increased ROS, endothelial leakage, extravasation, and inflammation are observed. It is thought that such damage, which recurs throughout life, leads to the loss of adrenocortical cells (Hornsby, 2002). A similar study reported that transient ischaemia can cause deterioration of the adrenal cortex over the years. In addition, selective apoptosis of ZR was thought to be related to increased expression of MHC-II and Fas (CD95) ligands with age, and cellular aging in general was thought to be the result of telomere shortening (Hornsby, 2004). In our study, spongiocyte degeneration and vessel wall separation were

observed. Disorders in the vessel wall disrupted the permeability of the endothelium and led to extravasation. In addition, stasis formations were also present in our study. It is possible that the endothelial cells lining the lumen in our study may contribute to circulatory disturbances by narrowing the vessel.

A study analyzing the ultrastructural structures of secretory cells in aging endocrine glands reported fibrillar formations in the nuclei of adrenal spongiocytes and mitochondrial cristae in both normal and reduced amounts (Shaposhnikov, 1985). In our study, darkening in the form of streaks was observed in the nuclei of some cells. Mitochondria in spongiocytes had both normal and degenerated appearance. In a study conducted in rats, it was reported that dead parenchymal cells protruded into the vessel lumen as a result of endothelial damage in adrenal glands (Chen-Pan et al., 1996). In our study, similar ruptures in the vessel wall and cell contents in the lumen were observed.

In a study in rats, hypertrophy was observed in ZF and ZR, which increased with age. Hypertrophy in ZF and ZR was associated with the proliferation of mitochondria and smooth ER involved in steroid hormone synthesis. Increased lipofuscin accumulation in the ZR with age and markedly increased lipid droplets in old ZF and ZR cells were reported (Rebuffat et al., 1992). A study conducted on males reported that ZR occupies a larger area in the adrenal cortex in young than in older animals. The ratio of ZF and ZG to the zona reticularis is lower in young than in old (Parker et al., 1997). We also observed the accumulation of lipofuscin and lysosomes in ZR cells. The increase in the number of mitochondria and lipid droplets in spongiocytes with age is in accordance with previous studies. We can conclude that the adrenal gland tries to compensate for the decreasing number of cells due to aging through cellular hypertrophy. Lipofuscin increase is an indicator of cellular aging in spongiocytes. As the spongiocytes respond to the decreasing number of cells by hypertrophy, they prolong cell life more than expected and the number of lipofuscin-like pigments also increases. The decrease in volume of the ZR may be due to its hypersensitivity to the decrease in blood supply due to endothelial damage. Because, as mentioned above, the vessels that come to the ZR come after passing through the ZG and ZF in the capsule part, a previous disturbance in the blood supply affects the ZR the most. Also, there are studies showing that changes in the endothelium with age impair blood supply (Scioli et al., 2014).

Decreased mitochondrial function with age leads to an increase in reactive oxygen species (ROS) and triggers vascular inflammation in endothelial cells (Ungvari et al., 2008). We also observed degeneration and cristae lysis of mitochondria in endothelial cells, some of which were accompanied by heterochromatin-rich regions in the nucleus and disruption of the perinuclear space. We hypothesized that all of this caused dysfunction in the endothelial cells, leading to circulatory disturbances. It can be concluded that circulatory disturbance leads to ischaemia-induced damage to adrenal cells and disruption of endocrine functions.

It has been described that age-related increases in cortisol and decreases in DHEA-S may be associated with the onset and progression of neurodegenerative diseases (Ferrari et al., 2001). It has been reported that aging affects glucocorticoid metabolism in a similar way to chronic stress, leading to neurological and cognitive changes, osteopenia, diabetes mellitus, visceral obesity, altered immune competence, and other disorders through cortisol (Yiallouris et al., 2019). We have mentioned that blood supply deteriorates with age. The effect of impaired circulation on the hypothalamus-pituitary-adrenal cortex axis may explain the changing hormone levels. In our study, we observed thickening of the basal lamina, deterioration of the endothelial cells, tears and separations in the vessel wall, and separations between the basal lamina and the intercellular spaces. These are all changes that can disrupt the exchange of substances between the spongiocytes and the circulation.

We did not find any gender differences in the changes that occur with the aging of the adrenal glands. However, we concluded that the degeneration of endothelial cells and vessels is relatively more severe in males. Although the reason for the difference between women and men is not well understood, it is known that the risk factor for estrogen-dependent cardiovascular disease is lower in women. However, we did not look at this in our study.

CONCLUSION

It is a fact that aging causes undesirable changes in the adrenal glands. It is known that some of these

changes are caused by cellular senescence, apoptosis, and telomere shortening that accompany aging. However, we can now say that changes occurring in the circulation as a result of endothelial aging are also responsible for these changes. Since the signals received by endocrine organs come from the blood and are secreted into the blood, they are directly affected by a circulatory disorder. In order to prevent age-related deterioration of the adrenal glands, blood flow must be fully guaranteed. In order to fully understand the effects of aging on the adrenal gland, designing and conducting a study to prevent endothelial damage will shed more light on the missing aspects of the subject.

REFERENCES

Augustin HG, Koh GY. Organotypic vasculature: From descriptive heterogeneity to functional pathophysiology. Science. 2017; 357(6353):2379.

Chen-Pan C, Yamamoto Y, Ito Y, Pan IJ, Hayashi Y. A new type of cell death, the protruded type, observed in the adrenal gland of normal rats. J Vet Med Sci. 1996; 58(4):373-376.

Costanzo L. Costanzo physiology. Philadelphia: Elsevier Health Sciences; 2021. pp.427-440.

Ferrari E, Cravello L, Muzzoni B, *et al.* Age-related changes of the hypothalamic pituitary adrenal axis: pathophysiological correlates. Eur J Endocrinol. 2001; 144(4):319-329.

Hall JE, Hall ME. Guyton and Hall textbook of medical physiology. Philadelphia: Elsevier Health Sciences; 2020. pp.955-972.

Hansen JT. Netter's clinical anatomy. Philadelphia: Elsevier Health Sciences; 2019. pp.203-204.

Hornsby PJ. Aging of the human adrenal cortex. Ageing Res Rev. 2002; 1(2):229-242.

Hornsby PJ. Aging of the human adrenal cortex. Sci Aging Knowledge Environ. 2004; 35:6.

Jameson JL, DeGroot LJ, De Kretser DM, *et al.* Endocrinology: adult and pediatric. 7th ed. Philadelphia: Elsevier Inc.; 2016; pp.2-26.

Kierszenbaum AL, Tres L. Histology and cell biology: An Introduction to Pathology. Philadelphia: Elsevier Health Sciences; 2019. pp. 593-601.

L Mescher A. (2013). Junqueira's Basic Histology: Text and Atlas. United States: McGraw-Hill Companies, Inc.; 2013; pp.354-359.

Parker CR, Mixon RL, Brissie RM, Grizzle WE. Aging alters zonation in the adrenal cortex of men. J Clin Endocrinol Metab. 1997; 82(11):3898-3901.

Rebuffat P, Belloni AS, Rocco S, *et al.* The effects of ageing on the morphology and function of the zonae fasciculata and reticularis of the rat adrenal cortex. Cell Tissue Res. 1992; 270(2):265-272.

Schaeffer M, Hodson DJ, Lafont C, Mollard P. (2011). Endocrine cells and blood vessels work in tandem to generate hormone pulses. J Mol Endocrinol. 2011; 47(2):59- 66.

Scioli MG, Bielli A, Arcuri G, Ferlosio A, Orlandi A. Ageing and microvasculature. Vasc Cell. 2014; 6:19.

Shaposhnikov VM. The ultrastructural features of secretory cells of some endocrine glands in aging. Mech Ageing Dev. 1985; 30(2):123-142.

Stupina AS, Shaposhnikov VM. Age and ultrastructural features of adrenal capillaries in rats following ACTH administration and in insulin shock. Biull Eksp Biol Med. 1981; 92(8):89-91.

Ungvari Z, Labinskyy N, Gupte S, *et al.* Dysregulation of mitochondrial biogenesis in vascular endothelial and smooth muscle cells of aged rats. Am J Physiol Heart Circ Physiol. 2008; 294(5):2121-2128.

Warde KM, Smith LJ, Basham KJ. Age-related Changes in the Adrenal Cortex: Insights and Implications. J Endocr Soc. 2023; 7(9):97.

Y**iallouris A, Tsioutis C, Agapidaki E, *et al.* (2019).** Adrenal aging and its implications on stress responsiveness in humans. Front Endocrinol (Lausanne). 2019; 10:54.

ACKNOWLEDGMENTS

We would like to thank Prof. Dr. Gülten Karabay and Prof. Dr. Attila Dağdeviren for guiding us in this study. We also thank the technicians at Başkent University Histology and Embryology Department for their help in laboratory studies.

Author contributions: NU designed the study. NU performed surgeries. NU and AŞ performed the literature review, interpretation, writing and editing, analysis, and critical review. NU and AŞ participated in drafting and revising the manuscript. NU: Nejat Ünlükal, AŞ: Abdullah Şentürk

Financial Disclosure: The authors declared that this study has received no financial support.

Conflict of Interests: The authors declared that there is no conflict of interests.

Additional information: All authors have read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to NU.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Original Article https://doi.org/10.47748/tjvr.1484775

TJVR 2024; 8 (2): 121-127

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Cochlear lateral wall changes secondary to hypercholesterolemia and noise exposure in the chinchilla model

Nevra Keskin Yilmaz[1,2] Rafael da costa Monsanto[2]

[1] *Department of Internal Medicine, Faculty of Veterinary Medicine, Ankara University, Ankara, Türkiye*
[2] *Department of Otolaryngology Head & Neck Surgery, University of Minnesota, Minneapolis, USA*

Correspondence: *Nevra Keskin Yilmaz (keskinnevra@gmail.com)*

Received: 16.05.2024 Accepted: 09.07.2024

ABSTRACT

Objective: To investigate the effects of hypercholesterolemia on the cochlear lateral wall structures in chinchillas and its impact on the susceptibility of the inner ear structures to noise exposure.

Materials and Methods: Fifteen chinchilla temporal bones were selected from the Animal Temporal Bone Collection of the Paparella Otopathology and Pathogenesis Laboratory at the University of Minnesota. The experimental group was subjected to 3-month 1% cholesterol diet, while the control group maintained a standard diet. After 3 months, the experimental group's left ears exposed to noise trauma for 10 minutes while right ears did not. One month later the animals were euthanized, and the temporal bones harvested from the animals underwent histopathological examination with morphometric assessments of stria vascularis (SV) and spiral ligament (SL).

Results: Histopathological analysis revealed no significant differences ($p>0.05$) in total SL area across cochlear turns between the experimental and control groups. However, distinct variations were observed in SV area within the lower basal ($p<0.01$) and upper basal ($p = 0.01$) turns of the hypercholesterolemia and noise-exposed group compared to the control group.

Conclusion: Hypercholesterolemia is one of the conditions that may contribute to sensorineural hearing loss. This study highlights its importance in auditory health by revealing the relationship between hypercholesterolemia and cochlear lateral wall structures, and increased susceptibility to noise-induced damage.

Keywords: *Hypercholesterolemia, Noise exposure, Cochlear lateral wall, Chinchilla*

INTRODUCTION

In recent years, hypercholesterolemia has emerged as a significant research topic, necessitating detailed investigation into the condition to uncover its growing prevalence and complex interaction with various health parameters. More than a century ago, Virchow's revelation that atheroma contains a yellow fatty substance, later identified as cholesterol by Windaus, pointed the involvement of lipids in the development of atherosclerosis (Mayerl et al., 2006). The early recognition of cholesterol's pivotal role in arterial plaques laid the foundation for the cholesterol hypothesis in understanding the pathogenesis of atherosclerosis (Axelsson and Lindgren, 1985). Numerous researchers exploring the potential link between high serum cholesterol levels and hearing loss have observed a correlation. These researchers suggest that elevated blood lipids may impact hearing by triggering premature widespread atherosclerosis in major arteries and vessels that

supply blood to the auditory organ (Lee et al., 2023).

In the late 1970s and early 1980s, Morizono and colleagues conducted studies investigating the effects of a high-cholesterol diet on auditory function in rabbits and chinchillas (Morizono and Paparella, 1978; Morizono and Sikora, 1978; Morizono et al., 1985). These studies found that animals subjected to a cholesterol-rich diet exhibited a progressive decline in auditory evoked potentials, particularly affecting higher frequencies.

The cochlear lateral wall, including the spiral ligament (SL) and stria vascularis (SV), plays a key role in maintaining cochlear fluid balance. This process involves four distinct capillary networks within the SL and SV, crucial for modulating cochlear endolymph homeostasis (Hosoya et al., 2023). These capillaries are essential for regulating sensory hair cell transduction by controlling endocochlear potential, ion transport, and maintaining fluid balance in the endolymph (Wangemann, 2006; Peeleman et al., 2020). Importantly, the cochlear wall is sensitive to noise exposure, primarily due to potential disruptions in cochlear microcirculation following acoustic trauma (Hirose and Liberman, 2003; Yu et al., 2021). However, no study has quantitatively analyzed changes in the cochlear lateral wall (to the best of our knowledge), impeding a complete understanding of the specific mechanisms underlying alterations in the cochlea due to hypercholesterolemia and noise exposure.

The main goal of this study was to evaluate the influence of hypercholesterolemia on cochlear lateral wall through histopathological analysis of the temporal bones in the chinchilla model. Additionally, this research aimed to conduct further analysis on cochlear changes in the context of exposure to acoustic trauma in the presented hypercholesterolemia model.

MATERIALS and METHODS

Animal temporal bone specimens

A total of 15 temporal bones from 10 adult chinchillas (*Chinchilla lanigera*) were selected from the Animal Temporal Bone Collection of the Paparella Otopathology and Pathogenesis Laboratory at the University of Minnesota.

In the presented study, the experimental group of animals had *ad-libitum* access to a diet comprising 1% cholesterol, which was incorporated into the standard Chinchow and custom-blended by the manufacturer (Ralston-Purina). This dietary regimen was maintained for a duration of 3 months, while the control group, consisting of 5 animals, was kept on a standard diet. After dietary intervention, the animals in the experimental group (n=5) were divided into two subgroups.

The right ears of these chinchillas constituted the first subgroup to explore the changes in hypercholesterolemia condition while the second group, including the left ears, were additionally exposed to noise.

For chinchillas included in the noise exposure subgroup, the animals were anesthetized with ketamine hydrochloride (20 mg/kg) and sodium pentobarbitol (30 mg/kg). Only the left ears of the animals in the experimental group underwent a 12-kHz pure-tone stimulation at 95 dB SPL for 10 minutes. One month later, serum samples were collected from all animals, and they were euthanized using a high dose of ketamin-HCl injection. Detailed information regarding our experimental methodology has been documented in a previous study conducted by our laboratory (Morizono and Sikora, 1982).

Following euthanasia, temporal bones underwent further processing, including perfusion, fixation, decalcification, embedding, and horizontal sectioning at a thickness of 20 μm. Every 10th section was stained with hematoxylin-eosin to enable histological analysis for comparisons among the high-cholesterol-fed group (Group 1), the high-cholesterol-fed and noise-exposed group (Group 2), and the control group (Group 3).

Analysis of the cochlear lateral wall

At the mid-modiolar level of all cochlear turns and the two neighboring sections, morphometric assessments of the SV and SL were conducted. Images were captured at either ×40 or ×100 magnification using a digital camera, and image analysis software (SPOT Advanced, SPOT Imaging Solutions, MI, USA) was employed on a computer to quantify the cut surface areas of the SV and SL.

Additionally, the average loss of fibrocytes in the SL on sections at the mid-modiolar level was assessed, following the techniques outlined by Hequembourg and Liberman (2001). A classification scale was utilized to evaluate fibrocyte loss in the SL, ranging from 0 for normal conditions (missing less than 1/3 of fibrocytes) to 3 for severe or complete loss of fibrocytes on mid-

modiolar-level sections. The calibrated image was captured at an original magnification of ×40.

Statistical analysis

The Kruskal-Wallis test was employed to assess group differences. Post-hoc pairwise comparisons using the Dunn-Bonferroni test were conducted to identify specific group differences in the significant results. All statistical analyses were carried out using SPSS 23.0 software for Windows (SPSS Inc., Chicago, IL), with statistical significance established at a P-value less than 0.05.

Ethical approval

Studies utilizing our archival collection are University of Minnesota Institutional Review Board and IACUC-exempt, as they originate from previously approved protocols (Decision ID: 00003249).

RESULTS

The serum cholesterol levels in the included animals exhibited a nearly 3.5-fold increase ($p<0.01$) within the high-cholesterol group compared to the control group after a 3-month dietary intervention. This finding suggests clinical hypercholesterolemia in the experimental group, consistent with a prior study conducted by our laboratory (Morizono and Sikora, 1982). Additionally, despite considerable variability in the obtained values, there were noteworthy elevations in glucose, alanine transaminase (ALT) and aspartate transaminase (AST)levels in the animal group fed with a cholesterol diet.

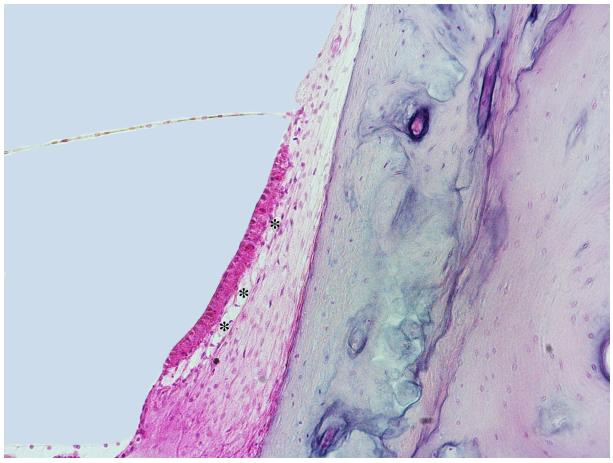

Figure 1. The separation of the SV and fibrous stroma (x20). *: shows the large empty-looking areas. Staining with hematoxylin-eosin (H&E)

Table 1. Median, minimum (min), and maximum (max) values of the total areas (μm²) for the Spiral ligament and Stria vascularis within each respective group. The rows in bold indicate the statistical significance ($p<0.05$) when compared to third group.

Cochlear turns	Groups	Spiral ligament			Stria vascularis		
		Median	min	max	Median	min	max
Lower basal	1	74152.89	68787.71	78639.86	4677.7	4150.25	5596.34
	2	74919.87	67693.44	76247.89	**4130.14**	**4061.07**	**4247.3**
	3	68694.6	56067.31	77573.12	5497.08	4350.72	6652.5
Upper basal	1	72717.63	60069.1	76886.04	4651.84	4042.18	5599.5
	2	72357.41	63218.35	75864.53	**4159.88**	**3564.33**	**5444.82**
	3	62589.19	51404.16	68905.71	5310.86	5039.23	5913.15
Lower middle	1	59295.31	49582.31	63436.09	4887.06	4289.66	5396.17
	2	56631.62	49099.46	63417.08	4044.67	3143.09	5557.34
	3	49761.68	45721.79	55008.83	5405.3	5405.3	6135.12
Upper middle	1	46668.39	41847.65	58347.41	3396.32	2736.86	4130.49
	2	48332.04	41882.49	56880.32	3385.65	2568.22	4531.77
	3	39097.88	33344.15	49961.94	4499.2	3685.54	4690.01
Apical	1	33291.01	25937.7	37786.27	2707.26	2499.89	3887.57
	2	25991.44	23259.39	40028.47	3649.16	2527.8	4330.89
	3	25417.55	23849.5	32271.02	3513.89	3396.63	4425.27

In our histopathological analysis, no significant differences ($p>0.05$) were identified between the groups regarding the total SL area or total fibrocyte loss across any cochlear turn (Table 1). In the first and second groups, large empty-looking areas surrounding Type I and type II fibrocytes were observed, leading to a reduction in intercellular contacts and the separation of the SV and fibrous stroma. This finding was evident in the lower basal turn, observed in 3 out of 5 samples in the first group and 4 out of 5 samples in the second group (Figure 1). Similarly, in the upper basal turn, the pathology was observed in 2 out of 5 samples in the first group, while it was evident in 4 out of 5 samples in the second group. Notably, no observable pathology was detected in any cochlear turns within the third group.

Furthermore, the mean area of SV was measured, and statistical analysis were performed among all three groups (Table 1). Specifically, we found significant differences in the lower basal ($p<0.01$) and upper basal ($p=0.01$) turns of the SV area within the second and third groups (Figure 2). No differences were observed in the remaining cochlear turns between these two groups. Additionally, the second group showed a significant decrease in the mean area of SV compared to those in the first group only in the lower basal ($p=0.03$) turn. Although a slight decline in the SV area was seen in the first group, no significant differences were found between the first and third groups.

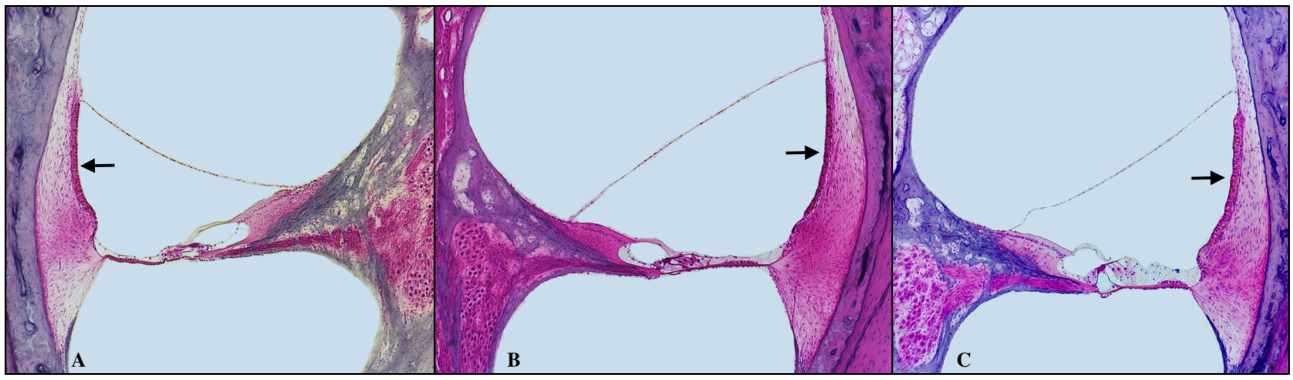

Figure 2. Photomicrographs showing the decrease in the mean area of SV within the representative basal turn of chinchilla temporal bones (x20). A: A section from hypercholesterolemia group. B: Section showing the decreased area in the hypercholesterolemia and noise exposed group. C: Section from non-diseased control group. The arrows specifically point to the SV. Staining with hematoxylin-eosin (H&E)

DISCUSSION

Hypercholesterolemia, characterized by elevated levels of cholesterol in the blood, can contribute to sensorineural hearing loss. The potential impact on auditory function involves multifaceted mechanisms, suggesting a complex interplay between elevated cholesterol levels and the development or exacerbation of sensorineural hearing impairment (Evans et al., 2006). Although prior research indicates that hyperlipidemia affects cochlear function and increases susceptibility to noise exposure, the exact mechanism explaining this correlation remains unclear (Morizono and Paparella, 1978; Sikora et al., 1986).

In this study, the chinchilla animal model was employed to induce a hypercholesterolemic condition. Hypercholesterolemia, a significant contributor to cardiovascular disease, not only affects the cardiovascular system but also has broader systemic impacts due to abnormal lipid levels (Kathak et al., 2022). In the presented study, in addition the hypercholesterolemia, increases in liver enzymes levels were observed. Serum ALT is a specific marker for hepatic damage primarily situated in the liver (Kathak et al., 2022), and AST, present in the cytoplasm of hepatocytes (Aulbach and Amuzie, 2017). The increases in these biomarkers indicate hepatic dysfunction due to dyslipidemia. Considering their role in energy metabolism, the close connection between glucose and lipid metabolism, and the elevated glucose level in the experimental group in the presented study, are not surprising. High glucose levels in hypercholesterolemia can result from insulin resistance, inflammation, beta cell dysfunction, oxidative stress, and fatty tissue dysfunction, collectively leading to increased blood sugar levels (Parhofer, 2015). These increases observed in serum analysis underline the complex relationship

between dyslipidemia and other systems in the presented model, providing valuable information about the broader effects of abnormal lipid levels.

In a previous study conducted in our laboratory investigating the cochlear effects of hypercholesterolemia utilizing the chinchilla model (Morizono et al., 1985), a noteworthy extension of P1 latency in the Auditory-Evoked Brainstem Response test was noted at various high frequencies in animals subjected to a cholesterol-rich diet, even without exposure to noise. Moreover, these cholesterol-fed animals exhibited increased vulnerability to noise-induced damage, particularly at extremely high frequencies, as evaluated through auditory perception. Our present research expands upon these earlier discoveries by investigating further into the impact on high cholesterol diet-fed animals. Notably, our investigation revealed specific changes in the cochlear lateral wall, particularly within the group exposed to noise.

Lateral wall structures take part in acoustic transduction in the mammalian cochlea. The cochlear lateral wall encompasses the SL and SV. The SL, comprising the fibrocytes, serves primarily as structural support for the cochlea (Peeleman et al., 2020) while SV plays an important role in preserving potassium homeostasis within the scala media in collaboration with SL (Locher et al., 2015). The diverse fibrocyte subtypes in the SL actively express various ion transporters and enzymes, contributing to K+ absorption and participating in an intricate gap junction network essential for the crucial K+ recycling process in endocochlear potential generation (Spicer and Schulte, 1996). In the presented study, while there was not a significant change in the mean area of SL between the groups, there was a significant pathology separating type I and type II fibrocytes and SV in the first and second groups compared to those in the third group. This anomaly presents itself as a pathological observation, with potential roots traced to the loss of basal cells that form the interface between the SV and SL, SV detachment, type I and type II fibrocyte loss in the SL, or the presence of edema. Nevertheless, across all scenarios, a diminished K+ supply to the SV emerges due to tight junctions hindering K+ movement from perilymph. This could pose a potential risk of compromising the K+ recycling pathway by disrupting the gap junction network that connects fibrocytes and the SV (Liang et al., 2005). Temporal bone studies, exemplified by Kusunoki et al. (2004), revealed a significant relationship between aging and fibrocyte loss in the basal turn in human temporal bones, potentially leading to hearing loss in the elderly. Furthermore, in a murine model, Schmutzhard et al. (2012) demonstrated that the impairment of type I fibrocytes in the SL may disrupt the blood labyrinth barrier, leading to the breakdown of the endocochlear potential and contributing to significant hearing loss. Moreover, exposure to loud noise then leads to an increase in the production of pro-inflammatory cytokines in type I and type II fibrocytes, suggesting that these cells play a vital role in regulating immune responses following exposure to loud sounds (Zhang et al., 2019). However, further investigation is needed to determine the contributing factors to this cochlear pathology in the context of elevated cholesterol diets and acoustic trauma.

The SV, a constituent of the lateral wall, accommodates crucial marginal, intermediate, and basal cells (Locher et al., 2015). The relationship between the SL and the SV is critical for establishing optimal conditions essential for cochlear hearing function (Peeleman et al., 2009). In the presented study, a notable reduction in the mean area of the SV was observed in the second group compared to that in the third group in the basal turn. In animal models, the isopotential curve of cochlear microphonics, action potential thresholds, and endocochlear potential showed no distinctions between the hypercholesterolemia and control groups. However, when subjected to moderately intense sound, the hypercholesterolemia group exhibited a significantly greater reduction in the thresholds (Morizono and Paparella, 1978; Morizono and Sikora, 1982). In a later study, consistent adherence to a high-cholesterol diet was reported to be associated with the likelihood of high-frequency hearing loss, potentially attributed to vascular pathology arising from a hyperlipidemic condition, indicating that hypercholesterolemia may play a role as a contributing factor in differential susceptibility to noise (Sikora et al., 1986). The strial degeneration observed in the basal turn in the presented study might help explain the threshold changes and high-frequency hearing loss identified in previous animal studies.

The development of hypercholesterolemia is linked to endothelial cell dysfunction, reduced vascular nitric oxide availability, increased oxidant stress, and the establishment of a highly pro-

inflammatory state (Zhang et al., 2019). In the case of noise exposure, cochlear microcirculation and lateral wall pathologies are influenced in varying ways depending upon the severity of the exposure, revealing associations with alterations in vasoactive factors and inflammatory responses within the cochlea (Shin et al., 2019). In the presented study, two conditions influencing cochlear microcirculation were assessed. However, it is crucial to note in the clinical context that various factors, including cardiovascular diseases and genetic disorders, may also impact the circulatory system.

This study is not without limitations. Firstly, the nature of the study (archival temporal bone samples) prevents the conduct of more comprehensive research. In addition, limited detailed procedural information regarding animal experiments in the archival files is restricting access to detailed data. Lastly, the unavailability of hearing analysis causes lack of detailed information to assess auditory function in the animals.

The data obtained by histopathological quantitative analyzes in this study has important outcomes, highlighting that noise exposure in the context of hypercholesterolemia induces changes in the lateral wall structure. The integrity of the lateral wall is critical for maintaining normal cochlear physiology and sensitivity. The histopathological alterations caused by noise in the lateral wall might play a crucial role in noise-related hearing loss. Attempting to understand the mechanisms that undergird damage to these lateral wall structures—a significant contributor to noise-induced hearing loss-may lead to innovative intervention approaches that extend beyond the preservation of hair cell integrity (Hirose and Liberman, 2003). This understanding has the potential to reshape our understanding of auditory vulnerabilities and present opportunities for preventive strategies against hearing loss stemming from excessive acoustic stimulation.

CONCLUSION

This study reveals remarkable histopathological changes in the cochlear lateral wall structures, highlighting the significant impact of hypercholesterolemia and its accompanying noise exposure. Understanding these changes may lead to new interventions targeting these structures and provide opportunities for innovative strategies to prevent noise-induced damage in the cochlea.

REFERENCES

Aulbach AD, Amuzie CJ. Biomarkers in nonclinical drug development. In: A comprehensive guide to toxicology in nonclinical drug development. Boston: Academic Press; 2017. pp.447-471.

Axelsson A, Lindgren F. Is there a relationship between hypercholesterolaemia and noise-induced hearing loss? Acta Otolaryngol. 1985; 100:379-386.

Evans MB, Tonini R, Shope CD, *et al.* Dyslipidemia and auditory function. Otol Neurotol. 2006; 27:609-614.

Hequembourg S, Liberman MC. Spiral ligament pathology: a major aspect of age-related cochlear degeneration in C57BL/6 Mice. JARO. 2001; 2:118-129.

Hirose K, Liberman MC. Lateral wall histopathology and endocochlear potential in the noise-damaged mouse cochlea. J Assoc Res Otolaryngol. 2003; 4:339-352.

Hosoya M, Iwabu K, Kitama T, Nishiyama T, Oishi N, Okano H, *et al.* Development of cochlear spiral ligament fibrocytes of the common marmoset, a nonhuman model animal. Sci Rep. 2023; 13:11789.

Kathak RR, Sumon AH, Molla NH, *et al.* The association between elevated lipid profile and liver enzymes: a study on Bangladeshi adults. Sci Rep. 2022; 12:1711.

Kusunoki T, Cureoglu S, Schachern PA, Baba K, Kariya S, Paparella MM. Age-related histopathologic changes in the human cochlea: a temporal bone study. Otolaryngol Head Neck Surg. 2004; 13:897-903.

Lee YY, Ha J, Kim YS, *et al*. Abnormal Cholesterol Metabolism and Lysosomal Dysfunction Induce Age-Related Hearing Loss by Inhibiting mTORC1-TFEB-Dependent Autophagy. Int J Mol Sci. 2023; 24:17513.

Liang F, Schulte BA, Qu C, Hu W, Shen Z. Inhibition of the calcium-and voltage-dependent big conductance potassium channel ameliorates cisplatin-induced apoptosis in spiral ligament fibrocytes of the cochlea. Neurosci. 2005; 135:263-271.

Locher H, de Groot JC, van Iperen L, Huisman MA, Frijns JH, Chuva de Sousa Lopes SM. Development of the stria vascularis and potassium regulation in the human fetal cochlea: Insights into hereditary sensorineural hearing loss. Dev Neurobiol. 2015; 75:1219-1240.

Mayerl C, Lukasser M, Sedivy R, Niederegger H, Seiler R, Wick G. Atherosclerosis research from past to present--on the track of two pathologists with opposing views, Carl von Rokitansky and Rudolf Virchow. Virchows Arch. 2006; 449:96-103.

Morizono T, Paparella MM. Hypercholesterolemia and auditory dysfunction. Annals Otol. Rhinol Laryngol. 1978; 87:804-814.

Morizono T, Sikora MA, Ward WD, Paparella MM, Jorgensen J. Hyperlipidemia and noise in the chinchilla. Acta Otolaryngol. 1985; 99:516-524.

Morizono T, Sikora MA. Experimental hypercholesterolemia and auditory function in the chinchilla. Otolaryngol Head Neck Surg. 1982; 90:814-818.

Parhofer KG. Interaction between Glucose and Lipid Metabolism: More than Diabetic Dyslipidemia. Diabetes Metab J. 2015; 39:353-362.

Peeleman N, Verdoodt D, Ponsaerts P, Van Rompaey V. On the role of fibrocytes and the extracellular matrix in the physiology and pathophysiology of the spiral ligament. Front Neurol. 2020; 11:580639.

Schmutzhard J, Kositz CH, Glueckert R, Schmutzhard E, Schrott-Fischer A, Lackner P. Apoptosis of the fibrocytes type 1 in the spiral ligament and blood labyrinth barrier disturbance cause hearing impairment in murine cerebral malaria. Malar J. 2012;11: 30.

Shin SA, Lyu AR, Jeong SH, Kim TH, Park MJ, Park YH. Acoustic trauma modulates cochlear blood flow and vasoactive factors in a rodent model of noise-induced hearing loss. Int J Mol Sci. 2019; 20:5316.

Sikora MA, Morizono T, Ward WD, Paparella MM, Leslie K. Diet-induced hyperlipidemia and auditory dysfunction. Acta Otolaryngol. 1986; 102:372-381.

Spicer SS, Schulte BA. The fine structure of spiral ligament cells relates to ion return to the stria and varies with place-frequency. Hear Res. 1996; 100:80-100.

Stapleton PA, Goodwill AG, James ME, Brock RW, Frisbee JC. Hypercholesterolemia and microvascular dysfunction: interventional strategies. J Inflamm (Lond). 2010; 7:54.

Wangemann P. Supporting sensory transduction: Cochlear fluid homeostasis and the endocochlear potential. J Physiol. 2006; 576:11-21.

Yu W, Zong S, Du P, *et al.* Role of the stria vascularis in the pathogenesis of sensorineural hearing loss: a narrative review. Front Neurosci. 2021; 15:774585.

Zhang G, Zheng H, Pyykko I, Zou J. The TLR-4/NF-kappaB signaling pathway activation in cochlear inflammation of rats with noise-induced hearing loss. Hear Res. 2019; 379:59-68.

ACKNOWLEDGMENTS

Authors would like to thank to Michael M. Paparella, MD and Sebahattin Cureoglu, MD for their support in the study.

Author contributions: NKY and RM, designed the study. NKY performed quantitative and statistical analysis. NKY and RM participated in drafting and revising the manuscript. NKY: Nevra Keskin Yilmaz, RM: Rafael da Costa Monsanto.

Financial Disclosure: We would like to acknowledge our funding sources: NIH NIDCD U24 DC020851-01, International Hearing Foundation, Lions 5m International, and Scientific and Technological Research Council of Türkiye (TUBITAK) (Scholarship for NKY).

Conflict of Interests: The authors declared that there is no conflict of interests.

Additional information: All authors have read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to NKY.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Original Article https://doi.org/10.47748/tjvr.1500593

TJVR 2024; 8 (2): 129-136

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

The genetic characterization of *Lake Sinai Virus* in colony losses apiaries in Türkiye

Dilek Muz[1] Mustafa Necati Muz[2]

[1] *Department of Virology, Faculty of Veterinary Medicine, Tekirdağ Namik Kemal University, Tekirdağ, Türkiye*
[2] *Department of Parasitology, Faculty of Veterinary Medicine, Tekirdağ Namik Kemal University, Tekirdağ, Türkiye.*

Correspondence: *Dilek Muz (dilekmuz@nku.edu.tr)*

Received: 13.06.2024 Accepted: 09.08.2024

ABSTRACT

Objective: Honeybees (Apis mellifera) have a unique role in natural pollination and maintaining biodiversity in the ecosystem. The alarming increase in unexpected colony losses, unexpected bee deaths, and the tragic extinction of entire colonies (Colony Collapsed Disorder- CCD) have sounded a global alarm, demanding immediate attention and collaborative action to address these critical challenges in bee breeding. Diseases, parasites, and pathogens significantly threaten colony health. Türkiye is a significant honey producer, providing an ideal environment for beekeeping due to its unique eco-geographical features. Unexpected colony losses and bee deaths are also questions of concern for beekeepers in Türkiye.

Materials and Methods: This study investigated honey bee viruses in apiaries experiencing sudden bee death losses and CCD-like symptoms in Türkiye (Bursa, Edirne, Kocaeli Mersin, Tekirdağ and Zonguldak), involving genetic analysis of the *Lake Sinai Virus* (LSV) RdRp gene region. The honeybee samples were obtained from 52 colonies in 26 apiaries complaining of unexpected bee deaths and CCD-like symptoms between May 2021 and September 2023.

Results: The results showed a high *Deformed wing virus* (DWV), *Black queen cell virus* (BQCV), and LSV prevalence, respectively. The sampled apiaries were infested mild-moderate- high grade with Varroa mites. Following PCR results, DWV, BQCV, LSV, *Israel acute bee paralysis virus*, *Chronic bee paralysis virus* and *Sacbrood virus* positivity was detected at 69.2% (n=18), 50% (n=13), 38.46% (n=10), 26.9% (n=7), 19.2% (n=5) and 3.8% (n=1), respectively. High rates of multiple virus coexisting and high varroa infestation were noted in colonies with heavy losses and CCD-like complaints. The RdRp gene from two LSV samples (TrLSV-6474, TrLSV-6517) was sequenced. Turkish LSV samples (TrLSVs) showed a 72.88% homology of each other and clustered LSV4 branches in the phylogenetic tree. Turkish LSV sequences showed a closer similarity rate than reference sequences in GenBank with Asian Korean, Chinese, and Japanese LSV sequences.

Conclusion: Further investigation is needed to comprehend the implications of elevated LSV populations on colony losses. The execution of genetic research with a more extensive sample size can significantly enhance the demonstration of species diversity and provide valuable insights into the influence of LSV variants on honeybee health and the management of diseases.

Keywords: *Honeybee, Virus, Lake Sinai virus, RdRp gene*

INTRODUCTION

Honeybees (*Apis mellifera*) have a unique role in natural pollination, contributing to sustainable biodiversity in the ecosystem. These incredible insects offer a range of highly sought-after products, including invaluable honey, the resinous compound propolis, the nutrient-rich bee bread known as Perga, the potent royal jelly, and the biologically active apilarnil. These products are of significant socioeconomic value and possess

remarkable medicinal properties, making honeybees an invaluable asset to humanity (Klein et al., 2007). However, the ability of honeybee colonies to sustain their productivity is intricately linked to the overall health and well-being of the bees themselves. The alarming increase in unexpected colony losses, mysterious bee deaths, and the tragic extinction of entire colonies (Colony Collapsed Disorder- CCD) have sounded a global alarm, demanding immediate attention and collaborative action to address these critical challenges in bee breeding and ensure the survival of these indispensable pollinators (Genercsh, 2010; Neumann and Carreck, 2010). Diseases, parasites, and pathogens significantly contribute to unexpected honey bee colony losses (Daniat et al., 2012). The *Varroa destructor* mite is a significant threat to honey bees at every stage of their development. The ectoparasitic mites cause extreme damage to colony health by feeding on bees' essential "fat body". Their excessive consumption behavior results in the fatality of developing bees within the confines of brood cells, and it facilitates the transmission of numerous bee viruses, thereby further compromising the colony's resilience (Ramsey et al., 2019). Varroa mite infestation has serious consequences for bee populations. The mites act as vectors for many viruses and put a lot of strain on colonies through their parasitic activities. This leads to a series of negative impacts, exacerbating existing environmental pressures worse and posing a significant threat to the delicate balance of beekeeping practices (Dainat et al., 2012).

To date, numerous viruses have been identified in bee colonies. Some viruses threaten sustaining colony health (Martin et al., 2013; McMenamin and Genersch, 2015). *Acute bee paralysis virus* (ABPV), *Black queen cell virus* (BQCV*), Chronic bee paralysis virus* (CBPV), *Deformed wing virus* (DWV), *Kashmir bee virus* (KBV), *Lake Sinai Virus* (LSV), and *Sacbrood virus* (SBV) are frequently detected in apiaries. These viruses have a single-stranded positive-sense RNA genome. ABPV, BQCV, KBV, and IAPV belong to the *Dicistroviridae* family in the *Picornavirales* order, while DWV and SBV are classified in the *Iflaviridae* family within the same order. CBPV is an unclassified RNA virus, sharing similarities with the *Nodaviridae* and *Tombusviridae* families. Bee viruses can remain in a bee's body without causing clinical symptoms through latent (covert) infection. Some viruses can only become harmful due to specific environmental stress factors, such as pesticides, food scarcity, and climate change (Genercsh and Aubert, 2010; Daniat et al., 2012).

Although viruses generally exist in the colony with latent (cover) infections, they can lead to epidemics (overt) disease when the colony is subjected to stress factors. However, highly virulent and contagious viruses can lead to unexpected colony losses, sudden deaths, and detrimental effects on colony health and productivity (Genersch and Aubert, 2010). The presence of co-pathogens and mixed infections can impact losses, and the pathogenic effects of many viruses, especially DWV, have been identified (Muz and Muz, 2023a; Muz and Muz, 2023b).

LSV was initially detected in honeybee colonies during a metagenomic survey in America (Runckel et al., 2011), there has been a notable increase in reports of LSV in colonies in recent years (Daughenbaugh et al., 2015; Ravoet et al., 2015; Tozkar et al., 2015). LSV has a single-stranded positive-sense RNA genome and is classified in the *Sinhaliviridae* family within the *Nodamuvirales* order (Walker et al., 2020). It demonstrates genetic diversity based on classifying the viral RNA-dependent RNA polymerase (RdRp) gene, LSV1-8 (Daughenbaugh et al., 2015). There is still a lack of information regarding the pathogenesis and clinical characteristics of LSV in bees. However, its presence has also been observed in colony losses and weak and collapsed colonies (Runckel et al., 2011; Cornman et al., 2012; Daughenbaugh et al., 2015; Glenny et al., 2017).

Türkiye is a significant honey producer, providing an ideal environment for beekeeping due to its unique eco-geographical features. Reports of colony losses, sudden deaths, and cases resembling CCD have been linked to pathogens, viruses, and Varroa mites (Muz and Muz, 2009; 2017; 2022; 2023b; Giray et al., 2010; Kalaycı et al., 2020). The occurrence of LSV has been reported by based PCR method in some studies in apiaries in Türkiye (Tozkar et al., 2015; Çağırgan et al., 2022; Mayack and Hakanoğlu, 2022; Muz and Muz, 2022; 2023a). There is still insufficient information about the genetic diversity of LSV field isolates, their virulence properties, and their effects on bee deaths and colony losses. In this study, we investigated several honey bee viruses in apiaries that had experienced unexpected colony losses between 2021 and 2023. The research involved the molecular characterization and phylogenetic

analysis of the LSV RdRp gene region obtained from these apiaries.

MATERIALS and METHODS

Sampling area and sample collection

The research focused on apiaries experiencing colony losses from six locations in Türkiye: Bursa, Edirne, Kocaeli, Mersin, Tekirdağ, and Zonguldak (Figure 1). The research sampling took place between May 2021 and September 2023. Sampling occurred in 26 apiaries where colony loss and unexpected bee death complaints were reported. In each apiary, 52 colonies were sampled, including one colony with symptomatic bees (deformed wings, inability to fly, trembling, paralysis, blackened bees) and one colony without symptomatic bees. Worker bees (n=10) and mite samples were collected from each colony. Moreover, it examined climatic data, breeding type, and feeding patterns when colony losses occurred. The bee samples designated for molecular testing were carefully stored at -80 °C until they were ready for analysis.

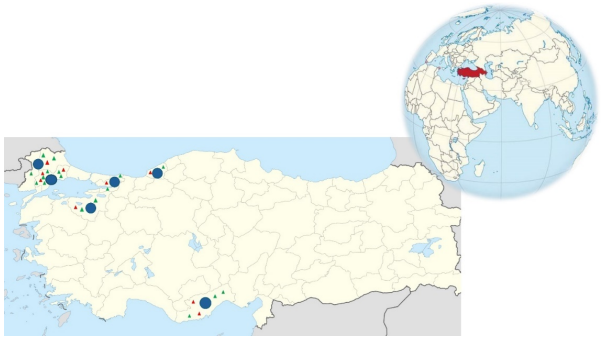

Figure 1. The map indicates the sampling provinces by blue circles. The sampled apiaries are denoted by triangles, and apiaries testing positive for LSV are marked in red.

Varroa destructor detection

The assessment of *Varroa destructor* infestation levels was conducted using two primary methods. Firstly, a sample of one hundred live honey bees was collected and introduced into a jar. Subsequently, 70% ethyl alcohol was added to the jar. The jar's contents were then thoroughly agitated to remove Varroa mites from bees. After a settling period, a meticulous count of the Varroa mites settled at the bottom of the jar was performed.

In addition, twenty sealed brood cells were carefully opened within each bee colony to inspect for the presence of Varroa mites visually. This two-pronged approach allowed for a comprehensive evaluation of mite infestation levels.

Based on the collected data, a classification system was employed to categorize the severity of infestation

* **High Infestation: Colonies exhibiting more than seven mites per one hundred bee sample.

* **Moderate Infestation: Colonies exhibiting four to six mites per one hundred bee sample.

* **Mild Infestation: Colonies exhibiting one to three mites per one hundred bee sample.

RNA extraction, Reverse Transcriptase-PCR and PCR

A total of 52 colonies from 26 apiaries were tested. Five bee pools were homogenized in PBS for a colony, and RNA was extracted from bee samples for molecular analysis. According to the kit's protocol, RNA samples were prepared using a commercial kit (GeneJET RNA Purification Kit, Thermo). RT-PCR was achieved using a cDNA Synthesis Kit (RevertAid First Strand, Thermo) according to the suggested protocol. PCR tests were applied to the cDNA samples obtained to detect honeybee viruses.

The reaction mixture was prepared, including 5u Taq polymerase, 10x Taq buffer, 3mM MgCl2, 300 pmol of dNTP mix, and sterile water in a 30 µl final volume. Virus-specific primer pairs for ABPV, BQCV, CBPV, DWV, IAPV, KBV, LSV, SBV were used for the mixture based on previously reported (Table 1). The reaction conditions involved previously reported Muz and Muz (2023a). After amplifying the PCR products, agarose gel electrophoresis was performed and the results were visualized using a UV transilluminator. To perform sequence analysis, positive DNA bands from the gel were purified using an extraction kit (GeneJet Gel Extraction Kit, Thermo) and then analyzed using an Applied Biosystems DNA analyzer. The sequences were examined using the BLAST tool, BioEdit program (v.7.2.5), and MEGA XI program. It was made with other strains deposited in the GenBank nucleotide database to compare and align sequences. The phylogenetic analysis utilized the neighbor-joining method with Kimura's two-parameter model (Kimura, 1980), and 1000 replicates were used for bootstrapping purposes (Tamura et al., 2021).

Table 1. The primer pairs utilized in the RT-PCR protocols within this study, along with the targeted genes and relevant literature information, are detailed in the provided table.

Virus	Amplified Region	Primer Sequence (5′-3′)	Length (bp)	Reference
ABPV	*Capsid protein*	F: GTGCTATCTTGGAATACTAC R: AAGGYTTAGGTTCTACTACT	618	Berenyi et al. 2006
BQCV	*Nonstructural polyprotein*	F: AGTAGTTGCGATGTACTTCC R: CTTAGTCTTACTCGCCACTT	472	Berenyi et al. 2006
CBPV	*RdRp*	F: TGTCGAACTGAGGATCTTAC R: GACCTGATTAACGACGTTAG	315	Berenyi et al. 2006
DWV	*Helicase*	F: ATCAGCGCTTAGTGGAGGAA R: TCGACAATTTTCGGACATCA	702	Chen et al. 2004
IAPV	*IGR*	F:GGTTGGCTGTGTGTCATCAT R:CGATGAACAACGGAAGGTTT	767	Palacios et al. 2008
KBV	*Nonstructural polyprotein*	F: GATGAACGTCGACCTATTGA R: TGTGGGTTGGCTATGAGTCA	415	Stoltz et al. 1995
LSV	*RNA dependent RNA polimerase*	F: CGTGCGGACCTCATTTCTTCATGT R: CTGCGAAGCACTAAAGCGTT	152	Daughenbaugh et al. 2015
SBV	*Structural protein*	F: ACCAACCGATTCCTCAGTAG R: CCTTGGAACTCTGCTGTGTA	487	Berenyi et al. 2006

RESULTS

As PCR results, in 26 apiaries tested, positivity for BQCV, DWV, CBPV, IAPV, LSV, SBV of the eight viruses investigated was detected. All apiaries were found negative for ABPV and KBV (Figure 2). The presence of DWV was detected in 18 (69.2%) of the apiaries with the highest positivity rate. Following this, BQCV, LSV, IAPV, CBPV, and SBV positivity was 50% (n=13), 38.46% (n=10), 26.9% (n=7), 19.2% (n=5) and 3.8% (n=1), respectively. LSV was detected during the experiment period.

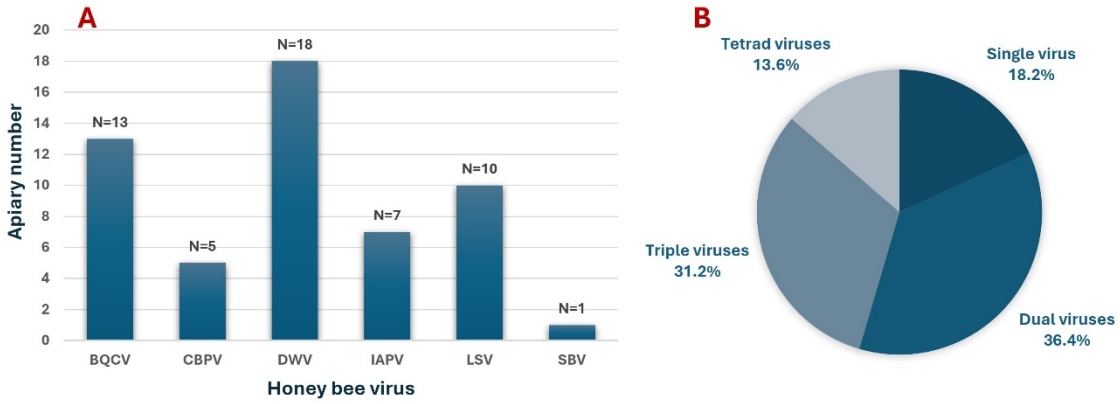

Figure 2. A: The honeybee virus positivity rates of the sampled apiaries are shown. B: The distribution and percent of single and multiple viruses coexist are shown.

All sampled apiaries were positive for varroa mites with changing intensity (mild, moderate, high infested) (Table 2). A mild varroa infestation was found in 12 apiaries, a moderate varroa infestation in 7 apiaries, and a high varroa infestation in 7 apiaries. Multiple viruses were also detected in apiaries with high varroa infestation. Complaints of bee deaths and colony loss were reported in all sampled apiaries. CCD-like symptoms were observed in 6 apiaries. High varroa infestation and multiple viruses were identified in apiaries with CCD-like symptoms. The existence of mixed infections, where more than one virus coexists, is noteworthy. 22 out of 26 sampled apiaries tested positive for at least one virus, which accounts for 84.61% of the total. The four (18%) apiaries were

positive for a single virus. These positivities were for DWV (n=3) and BQCV (n=1). In positive apiaries, dual, triple and tetrad virus positivity was detected in 8 (36.4%), 7 (31.2%) and 3 (13.6%) cases, respectively. Multiple virus positivity was also detected for at least two viruses in LSV-positive apiaries (n=10) (Table 2, Figure 2B).

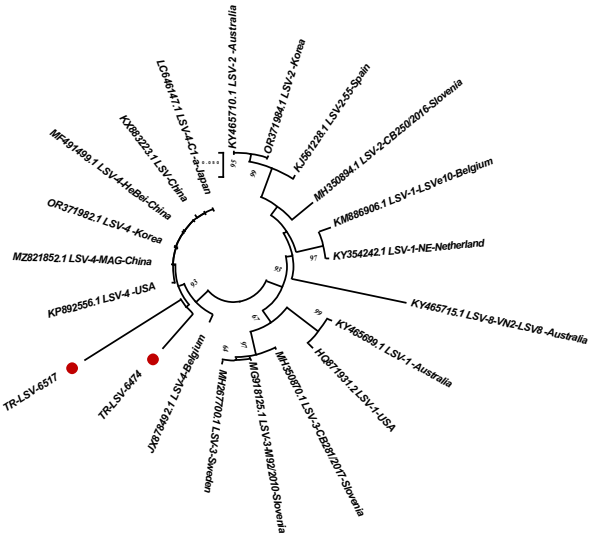

Figure 3. The phylogenetic tree of the LSV partial RdRp gene region was constructed using the neighbor-joining method and Kimura's two-parameter model with a 1000 bootstrap value. The tree highlights the TrLSVs identified in our study and is marked in red.

The RdRp gene from two samples tested positive for LSV through PCR (TrLSV-6474, TrLSV-6517) and was subjected to sequencing. These samples were taken from colonies that were experiencing colony losses. The sequences obtained from these Turkish LSV samples (TrLSVs) showed a 72.88% similarity. Furthermore, these TrLSVs were compared with sequences from Europe, America, and Asia deposited in GenBank. The TrLSVs exhibit similarities to other LSV sequences, ranging from 61.53% to 89.74%. Specifically, TrLSVs show higher (75.21-89.74%) similarity with LSV-4 sequences. For instance, TrLSV-6517 demonstrates a homology of 78.63% with Asian LSV-4 (Japanese-LC646147, Chinese- MF491499, MZ821852, Korean-OR371982) sequences, 75.21% with the Belgium sequence (JX878492), and 77.77% with the USA sequence (KP892556). Similarly, TrLSV-6474 displays 89.74%, 87.17%, and 88.88% homology with Asian (Japanese-LC646147, Chinese-MF491499, MZ821852, Korean- OR371982), European (Belgium-JX878492), and American LSV-4 (KP892556) sequences, respectively. In the phylogenetic tree analysis, the two TrLSV sequences cluster with LSV-4 sequences, using LSV1, LSV2, LSV3, LSV4, and LSV8 sequences from GenBank as reference (Figure 3).

Table 2. Detected viruses and varroa mite density in apiaries with unexpected colony losses and CCD-like symptoms. Varroa infestation intensity was graded (+: Mild infestation, ++: Moderate infestation, +++: High infestation).

Apiary No	Virus Positivity	Varroa Density	Colony Loss / CCD-like symptom
1	DWV, BQCV, IAPV	+++	+/+
2	BQCV, IAPV, LSV	++	+/-
3	-	+	+/-
4	-	+	+/-
5	DWV, BQCV, LSV	+++	+/-
6	BQCV	+	+/-
7	IAPV, LSV	+	+/-
8	-	+	+/-
9	DWV, BQCV, CBPV	+++	+/+
10	DWV, BQCV, CBPV	+++	+/+
11	IAPV, LSV	++	+/-
12	DWV, LSV	++	+/-
13	DWV, BQCV, CBPV, LSV	+++	+/+
14	DWV, BQCV, IAPV, LSV	+++	+/+
15	DWV, BQCV	+	+/-
16	DWV, CBPV, IAPV	++	+/-
17	DWV, IAPV	+	+/-
18	DWV, BQCV, LSV	+	+/-
19	DWV	++	+/-
20	DWV, BQCV, CBPV, LSV	+++	+/+
21	DWV, LSV	++	+/-
22	DWV	+	+/-
23	DWV	+	+/-
24	DWV, BQCV	++	+/-
25	-	+	+/-
26	DWV, BQCV	+	+/-

DISCUSSION

Türkiye with its diverse eco-geographic features, serves as a vital bridge connecting the Asian, European, and African continents. The country's seven geographical regions, each with distinct climate characteristics, contribute to the ecosystem's sustainable dynamics of living organisms. Changes in environmental conditions, animal and human population movements, and habitat alterations can impact the species diversity of living populations. Honeybees significantly contribute to species diversity within the ecosystem (Klein et al., 2007). Colony losses, unexpected bee deaths, and CCD may also occur in apiaries due to the presence of many bee viruses, parasites, and pathogens. The spread of

pathogens, variations in virulence, and the compounding effects of multiple stress factors negatively impact colony health, leading to a rise in colony losses (Genersch, 2010; McMenamin and Genersch, 2015). This issue has developed into a global crisis (Muz, 2008). Apiculture is facing a formidable challenge due to honey bee colony losses, with diseases, parasites, and pathogens significantly contributing to this alarming phenomenon (Daniat et al., 2012). The presence of various viruses in colonies of young honeybees can lead to complications in their field activities and other biological functions. These complications may manifest as wing deformities, paralysis, and flight issues (Muz et al., 2019). Additionally, a lack of timely or sufficient feed, like nectar and pollen, can cause a decrease in population density towards the end of winter, resulting in missed opportunities to capitalize on the advantages of early spring. Since LSV has recently been identified in honey bees, its effect on colonies as a bee pathogen has been intriguing. Knowledge of the pathogenicity, genetic diversity, and effect of LSV on colony losses is still insufficient. More detailed information on bee pathogens is one of the strategies to be used in planning colony management to prevent losses in apiaries. This study investigated LSV positivity and genetic structure in apiaries with colony losses, unexpected bee deaths and CCD-like complaints. It was also analyzed regarding common viruses, including DWV, BQCV, CBPV, IAPV, KBV, ABPV, LSV, and SBV, and mite infestation in apiaries. The findings revealed that varroa infestation was widespread in all apiaries, with viruses detected in 84.61% of the cases. Apiaries with heavy losses and CCD-like complaints showed elevated rates of both multiviruses and high varroa. Furthermore, DWV and BQCV were identified as the most prevalent viruses, followed by LSV the apiaries.

Apiaries that suffered colony losses and displayed symptoms similar to CCD were found to have been affected by multiple viruses and high varroa mite infestation. Varroa mites feed on bees' tissue fluids, fatty tissue, and hemolymph, which can diminish the flight performance, sperm quantity, and quality of male honey bees (Rinkevich et al., 2017). Furthermore, high varroa infestations threaten colony health by transmitting viruses (Muz 2008; Ramsey et al., 2019). Research indicates that due to the high mite infestation of honey bees engaged in field activities, the offspring's ability in orientation flights and worker bees' ability to return to the hive are impaired. As a result, affected bees require more time and food to return to the colony, or they may be unable to return (Peck et al., 2016). These factors may contribute to CCD-like symptoms.

The LSV was initially discovered in the USA through a metagenomic analysis (Runckel et al., 2011), and subsequent reports have shown its widespread prevalence in honeybee populations across various continents in subsequent years (Corman et al., 2012; Daughenbaugh et al., 2015; Amakpe et al., 2016; Robert et al., 2017; Simenc et al., 2020; Kitamaru et al., 2022; Nguyen et al., 2024). Its genetic variants and global prevalence suggest a longstanding and stable relationship with *A. melifera*. While the precise role of LSV in colony collapses and its pathogenesis in bees remains incompletely understood, numerous studies have suggested its association with weakened colonies (Glenny et al., 2017; Faurot Daniels, 2020). Furthermore, its high prevalence has been linked to losses (Cepero et al., 2014), with LSV2 abundance specifically associated with weaker and collapsed colonies (Corman et al., 2012; Daughenbaugh et al., 2015; Faurot-Daniels et al., 2020). Although LSV2 and LSV3 have been reported as more prevalent in Korean apiaries, the presence of LSV2, LSV3, and LSV4 has been established. LSV has been identified as the virus with the highest prevalence among other viruses in severe winter losses in Korean apiaries (Nguyen et al., 2024). Conversely, a study in Belgium reported no association with winter deaths. Additionally, research conducted in China (Hue et al., 2023) suggested that the seasonal prevalence of LSV is irregular and may exhibit seasonal patterns alongside other honeybee pathogens. Our own findings indicate that in apiaries experiencing colony losses and showing symptoms reminiscent of CCD, the higher diversity of viruses, including LSV, suggests a possible role of virulence in these instances after DWV and BQCV.

The prevalence of LSV in apiaries in this study was recorded at 38.46%. Colony losses and varroa infestations were also documented. Previous studies in Türkiye have reported LSV prevalence ranging from 40% to 56.5% (Çağırgan et al., 2022; Mayack and Hakanoğlu, 2022; Muz and Muz, 2022). However, a ten-year study across different regions of Türkiye reported a lower prevalence of 30% (Muz and Muz, 2023a). Globally, the prevalence of LSV varies between countries (Simenc et al., 2020; Cukanova et al., 2022; Hue et

al., 2023; Nguyen et al., 2024) and is considered a common honey bee virus. Stress factors can influence honeybee viruses' seasonal dynamics, diversity, and burden (D'Alvise et al., 2019). Similar to DWV genotypes, Varroa mites can contribute to increased viral load and genetic diversity of viruses through vectoring. This can potentially lead to greater damage to bee colonies. However, the prevalence of DWV, BQCV, and CBPV has increased mite's existence (Ryabov et al., 2014; Robert et al., 2017), but no evidence of LSV has been demonstrated. The impact of mite infestation on LSV population expansion remains uncertain. Environmental factors such as migratory beekeeping and abundant food resources are also effective bee pathogens and disease transmission (Hue et al., 2023). Further comprehensive research will help fully understand the effects of high LSV populations on colony losses.

Analyzing a wide range of LSV variants is crucial for identifying strains that affect honeybee health. Our study identified LSV-4 in apiaries where colony losses and unexpected bee deaths occurred, based on RdRp gene region sequences. Turkish LSV sequences showed a closer relationship with Asian Korean, Chinese, and Japanese LSV sequences. Although a metagenomic study reported the presence of LSV1 and LSV2 (Tozkar et al., 2015) from Türkiye, further classification and characterization of LSV field variants are needed.

CONCLUSION

It is crucial to gather more information about genetic diversity and the pathogenesis of field viruses to develop strategies to manage colony losses. Conducting genetic research with a larger sample size can help demonstrate species diversity more effectively, shedding light on the diversity and impact of LSV variants on honeybee health and disease management. Further investigation is needed to comprehend the implications of elevated LSV populations on colony losses. The execution of genetic research with a more extensive sample size can significantly enhance the demonstration of species diversity and provide valuable insights into the influence of LSV variants on honeybee health and the management of diseases.

REFERENCES

Amakpe F, De Smet L, Brunain M, *et al.* Discovery of Lake Sinai virus and an unusual strain of acute bee paralysis virus in West African apiaries. Apidologie. 2016; 47:35-47.

Berenyi O, Bakonyi T, Derakhshifar I, Koglberger H, Nowotny N. Occurrence of six honeybee viruses in diseased Austrian apiaries. Appl Environ. Microbiol. 2006; 72(4):2414-20.

Cepero A, Ravoet J, Gómez-Moracho T, *et al.* Holistic screening of collapsing honey bee colonies in Spain: A case study. BMC Res Notes. 2014; 7(1):649

Chen Y, Zhao Y, Hammond J, Hsu HT, Evans J, Feldlaufer M. Multiple virus infections in the honey bee and genome divergence of honey bee viruses. J Inverteb Pathol. 2004; 87(2-3):84-93.

Cornman RS, Tarpy DR, Chen Y, *et al.* Pathogen webs in collapsing honey bee colonies. PLoS One. 2012; 7(6):e43562.

Čukanová E, Moutelíková R, Prodělalová J. First detection of Lake Sinai virus in the Czech Republic: a potential member of a new species. Arch Virol. 2022; 167(11):2213-2222.

Çağırgan AA, Kaplan M, Pekmez K, Arslan F. Türkiye'de Varroa akarlarında Lake Sinai Virus (LSV)' un ilk tespiti. Atatürk Üniv Vet Bil Derg. 2022;17(1):16-19.

D'Alvise P, Seeburger V, Gihring K, Kieboom M, Hasselmann M. Seasonal dynamics and co-occurrence patterns of honey bee pathogens revealed by high-throughput RT-qPCR analysis. Ecol Evol. 2019; 9(18):10241-10252.

Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. Predictive Markers of Honey Bee Colony Collapse. PloS One. 2012; 7:0032151.

Daughenbaugh KF, Martin M, Brutscher LM, *et al.* Honey bee infecting Lake Sinai viruses. Viruses. 2015; 7(6):3285-3309.

Faurot-Daniels C, Glenny W, Daughenbaugh KF, McMenamin AJ, Burkle LA, Flenniken ML. Longitudinal monitoring of honey bee colonies reveals dynamic nature of virus abundance and indicates a negative impact of Lake Sinai virus 2 on colony health. PLoS One. 2020; 15(9):e0237544.

Genersch E. Honey bee pathology: current threats to honey bees and beekeeping. Appl Microbiol Biotechnol. 2010; 87:87-97.

Genersch E, Aubert M. Emerging and re-emerging viruses of the honey bee (Apis mellifera L.). Vet Res. 2010; 41(6):54.

Giray T, Kence M, Oskay D, Döke MA, Kence A. Scientific note: colony losses survey in Turkey and causes of bee deaths. Apidologie. 2010; 41;451-453.

Glenny W, Cavigli I, Daughenbaugh KF, Radford R, Kegley SE, Flenniken ML. Honey bee (*Apis mellifera*) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. PLoS One. 2017; 12:e0182814.

Hou C, Liang H, Chen C, *et al.* Lake Sinai virus is a diverse, globally distributed but not emerging multi-strain honeybee virus. Mol Ecol. 2023; 32(14):3859-3871.

Kalaycı G, Cagirgan AA, Kaplan, *et al.* The role of viral and parasitic pathogens affected by colony losses in Turkish apiaries. Kafkas Univ Vet Fac Derg. 2020; 26(5):671-677.

Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980; 16:111-120.

Klein AM, Vaissière BE, Cane JH, *et al.* Importance of pollinators in changing landscapes for world crops. Proc Biol Sci. 2007; 274(1608):303-313.

Martin SJ, Ball BV, Carreck NL. Te role of deformed wing virus in the initial collapse of varroa infested honey bee colonies in the UK. J Apic Res. 2013; 52:251-258.

Mayack C, Hakanoğlu H. Honey bee pathogen prevalence and interactions within the Marmara Region of Turkey. Vet Sci. 2022; 9:573.

McMenamin AJ, Genersch E. Honey bee colony losses and associated viruses. Curr Opin Insect Sci. 2015; 8:121-129.

Muz MN. Colony collapse disorder in honeybees. T Parazitol Derg. 2008; 32(3):271-275.

Muz D, Muz MN. Survey of the occurrence of Deformed Wing Virus and multiple parasites of queens (Apis mellifera L.) in apiaries with collapsed colonies in Hatay, Turkey. J Apicult Res. 2009; 48(3):204-208.

Muz D, Muz MN. Investigation of some pathogens "Colony Loss Syndrome" resembled losses apiaries in Tekirdağ. Kocatepe Vet J. 2017; 10(1):21-28.

Muz MN, Özdemir N, Muz D. Global honeybee health and veterinary medicine. Bulletin of Veterinary Pharmacology and Toxicology Association. 2019; 10(1):24-30.

Muz D, Muz MN. Investigation of honey bee colony losses in Thrace region. 4th International Eurasian Conference on Science, Engineering and Technology. December 14-16; 2022.

Muz D, Muz MN. Israel Acute Bee Paralysis Virus prevalence in apiaries with colony loss in Türkiye. Uludağ Bee J. 2023a; 23(1):105-114.

Muz D, Muz MN. Acute bee paralysis virus field isolates from apiaries suffering colony losses in Türkiye. J Apic Res. 2023b; 20:1-7.

Neumann P, Carreck N. Honey bee colony losses. J Apicul Res. 2010; 49(1):1-6.

Nguyen TT, Yoo MS, Truong AT, *et al.* Prevalence and genome features of lake sinai virus isolated from *Apis mellifera* in the Republic of Korea. PLoS One. 2024; 19(3):e0299558.

Peck DT, Smith ML, Seeley TD. *Varroa destructor* mites can nimbly climb from flowers onto foraging honey bees. PLoS One. 2016; 11:e0167798.

Palacios G, Hui J, Quan PL, *et al.* Genetic analysis of Israel acute paralysis virus: distinct clusters are circulating in the United States. J Virol. 2008; 82(13):6209-6217.

Ramsey SD, Ochoa R, Bauchan G, *et al.* *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. Proc Natl Acad Sci USA. 2019; 116:1792-1801.

Ravoet J, De Smet L, Wenseleers T, De Graaf DC. Genome sequence heterogeneity of Lake Sinai virus found in honey bees and Orf1/RdRP-based polymorphisms in a single host. Virus Res. 2015; 201:67-72.

Rinkevich FD, Danka RG, Healy KB. Influence of Varroa mite (*Varroa destructor*) management practices on insecticide sensitivity in the honey bee (*Apis mellifera*). Insects. 2017; 8:9.

Runckel C, Flenniken ML, Engel JC, *et al.* Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, *Nosema*, and *Crithidia*. PLoS One. 2011; 6(6):e20656.

Ryabov EV, Wood GR, Fannon JM, *et al.* A virulent strain of Deformed Wing Virus (DWV) of honeybees (Apis mellifera) prevails after Varroa destructor-mediated, or in vitro, transmission. PLoS Pathogens. 2014;10.

Stoltz D, Shen XR, Boggis C, Sisson G. Molecular diagnosis of Kashmir bee virus infection. J Apicul Res. 1995; 34(3):153-160.

Tamura K, Stecher G, Kumar S. MEGA 11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021; 38(7):3022-3027.

Tozkar CO, Kence M, Kence A, Huang Q, Evans JD. Metatranscriptomic analyses of honey bee colonies. Front Genet. 2015; 6:100.

Walker PJ, Siddell SG, Lefkowitz EJ, *et al.* Changes to virus taxonomy and the statutes ratified by the International Committee on Taxonomy of Viruses (2020). Arch Virol. 2020; 165(11):2737-2748.

ACKNOWLEDGMENTS

Author contributions: DM, MNM designed the study. DM and MNM performed laboratory analysis. DM and MNM participated in drafting and revising the manuscript. DM: Dilek Muz, MNM: Mustafa Necati Muz

Financial Disclosure: The authors declared that this study has received no financial support.

Conflict of Interests: The authors declared that there is no conflict of interests.

Additional information: All authors have read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to DM.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Original Article https://doi.org/10.47748/tjvr.1511798

TJVR 2024; 8 (2): 137-142

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Retrospective evaluation of pelvic fractures in cats: 144 cases (2018-2023)

Ali Gülaydın[1] Mustafa Barış Akgül[1] Nihat Şındak[1] Müzzemil Hattap Soysal[2] Onur Yıldırım[1] Sevdet Kılıç[1] Maruf Yılmaz[1] Bahar Erden[1]

[1] *Department of Surgery, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye*
[2] *Department of Veterinary Surgery, Institute of Health Sciences, Siirt University, Türkiye.*

Correspondence: *Ali Gülaydın (a.gulaydin@siirt.edu.tr)*

Received: 06.07.2024 Accepted: 15.08.2024

ABSTRACT

Objective: This study aims to examine pelvic fractures in cats brought to the Siirt University Animal Health and Research Hospital between 2018 and 2023.

Materials and Methods: Using a retrospective design, the study analyzed data from 144 cats.

Results: The data revealed that pelvic fractures generally occurred as a result of traumatic events such as motor vehicle accidents and falls from heights. Of the 259 fracture cases studied, it was determined that 24.32% were pubic fractures, 23.54% were sacroiliac separations, 19.69% were ischial fractures, 12.74% were ilial fractures, 10.42% were symphysis pelvis separations, 7.72% were acetabular fractures, and 1.54% were sacral fractures. Patients in the study were treated conservatively or operatively (using methods such as iliosacral screws, acetabular C plates, locked plates, cerclage wires, and dorsal laminectomy).

Conclusion: In conclusion, operative treatment plays a significant role in the management of pelvic fractures, and the suitability of surgery should be carefully evaluated. Clinical outcomes indicated that cats undergoing operative treatment achieved more favorable results and higher discharge rates.

Keywords: *Feline, Pelvic fractures, Iliosacral screw, Cerclage wires, Locked plates*

INTRODUCTION

The complex structure of the Os coxae consists of three separate bones. The ilium is located craniodorsally, the pubis is located cranioventrally, and the ischium is located caudoventrally. The Os coxae are formed by the fusion of the pubis and ischium at the midline of symphysis pelvis (Dursun, 2008; Bahadır and Yıldız, 2010).

Pelvic fractures in cats are commonly associated with motor vehicle accidents, falls from heights, blunt force trauma, gunshot wounds, and attacks by other animals such as dogs (Bookbinder and Flanders, 1992; Meeson and Geddes, 2017; Bourbos et al., 2020; Yurtal et al., 2022; Çatalkaya et al., 2024). Rarely, fractures in this region can also occur due to factors like neoplastic lesions and compression (Bookbinder and Flanders, 1992; Yurtal et al., 2022; Çatalkaya et al., 2024). Pelvic fractures account for approximately 20% to 30% of all fracture cases in cats (Altunatmaz et al., 2004; Draffan et al., 2009; Sadan et al., 2016). Among pelvic fractures, the most common are fractures of the pubis, which forms the pelvic floor, followed by sacroiliac luxations (Bookbinder and Flanders, 1992; Aksoy and Özsoy, 2003; Sadan, 2016).

Due to the anatomical structure of the pelvis, trauma can result in multiple fractures. Additionally, the major muscle groups in the region provide extra stability to the pelvic bones (Piermattei and Johnson, 2004; Harasen, 2007; Çatalkaya et al., 2024). Treatment of pelvic

fractures can involve operative intervention, cage rest, and medical management, taking into account various factors (Altunatmaz et al., 2004; Çatalkaya et al., 2024). Surgical intervention is recommended within 48-72 hours to minimize the risk of early fibrous union and muscle contraction. However, it is crucial to carefully assess the patient's condition during this period to determine suitability for surgery (Piermattei and Johnson, 2004; DeCamp, 2016).

Pelvic fractures have been successfully treated using various osteosynthesis materials in operative interventions (Bookbinder and Flanders, 1992; Altunatmaz et al., 2004; Schmierer et al., 2015; DeCamp, 2016). Methods such as two iliosacral lag screws and combinations of iliosacral screws with intramedullary pins are utilized for sacroiliac luxations (DeCamp, 2016). Plate osteosynthesis is predominantly used in the treatment of ilium fractures (Altunatmaz et al., 2004; Langley-Hobbs et al., 2009; DeCamp et al., 2016; Scrimgeour et al., 2017), while pins and compression screws (DeCamp et al., 2016) and T plates (Scrimgeour et al., 2017) can also be applied. For sacrum fractures, dorsal laminectomy, tail amputation, and pin and screw applications are employed (Smeak et al., 1985). Conservative treatment is typically preferred for pubis and ischium fractures (Tomlinson, 2003; DeCamp, 2016). During the conservative treatment of pelvic fractures, appropriate cage rest is applied at suitable intervals, and practices such as adequate bedding, local massage, and repositioning of the patient are crucial in preventing wound formation. Additionally, regular monitoring of urination and defecation, assessment of nerve damage, and management of additional injuries are necessary (Arıcan, 2019a).

The aim of this study was to retrospectively evaluate pelvic fractures diagnosed and treated in cats brought to Siirt University Animal Health Application and Research Hospital between 2018 and 2023.

MATERIALS and METHODS

This study retrospectively evaluated 144 cats diagnosed with pelvic fractures based on clinical and radiological examinations at Siirt University Animal Health Application and Research Hospital between 2018 and 2023. Data included demographic information such as age, breed, gender, and weight of the patients, along with the etiology and localization of the identified pelvic fractures. Treatment options applied were also assessed within this context.

In the study, the etiology of fractures was classified as traffic accidents, falls from height, and unknown causes. The localization of fractures was categorized as symphysis pelvis separation, acetabular fracture, sacroiliac separation, ilium fracture, ischium fracture, pubis fracture, and sacrum fracture. Treatment options were evaluated as operative and conservative.

Ethical approval

This study was approved with the decision of the Local Ethics Committee of Animal Experiments at Siirt University, dated 31/05/2024, and numbered 2024/05/30.

RESULTS

During the study period, out of 1524 cats brought to Siirt University Animal Health Application and Research Hospital for diagnosis and treatment purposes, pelvic fractures were diagnosed in 144 (9.44%) cases. The annual distribution of cases during the study period revealed that 12.6% (n=18) were diagnosed in 2018, 29.2% (n=42) in 2019, 15.2% (n=22) in 2020, 20.2% (n=29) in 2021, 12.6% (n=18) in 2022, and 10.5% (n=15) in 2023.

Of the 144 cats included in the study, 57.6% (n=83) were male and 42.4% (n=61) were female. The age of the cats ranged from 20 days to 2 years, with a mean age of 13.1 months determined. Among the cats, 59.02% (n=85) were in the age range of 0-1 year, 33.33% (n=48) were in the age range of 1-2 years, and 7.63% (n=11) were older than 2 years. The majority of cats, 87.5% (n=126), were mixed breed, while 5.5% (n=8) were British Shorthair, 4.86% (n=7) were Scottish Fold, and 0.69% (n=1) each were Van cat, Ankara cat, and Persian cat.

Upon evaluating the etiology of cases, it was found that 65.97% (n=95) were attributed to traffic accidents, 29.16% (n=42) to falls from height, and 4.86% (n=7) to trauma of unknown origin.

In 144 cats diagnosed with pelvic fractures, a total of 259 fractures were identified. Specifically, fractures included pubic fractures in 24.32% (n=63) of cases, sacroiliac separations in 23.5% (n=61) [bilateral sacroiliac separation 10.4% (n=27), left sacroiliac separation 7.3% (n=19), right sacroiliac separation 5.7% (n=15)], ischial fractures in 19.6% (n=51), ilial fractures in 12.7% (n=33), symphysis pelvis separation in 10.4% (n=27), acetabular

fractures in 7.7% (n=20), and sacral fractures in 1.5% (n=4) of cases. The results were given in detail in Table 1.

Table 1. Demographics, aetiology, and localization of fractures in cats included in the study (n=144).

Demographics	**n**	**%**
Male	83	57.6
Female	61	42.4
0-1 age	85	59.02
1-2 age	48	33.33
>2 age	11	7.63
Mixed Breed	126	87.5
British Shorthair	8	5.5
Scottish Fold	7	4.86
Van Cat	1	0.69
Ankara Cat	1	0.69
Iran Cat	1	0.69
Etiology of fractures		
Traffic Accidents	95	65.97
Fall from high	42	29.16
Unknown	7	4.86
Lokalization of fractures		
Acetabular fractures	20	7.72
Right sacroiliac fractures	15	5.79
Left sacroiliac	19	7.33
Bilateral sacroiliac fractures	27	10.42
Ilium fractures	33	12.74
Symphisis pelvis separations	27	10.42
Ischium fractures	51	19.69
Pubis Fractures	63	24.32
Sacrum fractures	4	1.54
Total	**259**	**100**

Out of a total of 144 cats diagnosed with pelvic fractures, 86.1% (n=124) underwent surgical treatment, while 13.9% (n=20) received conservative treatment with cage rest only. The surgical methods applied according to fracture classification were detailed in Table 2. Radiographic images of the some of the applied methods were shown in Figure 1 and 2. Of the patients, 73.61% (n=10) were discharged after treatment, while 26.38% (n=38) died due to various reasons.

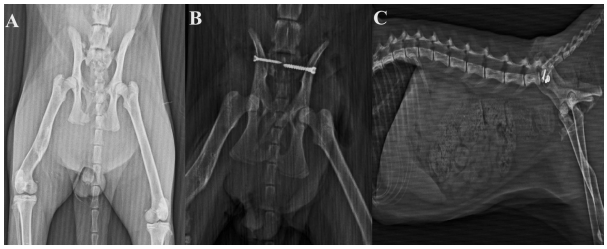

Figure 1. Radiographic image of the treatment of bilateral sacroiliac separation with iliosacral screwing method (A: Pre-op VD, B: Post-op VD, C: Post-op LL).

Figure 2. Radiographic image of the treatment of a corpus ilium fracture case with locking plate osteosynthesis method (A: Pre-op VD, B: Post-op VD, C: Post-op LL).

Table 2. Treatment options applied according to the type of fracture.

Fractures	**n**	**Operative treatment Options**	**Operative treatment n (%)**	**Cage rest (conservative treatment) n (%)**
Right sacroiliac separations	15	Iliosacral screw	50 (81.96)	11 (18.03)
Left sacroiliac separations	19			
Bi lateral sacroiliac separations	27			
Acetabular fractures	20	Acetabular C plate	11 (55)	4 (20)
		Excision arthroplasty	5 (25)	
Ilium fractures	33	Locking plate + Cerclage wire	27 (81.81)	6 (18.18)
Symphysis pelvis separation	27	Cerclage wire	10 (37.03)	17 (62.96)
Ischial fractures	51	Cerclage wire	20 (39.21)	31 (60.78)
Pubic fractures	63	-	-	63 (100)
Sacral fractures	4	Dorsal laminectomy	1 (25)	3 (75)
Total	259			

DISCUSSION

This study examines the pelvic fractures of cats that presented to the Siirt University Veterinary Health Application and Research Hospital over a five-year period, providing significant material statistically analyzing pelvic fractures in cats. Unlike previous studies (Yurtal et al., 2022; Çatalkaya et al., 2024) in this field, this study contains a large data set that focuses exclusively on cats.

Previous studies have reported that pelvic fractures are mostly encountered in young animals (1-3 years) (Bennet, 1975; Bookbinder and Flanders, 1992; Sadan et al., 2016; Altunatmaz et al. 2004; Bourbos et al., 2020; Yurtal et al., 2022; Çatalkaya et al., 2024). Similarly, in this study, it was determined that the average age of animals with pelvic fractures was 13.1 months. It is thought that the reason for this could be that young animals are more active and have difficulties living on the street due to a lack of life experience.

When evaluating the gender distribution of animals diagnosed with pelvic fractures, previous studies have indicated a higher incidence in male cats compared to female cats (Bennet, 1975). Contrarily, Ünsaldı (1995) reported a higher prevalence of pelvic fractures in female cats. The present study corroborates Bennet's findings, revealing a greater occurrence of pelvic fractures in male cats. These divergent findings highlight the necessity for more comprehensive research to understand the factors influencing the incidence of pelvic fractures across different genders.

Yurtal et al. (2022) reported no statistically significant relationship between pelvic fractures and cat breeds in their study. In contrast, Solak (2021) found that mixed-breed cats were more affected by pelvic fractures. In the present study, the majority of the cats were of mixed breed. This suggests that the prevalence of pelvic fractures in these animals is likely due to the high population of mixed-breed stray cats in Siirt region rather than a specific breed predisposition. Consequently, these cats are more susceptible to traffic accidents, resulting in pelvic fractures.

Studies have indicated that the etiology of pelvic fractures is associated with factors such as traffic accidents, falls from heights, blunt force trauma, dog attacks, and neoplastic lesions (Altunatmaz et al., 2004; Sağlam et al., 2016; Meeson and Geddes, 2017; Yurtal et al., 2022; Çatalkaya et al., 2024). Traffic accidents have been highlighted as the most common cause of pelvic fractures in numerous studies (Altunatmaz et al., 2004; Meeson and Geddes, 2017; Yurtal et al., 2022). Researchers have emphasized that this prevalence is due to the complex structure of large cities (Meeson and Geddes, 2017). Similarly, in this study, it was determined that cases resulting from motor vehicle accidents accounted for over 65% of all incidents, making it the most common cause. The second most common cause identified was falls from heights.

Studies have indicated that the proportion of pelvic fractures to total fractures ranges from 20-30% (Altunatmaz et al., 2004; Draffan et al., 2009; Sadan et al., 2016; Bourbos et al., 2020; Cinti et al., 2020). However, when the data obtained in this study were examined, it was seen that 144 (9.44%) of the 1524 fracture cases were pelvic fractures.

In general, the most frequently encountered type of fracture in pelvic fractures is reported to be pubic fractures, which constitute the pelvic floor (Bookbinder and Flanders, 1992; Sadan, 2016). In a study conducted by Aksoy and Özsoy (2003) on 239 ossa coxae fractures, they diagnosed 32.2% pubic fractures, 28.8% iliac fractures, 21.7% ischial fractures, and 17.1% acetabular fractures. Similarly, Yurtal et al. (2022) identified 88 iliac fractures, 90 ischial fractures, 60 pubic fractures, 54 acetabular fractures, 139 sacroiliac separations, and 43 symphysis pubis separations in 183 cases of pelvic fractures. The data from our study indicate that pubic bone fractures, with a frequency of 24.32%, are the most common type of pelvic fracture, followed by sacroiliac separation at 23.5%, ischial fractures at 19.6%, iliac fractures at 12.7%, symphysis pubis separations at 10.4%, acetabular fractures at 7.7%, and sacral fractures at 1.5%. These results are consistent with the general literature.

In the studies by Bookbinder and Flanders (1992) and Piermattei and Johnson (2004), it was emphasized that due to the box-like nature of the pelvic structure, a fracture in one bone could lead to a fracture in another bone. In the present study, the fact that at least two pelvic fractures were detected in 88 out of 144 animals (61.1%) supports this theory.

Literature reviews have indicated that sacral fractures, iliac fractures, and acetabular fractures, as well as fractures that cause more than 50% narrowing of the pelvic canal-essentially the bones

that bear more body weight-require surgical treatment (Meeson and Geddes, 2017; Arıcan, 2019a; Arıcan, 2019b; Çatalkaya et al., 2024). Conversely, fractures for which conservative treatment is more often chosen include pubic, ischial, and posterior one-third acetabular fractures (Arıcan, 2019a; Arıcan, 2019b; Çatalkaya et al., 2024).

When evaluating the treatment methods applied to cases in this study, it was observed that 50 sacroiliac separations, 16 acetabular fractures, 27 iliac fractures, 10 symphysis pelvis separations, and 1 sacral fracture were treated surgically (86.1% of 144 cats), while 11 sacroiliac separations, 4 acetabular fractures, 6 iliac fractures, 17 symphysis pelvis separations, 31 ischial fractures, 63 pubic fractures, and 3 sacral fractures were treated conservatively (13.9% of 144 cats).

Thus, it is accurate to state that the treatment options throughout the study were determined in accordance with the literature. Furthermore, we believe that adhering to the principle of first stabilizing the patient's vital functions, as supported by the literature (Kim et al., 2011; Meeson and Cor, 2011; Sadan et al., 2016; Gant and Asztalos, 2019; Parlak et al., 2021; Çatalkaya et al., 2024), played a significant role in the recovery processes of the patients.

CONCLUSION

This study provided significant findings on the etiology and treatment options of pelvic fractures in cats. The analyses revealed that pelvic fractures predominantly occur as a result of traumatic events such as motor vehicle accidents and falls from heights.

The study determined that operative intervention plays a crucial role in the treatment of pelvic fractures. More favorable outcomes and higher discharge (recovery) rates were observed in cats that underwent surgical treatment. However, it was also considered that in some cases, surgery may not be appropriate, and medical management should be employed.

In conclusion, this study highlights the potential significance of surgical intervention in the management of pelvic fractures in cats and underscores the necessity of carefully evaluating the appropriateness of such interventions. Furthermore, adopting a standardized approach to the treatment of pelvic fractures could contribute to better outcomes in clinical practice. Future research will provide valuable insights into the development of treatment strategies for pelvic fractures in cats.

REFERENCES

Aksoy O, Özsoy S. Clinical evaluation of ossa coxae fractures in cats and dogs and clinical evaluation of complications arising from these. Kafkas Univ Vet Fak Derg. 2003; 9(2):187-202.

Altunatmaz K, Aksoy Ö, Özsoy S. Radiographic evaluation of ossa coxae fractures and concomitant orthopedic lesions in cats and dogs (1992-2002): 621 cases. Istanbul Univ Vet Fak Derg. 2004; 30(1):1-9.

Arıcan M. Evaluation criteria for pelvic fractures. Caging or surgery? KHVDH International Continuous Education Congress. November 8-10, Istanbul, 2019a.

Arıcan M. Surgical approaches to pelvic fractures. KHVDH International Continuous Education Congress. November 8-10, Istanbul, 2019b.

Bahadır A, Yıldız H. Veterinary anatomy of the musculoskeletal system and internal organs. İstanbul: Güven Mücellit Matbaacılık Ltd. Şti.; 2010. pp.74-80.

Bookbinder PF, Flanders JA. Characteristics of pelvic fracture in the cat. Vet Comp Orthop Traumatol. 1992; 5(03):122-127.

Bourbos A, Cinti F, Sergiampietri F, Pisani G. The use of an intraoperative skeletal traction device for delayed reduction in pelvic fractures in two dogs. Vet Comp Orthop Traumatol. 2020; 3(01):40-45.

Cinti F, Cavaliere L, Degna MT, Rossi F, Pisani G. Triple pelvic osteotomy fixed with lag screw for the treatment of pelvic canal stenosis in five cats. Vet Comp Orthop Traumatol. 2020; 33(5):363-369.

Çatalkaya E, Yayla S, Altan S, *et al.* A retrospective study on pelvic fractures in cats and dogs (2020-2022). Kocatepe Vet J. 2024; 17(1):15-21.

DeCamp CE. Fractures of the pelvis. In: DeCamp CE, Johnston SA, Dejardin LM, Schaefer SL, eds. Brinker Piermattei, and Flo's Handbook of Small Animal Orthopedics and Fracture Repair. 5th ed. St Louis: Elsevier; 2016. pp.437-467.

Draffan D, Clements D, Farrell M, Heller J, Bennett D, Carmichael S. The role of computed tomography in the classification and management of pelvic fractures. Vet Comp Orthop Traumatol. 2009; 22(3):190-197.

Dursun N. Veterinary anatomy I. 12th ed. Ankara: Medisan Publishing; 2008.

Gant P, Asztalos I. Retrospective evaluation of factors influencing transfusion requirements and outcome in cats with pelvic injury (2009-2014): 122 cases. J Vet Emerg Crit Care. 2019; 29(4):407-412.

Harasen G. Pelvic fractures. Canadian Vet J. 2007; 48(4):427-428.

Kim KH, Lee J, Yoon HY, Jeong SW. Clinical analysis of pelvic fracture in 54 dogs. J Vet Clin. 2011; 28(5):467-472.

Langley-Hobbs SJ, Meeson RL, Hamilton MH, Radke H, Lee K. Feline ilial fractures: a prospective study of dorsal plating and comparison with lateral plating. Vet Surg. 2009; 38(3):334-342.

Meeson R, Corr S. Management of pelvic trauma: Neurological damage, urinary tract disruption and pelvic fractures. J Feline Med Surg. 2011; 13(5):347-361.

Meeson RL, Geddes AT. Management and long-term outcome of pelvic fractures: a retrospective study of 43 cats. J Feline Med Surg. 2017; 19(1):36-41.

Parlak K, Zamirbekova N, Uzunlu EO, Akyol ET, Yavru N. Comparison of the focused assessment with sonography for trauma protocol and animal trauma triage scoring system in traumatized dogs. Kafkas Univ Vet Fak Derg. 2021; 27(4):439-444.

Piermattei DL, Johnson KA. An atlas of surgical approaches to the bones and joints of the dog and cat. 4th ed. Philadelphia: Saunders; 2004.

Sadan MA, von Pückler K, Kramer M. Pelvic floor fractures in 55 dogs and 39 cats: CT and X-Ray findings. IJVRS. 2016; 2(1):1-7.

Sağlam M, Çalık EY. Clinical and radiological evaluation of traumatic lesions of the pelvis and hip joint in cats. Vet Hekim Der Derg. 2016; 87(1):34-43.

Schmierer PA, Kircher PR, Hartnack S, Knell SC. Screw loosening and pelvic canal narrowing after lateral plating of feline ilial fractures with locking and nonlocking plates. Vet Surg. 2015; 44(7):900-904.

Scrimgeour AB, Craig A, Witte PG. Locking T-Plate repair of ilial fractures in cats and small dogs. Vet Comp Orthop Traumatol. 2017; 30(6):453-460.

Smeak DD, Olmstead ML. Fracture/Luxations of the sacrococcygeal area in the cat: A retrospective study of 51 cases. Vet Surg. 1985; 14:319-324.

Solak AG. Retrospective evaluation of pelvic injuries in cats brought to the surgery clinic between 2018-2020. [Master Thesis]. Balıkesir: Balıkesir University, Health Science Institute; 2021.

Tomlinson JL. Fractures of the pelvis. In: Textbook of Small Animal Surgery. 3rd ed. Philadelphia: WB Saunders Co.; 2003. pp.1989-2001.

Ünsaldı E. Treatment of pelvic fractures with osteosynthesis in cats. Ankara Univ Vet Fac Derg. 1995; 42(2):129-138.

Yurtal Z, Deveci MZY, Alakuş İ, *et al.* Prevalence of pelvic fractures in cat and dogs: A retrospective study in 183 cases (2016-2020). J Adv VetBio Sci Tech. 2022; 7(1):109-114.

ACKNOWLEDGMENTS

Author contributions: AG MBA designed the study, performed surgeries, collected data and participated in writing original draft and critical revisions. NŞ participated in writing original draft and critical revisions. MHS collected data, participated in drafting the manuscript. OY, SK, MY and BE performed surgeries and collected data. AG: Ali Gülaydın, MBA: Mustafa Barış Akgül, NŞ: Nihat Şındak, MHS: Müzzemil Hattap Soysal, OY: Onur Yıldırım, SK: Sevdet Kılıç, MY: Maruf Yılmaz, BE: Bahar Erden

Financial Disclosure: The authors declared that this study has received no financial support.

Conflict of Interests: The authors declared that there is no conflict of interests.

Additional information: All authors have read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to AG.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Review https://doi.org/10.47748/tjvr.1421153

TJVR 2024; 8 (2): 143-150

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Digestion and importance of starch in ruminants

Şevket Evci[1]

[1] *Delice Vocational School, Kırıkkale University, Kırıkkale, Türkiye.*

Correspondence: *Şevket Evci (sevketevci@kku.edu.tr)*

Received: 17.01.2024 Accepted: 25.03.2024

ABSTRACT

Ruminants have a unique digestive physiology that heavily relies on microbial fermentation specifically in the rumen. The rumen, a complex microbial ecosystem, is the primary site for starch digestion. Enzymatic hydrolysis and microbial fermentation of starch in this compartment produce important by products, such as volatile fatty acids (VFAs) and microbial proteins. These by products are crucial sources of energy and protein, which affect the overall metabolic dynamics of ruminants. It is essential to have a comprehensive understanding of the factors that influence starch digestion rates to optimize ruminant nutrition. In this review, the complex mechanisms of starch digestion in ruminants and the various factors involved in starch digestion, including feed composition, microbial population and enzymatic activity, and how these contribute to the digestive process, are examined and its important role in shaping the nutritional environment is attempted to be explained. Additionally, identifying and characterizing starch fractions in concentrated feed sources is crucial for formulating well-balanced rations. In conclusion, this review synthesizes current knowledge on starch digestion in ruminants, offering insights into the complexities of the process. The collected information not only contributes to academic understanding but also has practical implications for optimizing feeding strategies, enhancing nutrient utilization, and promoting the overall well-being of ruminants.

Keywords: *Ruminants, Starch, Starch digestion*

INTRODUCTION

The main organic components of ruminant diets are carbohydrates, fats and proteins. Among these, carbohydrates play an important role for ruminants both as a source of energy and ballast and because they are present in almost 70% of the rations. Among carbohydrates, cellulose and starch are the most important components for ruminants (Saha et al., 2021; Wang et al., 2021). In modern farms, the cellulose content is kept low and the starch content is kept high in order to achieve and maintain high milk or meat production. In our country, barley and wheat are used as starch sources in rations, and maize has also started to take an important place. However, it is known that due to the chemical properties of the starches they contain, care must be taken when using the otherwise they may cause serious metabolic problems such as acidosis in animal health. Carbohydrates are important for the health, energy and milk yield of ruminants in general and starch in particular (Hall, 2006a; Biliaderis, 2009).

1. Importance of carbohydrates in ruminant diets

The energy required by the animal's body is released when ingested nutrients are converted to carbon dioxide (CO_2) and water (H_2O) by burning oxygen in the body. Energy is obtained as a result of the metabolism of carbohydrates, fats, and proteins in the body. Among these carbohydrates, formaldehyde is produced from water and CO_2 by

photosynthesis with the action of sunlight and chlorophyll in green plants. Formaldehyde is converted to carbohydrates, which make up about 75% of plants (Baldwin and Connor, 2017). The carbohydrates found in plants are polysaccharides, cellulose, hemicellulose, pectins, fructans, and starches (Saha et al., 2021).

Although carbohydrates are present at the level of 1-1.5% in the animal body, they have an important place both as a source of energy in tissues and as an economic source of energy (Ergün et al., 2020).

In ruminants, protozoa and bacteria in the rumen microflora break down carbohydrates in an anaerobic environment to produce glucose. As a result of glucose degradation, CO_2, methane (CH_4), H_2O and volatile fatty acids (VFAs) are released (Saha et al., 2021). Volatile fatty acid, consisting of acetic acid, propionic acid, and butyric acid, each carry 2, 3, 4 carbon (C) atoms. In addition to providing approximately 70% of the energy requirements of ruminants, these VFAs also contribute in rumen maturation and digestion of carbohydrates such as cellulose and hemicellulose. Daily 2-4 kg of VFA are synthesized in a cattle rumen (Ergün et al., 2020).

2. Carbohydrate Types and Metabolism

Carbohydrates represent approximately 70% and above of the dry matter content of ruminant diets. These organic substances are biochemically classified into 4 types according to the number of simple sugars (monosaccharides) they contain: monosaccharides, disaccharides, oligosaccharides and polysaccharides. They are also classified as structural (cellulose, hemicellulose, lignin, beta-glucans, pectins), non-structural (starch, fructans, organic acids, mono-, di- and oligosaccharides) and non-starchy (cellulose, hemicellulose, lignin, fructans, beta-glucans, pectics). However, in 2001, the National Research Council (NRC) divided dietary carbohydrate sources into two categories: neutral detergent fiber (NDF) and non-fiber carbohydrates (NFC). Together, these two carbohydrates provide 70% or more of the dry matter and most of the energy in the diet. NDF consists of cellulose, lignin, and hemicelluloses, while NFC includes sugars (glucose, fructose, sucrose, lactose), starch, fructans, pectins, beta-glucans, galactans, and other carbohydrates (NRC, 2001; Hall, 2002; Hall, 2007a;) (Figure 1).

2.1. Non-Fiber carbohydrates (NFC)

Carbohydrates in this category include organic acids, sugars (monosaccharides and some oligosaccharides), starch, and neutral detergent soluble fiber (NDSF) (Figure 1) (Hall, 2007a).

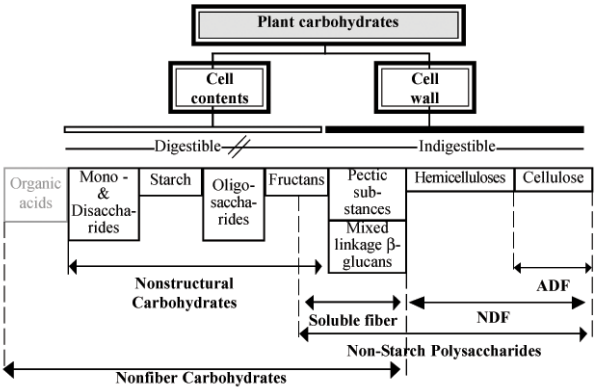

Figure 1. Carbohydrate components in plants (Hall 2007b).

2.1.1. Organic acids

These include fermentation acids found in silage (acetate, propionate, butyrate, lactate) and plant organic acids found in fresh forage and hay (malate, citrate, quinate, etc.). They are not carbohydrates, but are included in this group because they are included in the NFC calculations (Hall, 2007a).

2.1.2. Sugars (monosaccharides and disaccharides)

This includes both simple sugars (glucose, fructose, etc.) and disaccharides (sucrose, lactose). In plants, the main sugars are glucose, fructose, and sucrose. Lactose is found only in milk products. Sugars can form lactic acid as a result of fermentation, as well as butyrate more than other non-fibre carbohydrates (NFCs) and propionate near the starch level (Bhandari et al., 2023). Some sugars are also converted to microbial glycogen in the rumen (Weinert-Nelson et al., 2023).

2.1.3. Starch

Starch is composed of alpha-linked glucose chains stored by plants in crystalline granules. Starch digestion occurs in a wide range of species, from microorganisms to animals. However, there are large variations in the rate of fermentation or digestion depending on the processing, storage method or plant source of the digested starch. The finer the particle size of the forage, the faster the fermentation (Hall and Zanton, 2022). Smaller grains such as wheat, barley, and oats tend to ferment faster than coarser grains such as corn or sorghum (Slafer and Savin, 2023). Starch fermentation rates may increase as the starch content of the diet increases (Oba and Allen, 2003).

2.1.4. Soluble fibers

These include pectins, beta glucans, fructans and other non-starch polysaccharides not found in NDF. These carbohydrates cannot be digested by mamalian digestive systems, but can be digested by microbes. Soluble fibers tend to ferment very rapidly. Pectins, the major type of soluble fiber found in legume feeds, citrus pulp, and sugar beet pulp, can produce more acetate than other NFC (Bhandari et al., 2023). With the exception of fructans, little or no lactate is produced as a result of soluble fiber fermentation. Fermentation is also inhibited when the rumen pH is more acidic. Common sources of soluble fiber include legume forages, citrus pulp, beet pulp, soybeans, and soybean meal (Ma et al., 2021).

2.2. NDF

NDF is a carbohydrate source that cannot be digested by digestive enzymes but can be digested by rumen microorganisms and is essential for maintaining rumination and rumen function. NDF is composed of hemicellulose, cellulose and lignin. The cellulose and lignin portion is called acid detergent fiber (ADF). Volatile fatty acid is formed by the breakdown of cellulose. In addition, cellulose increases salivation and helps maintain optimal rumen pH. Hemicellulose also plays an important role in the development of rumen papillae and the formation of VFA. Lignin, on the other hand, is a substance that is not a true carbohydrate and is nearly indigestible, and its excess in feedstuffs reduces feed utilization. Dietary NDF is an important component of rumen pH, milk fat content, and dry matter intake in cattle (Hall, 2002; Hall, 2007a).

2.3. Carbohydrate metabolism in ruminants

Cellulose, hemicellulose, starch, sugars, pentosans, fructans, and pectins in forages are carbohydrate sources for ruminants. Cellulolytic bacteria use their enzymes to break down cellulose into glucose, cellobiose and short-chain oligosaccharides. Rumen microorganisms have enzymes that break down pentosan and hemicelluloses to xylose, arabinose, mannose and galactose. Starch, fructose and sugars ferment rapidly. Pectins are degraded to methanol and pectic acid by microbial enzymes. Lignin cannot be digested by rumen microorganisms (Dijkstra et al., 2005; Grev et al., 2017).

The end products of the main fermentation of carbohydrates in ruminants are VFA, CO_2 and CH_4. Acetic acid is produced from pyruvic acid, propionic acid is mostly produced by the reduction of lactic acid, and butyric acid is produced by the condensation of acetic acid and acetyl-CoA. Of the gases produced in the rumen, 70% is CO_2 and 30% is methane gas. Most of the methane gas is excreted through the rumen (Einsmenger et al., 1990; Dijkstra et al., 2005,).

Acetic acid and butyric acid from VFA are converted to acetyl-CoA in the liver. Acetyl-CoA is involved in the formation of milk fat and other fats in the body. Acetic acid and butyric acid make up half of milk fat, and triglycerides absorbed from the intestines make up the other half. Propionic acid is converted in the liver to oxalacetic acid and glucose. Glucose is used as a source for the synthesis of lactose from mammary tissue. Approximately 2 kg of lactose must be produced in the liver for 20 kg of milk per day. Therefore, the amount of glucose produced in the liver plays an important role in the amount of milk produced per day. In addition, in the rumen of cattle, propionic acid is converted to propionate, which is the main substance for gluconeogenesis. Approximately 30-70% of the energy source glucose is provided by propionate (Hall, 2006a; Hall, 2007a).

The ratio of roughage to concentrates in ruminant diets significantly affects the ratio of VFA between them. In ruminants fed roughage, VFA is composed of 60-70% acetic acid, 15-20% propionic acid, and 10-15% butyric acid. As the amount of degradable carbohydrates in the diet increases, the amount of acetic acid and propionic acid reaches 40%, so the amount of propionic acid increases and the amount of acetic acid decreases. While the decrease in the amount of acetic acid causes a decrease in the fat content of the milk, the increase in propionic acid reduces the energy loss during rumen fermentation in fattening cattle. Therefore, an increase in propionic acid is desirable in beef cattle, while a decrease in acetic acid is undesirable in dairy cattle. In addition, when roughage is added to the ration in finely ground form, the amount of acetic acid decreases, resulting in a decrease in the fat content of the milk. However, in high yielding cows, it is not possible to meet the nutritional needs of the animals with large amounts of roughage, so it is recommended that the ration be adjusted so that the roughage content is not less than 40% (Hall, 2006a; Hall, 2007a).

3. Starch Types and Their Digestion Mechanism in Rumen

3.1. Importance of starch and starch digestion

Starch is stored in plant organs such as roots, seeds, and fruits and makes up about 80% of these organs. It is found in smaller amounts in the stems and leaves of plants. Starch particles consist of 20-30% amylose and 70-80% amylopectin. The amount of amylose increases as the plant matures. In amylose, the glucose units are linked by α-1,4 glycosidic linkages and in amylopectin, they are linked by α-1,4 linkages to the glucoses in the backbone chain and by α-1,6 linkages at the branching points and are the most abundant part of starch. From Perez et al. (2009) chemical structure of amylose and amylopectin in starch has shown in Figure 2. Compounds with α-1,4 bonds are degraded to maltose, those with α-1,6 bonds to isomaltose, and the end product is usually glucose. Since all α-1,4 bonds in plants can be degraded by the enzyme α-amylase, all amylose can be degraded, but only 60% of amylopectin can be degraded (Tester et al., 2004; Perez et al., 2009; Wang et al., 2024).

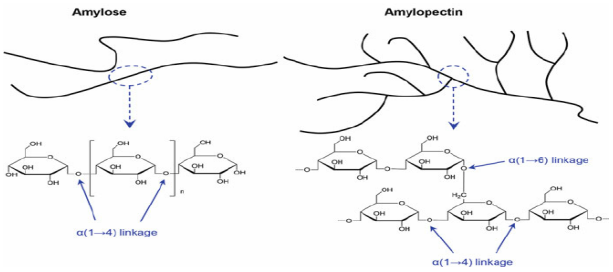

Figure 2. Chemical structure of amylose and amylopectin in starch (Perez et al., 2009).

Starch is deposited in granules within the endosperm. Depending on the grain type, granules vary widely in shape (round, lenticular, polygonal), size distribution (unimodal or bimodal), and whether they are single (simple) or clusters of granules (compound) (Tester et al., 2004). Starch granules are formed by the accumulation of growth rings composed of alternating semi-crystalline and amorphous sheets. These rings extend from the center of the granule (hilum) to the surface of the granule, similar to the layers of an onion. The amorphous regions in starch granules are thought to represent the branching points of amylopectin, while the crystalline region is thought to represent the more compact double helix structure of amylopectin. The semicrystalline regions are more abundant in amylopectin and are resistant to enzymatic degradation because they are resistant to water ingress. In contrast, amylose has more amorphous layers and is therefore more susceptible to enzymatic degradation and water ingress. Starches are defined as waxy when the ratio of amylose to amylopectin is <15%, normal when the amylose content is 16-35% of the granule, and high amylose when the amylose content exceeds 36% of the granule (Svihus et al. 2005; Perez et al., 2009).

Starch digestion varies with grain type, grain processing methods, preservation methods, diet composition, and animal species. Starch from barley, wheat and oats is more rapidly digested than starch from corn, while starch from sorghum is the most resistant to digestion. These differences are due to the structure of the endosperm rather than the amylose and amylopectin in the starch. Mealy and glassy endosperm are both resistant to enzymes and digestion, but for different reasons: mealy endosperm contains soluble proteins, while glassy endosperm contains insoluble proteins called prolamins (Hoffman and Shaver, 2010; Trotta et al., 2021). Starch sources vary according to the amount and proportion of germ and vitreous endosperm, and there are significant differences between vitreous endosperm in some cereal species. For example, vitreous endosperm in dried corn ranges from 0% to 75%, and corn with more vitreous endosperm is more resistant to grinding and digestion than corn with waxier endosperm. Vitreous content increases as the crop dries in the field, so the difference between field-dried hybrid corn is large. Because corn silage is harvested earlier than high moisture corn, the kernels will be moister and have less vitreous endosperm. However, for similar products, there can be a 30-40% difference in dry matter when harvesting corn silage with high vitreous endosperm and a 60-75% difference in dry matter when harvesting high moisture corn (Hoffman and Shaver, 2010; Fernandes et al., 2021).

When grain is ensiled, starch fermentation in the rumen is affected by both the moisture concentration in the grain and the storage time. This is because ensiling dissolves endosperm proteins over time. This risk is greater in high moisture grains such as corn silage. Therefore, corn silage should be stored in the silo for several months before feeding (Allen, 1998; Allen et al., 2003).

Cattle can digest about 90% of starch, depending on the rate at which it passes through the rumen and the amount in the ration. Starch is a

carbohydrate that can ferment quickly without stimulating too much rumen movement and without remaining in the rumen for a long time. For these reasons, if the ration contains high amounts of easily digestible carbohydrates such as starch and molasses, cellulose digestion in the rumen is reduced, and therefore the amount of milk fat is reduced. In addition, lactic acid formation increases along with propionic acid in ruminants fed high starch diets, leading to problems such as acidosis and laminitis. If starch reaches the large intestine without being fermented in the rumen, it is fermented there and can cause diarrhea because the VFAs stimulate the intestinal wall (Du et al., 2021; Palmonari et al., 2021).

The outer surface of cereal grains consists of a thick, multilayered pericarp that protects the inner layers of the grain and endosperm from microbial attack. In addition to the pericarp, which accounts for 3% to 8% of the total kernel weight, barley and oats have a fibrous stem, or tegument, which accounts for up to 25% of the total kernel weight (Evers et al., 1999). Chemically, about 90% of the pericarp and tegument are fibers, and their digestibility is about 40% due to their lignified structure (Van Barneveld, 1999). The ruminal digestibility of the hull and pericarp is further reduced by the low ruminal pH (<6.2) associated with high grain diets.

The endosperm consists of two distinct tissues, the starchy endosperm and the aleurone. The aleurone consists of 1 to 3 layers, depending on the type and genetics of the grain (Narwal et al., 2020). The endosperm cell walls of wheat and maize consist mainly of arabinoxidants, while those of oats and barley consist mainly of β-glucans. Endosperm cell walls are largely devoid of lignin and, given the high arabinoxylanase and β-glucanase activity of rumen microorganisms, are not a significant barrier to starch digestion (McAllister et al., 2001).

Endosperm cell walls surround starch granules embedded in a protein matrix. The endosperm has two distinct regions in both maize and sorghum grain. In the vitreous endosperm region, starch granules are densely packed within a protein matrix, whereas in the endosperm region, starch granules are loosely associated with the protein matrix. In maize, the starch granules are very closely associated with the protein. In barley and wheat, the protein matrix is loosely associated with starch granules throughout the endosperm (Ergün et al., 2020).

3.2. Effects on rumen digestion mechanism

Rumen bacteria are responsible for the majority of starch digestion in the rumen. *Streptococcus bovis*, *Ruminobacter amylophilus*, *Prevotella ruminicola*, *Butyrivibrio fibrisolves*, *Succinimonas amylolytica* and *Selenomonas ruminantium* are the major starch digesters (Trotta et al., 2021; Wang et al., 2022). Although TIC enzymes have a significant effect on the ability to digest starch (Zhang et al., 2006), this change in digestion is less pronounced when isolated starch granules are subjected to digestion by a mixed rumen microbial population (Fondevila and Dehority 2001; Iommelli et al., 2022).

Microbial digestion of wheat and barley starch granules spreads inward from the microbial attachment point on the surface of the granule. In contrast, corn starch granules are digested from the inside out by tunneling amylolytic bacteria. As a result, when digestion is complete, the interior of the granule is empty, leaving only the outer surface layer. In addition, using very high concentrations of grain in the diet can cause a decrease in the diversity and number of protozoa, which can lower ruminal pH and increase the risk of acidosis in cattle (Faichney et al., 1997).

The structure of the protein matrix surrounding the starch granules of cereals commonly used in cattle diets has a much greater effect on the rate and amount of starch digestion than the properties of the starch itself (Iommelli et al., 2022). In hard maize, rumen bacteria preferentially colonize around starch granules within the glassy protein matrix. As digestion progresses, they hydrolyze the starch granules, passing them into the endosperm cells but leaving the protein matrix intact. As a result, with prolonged exposure to rumen bacteria, all starch granules are digested, leaving only the surrounding protein matrix and endosperm cell wall (Fernandes et al., 2021). Many of the differences in digestion between more slowly fermented grains (e.g. maize, sorghum) and more rapidly fermented grains (e.g. wheat, barley) may depend on the properties of the protein matrix between these grains (Khan et al., 2015).

3.3. Starch' importance in ruminant diets

It may seem logical to increase the energy content of the ration by feeding more cereals and less roughage in order to increase milk production and thus meet the nutritional needs of cows. However, instead of improving performance, this situation leads to low milk fat, acidosis, decreased milk production, digestive disorders, laminitis and

situations that can lead to death. The starch in wheat ferments faster than the starch in corn. Therefore, when fed in excess, it causes digestive upset, decreased rumen pH, acidosis, laminitis, decreased feed digestibility, diarrhea, and decreased feed intake (Hall, 2006b).

4. Applications to Modify Starch Structure

Two basic processes are used to increase the digestibility of starch in feed ingredients in the ration.

4.1. Gelatinization

Permanent modification of the granular structure by breaking hydrogen bonds. During gelatinization, starch absorbs water, expands, breaks hydrogen bonds, releases some amylose, and thus becomes more soluble and subject to more enzyme activity (Pan et al., 2021). In water, most starches gelatinize at temperatures above 80°C. The gelatinization temperature is higher for small starch granules. Grains rich in amylose are more resistant to gelatinization than grains with normal and high amylopectin content (Svihus et al., 2005).

4.2. Retrogradation

It is the reversible conversion of the dissolved, dispersed or amorphous form of starch into the crystalline or insoluble form that limits starch digestibility. Retrogradation is a desirable process in certain applications, including the production of breakfast cereals, parboiled rice, dehydrated mashed potatoes, and Chinese rice vermicelli, because it modifies their structural, mechanical, and sensory properties. The retrogradation of starch is desirable for nutritional reasons because it slows down the enzymatic digestion of starch and moderates the release of glucose into the blood stream. In Western countries, starch contributes to over 50% of the average caloric intake, and up to 90% in the developing world. As one of the major carbohydrate components in many foods, the digestion of starch has significant health implications. As discussed later, the digestibility of starch is of nutritional interest in relation to the rising incidence of obesity and diet-related diseases (Wang et al., 2015). In short, it is the recrystallization of starch. Amylose is the main component that facilitates retrogradation (Biliaderis, 2009).

CONCLUSION

As a result, determining the starch content, fractions and degradation rates in dense feed sources used in ruminant diets is of great importance in terms of healthy animal growth, productive metabolism and overall implementation of an optimal nutritional strategy. The digestive systems of ruminants allow them to obtain energy-rich substances by effectively breaking down starch, particularly through the microorganisms in their stomachs. This process plays a vital role in ensuring that animals meet their energy requirements and have a healthy growth process. In addition, determination of starch fractions in concentrated feed sources provides guidance for optimizing the nutrient content of rations and meeting the specific nutritional needs of animals. These analyses ensure that animals receive the nutrients they need in the most effective way, thereby improving their overall health. In-depth knowledge of starch content, fractions and degradation rates in ruminant diets is critical from a sustainable agriculture and animal welfare perspective. As a result, this analytical information plays a key role in the development of ruminant feeding strategies, a better understanding of the nutritional needs of animals, and improving the productivity of the livestock industry in general.

REFERENCES

Allen MS, Grant RJ, Weiss WP, Roth GW, Beck JF. Effects of endosperm type of corn grain on starch degradability by ruminal microbes in vitro. J Dairy Sci. 2003; 86:61.

Baldwin RL, Connor EE. Rumen function and development. Vet Clin North Am Food Anim Pract. 2017; 33 (3):427-439.

Bello-Pérez LA, Rodríguez-Ambriz SL, Agama-Acevedo E, Sanchez-Rivera MM. Solubilization effects on molecular weights of amylose and amylopectins of normal maize and barley starches. Cereal Chem, 2009. *86*(6), 701-705.

Bhandari KB, Rusch HL, Heuschele DJ. Alfalfa stem cell wall digestibility: Current knowledge and future research directions. Agronomy. 2023; 13(12):2875.

Biliaderis CG. Structural transitions and related physical properties of starch. In: Be Miller J, Whistler R, eds. Starch: Chemistry and Technology. 3th ed. USA: Academic Press; 2009. pp.293-372.

Dijkstra J, Forbes JM, France J. Quantitative aspects of ruminant digestion and metabolism. 2nd ed. UK: CABI Publishing; 2005. pp.157-170.

Du C, Ma L, Zhen YG, Kertz AF, Zhang WJ, Bu DP. Effects of different physical forms of starter on digestibility, growth, health, selected rumen parameters and blood metabolites in Holstein calves. Anim Feed Sci Tech, 2021; *271*, 114759.

Ergün A, Tuncer ŞD, Çolpan İ, *et al.* Hayvan besleme ve beslenme hastalıkları. 8. Baskı. Ankara: Elma Teknik Basım Matbaacılık; 2020. ss.33-50.

Evers AD, O'Brien L, Blakeney AB. Cereal structure and composition. Aust J. Agric Res. 1999; 50:629-650.

Faichney GJ, Poncet C, Lassalas B, *et al.* Effect of concentrates in a hay diet on the contribution of anaerobic fungi, protozoa and bacteria to nitrogen in rumen and duodenal digesta of sheep. Anim Feed Sci Technol. 1997; 64:193-213.

Fernandes J, da Silva EB, de Almeida Carvalho-Estrada P, Danil JLP, Nussio LG. Influence of hybrid, moisture, and length of storage on the fermentation profile and starch digestibility of corn grain silages. Anim Feed Sci Technol. 2021; 271:114707.

Fondevila M, Dehority BA. In vitro growth and starch digestion by *Entodinium exiguum*as influenced by the presence or absence of live bacteria. J Anim Sci. 2001; 79:2465-2471.

Grev AM, Wells MS, Samac DA, Martinson KL, Sheaffer CC. Forage accumulation and nutritive value of reduced lignin and reference alfalfa cultivars. Agron J. 2017; 109(6), 2749-2761.

Hall MB. Working with Non-NDF carbohydrates with manure evaluation and environmental considerations. Proc Mid South Ruminant Conf, Texas; April 24-25 2002.

Hall MB. Dietary carbohydrate impact on milk components. Proceedings of the Four-State Dairy Nutrition and Management Conference, Dubuque, Iowa; 2006a June 15. pp.79-83.

Hall MB. Rumen acidosis: Carbohydrate feeding considerations. Penn State: Dairy Cattle Nut Proc; 2006b. pp.1-9.

Hall MB. Carbohydrate nutrition and manure scoring. Part I: Carbohydrates. Proceedings of the Minnesota Dairy Health Conference, St. Paul, Minnesota. May 15; 2007a. pp.69-79.

Hall MB. Methodological challenges in carbohydrate analysis. Proceedings of the 44th Annual Meeting of the Brazilian Society of Animal Science. Brazilian J Anim Sci (supplement). 2007b; 36:359-367.

Hall MB, Zanton GI. Substitution of cane molasses for corn grain at two levels of degradable protein. I. Lactating cow performance, nutrition model predictions, and potential basis for butterfat and intake responses. J Dairy Sci. 2022; 105(5):3939-3953.

Hoffman P, Shaver R. Improving and evaluating starch digestibility for lactating dairy cows. In Four-State Dairy Nutrition and Management Conference 2010 (p. 32).

Iommelli P, Zicarelli F, Musco N, *et al.* Effects of cereals and legumes processing on in situ rumen protein degrability: A review. Fermentation. 2022; 8(8):363.

Kalaycıoglu L, Serpek B, Nizamlıoğlu M, Başpınar N, Tiftik AM. Biyokimya. Ankara: Nobel; 2000. pp.412-420.

Khan NA, Yu P, Ali M, Cone JW, Hendriks WH. Nutritive value of maize silage in relation to dairy cow performance and milk quality. J Sci Food Agricul. 2015; 95(2):238-252.

Ma C, Li Y, You H, *et al.* Nonstructural carbohydrates, carbon and nitrogen concentrations in fine roots of Quercus variabilis secondary forests after two different periods of regeneration. Forest Systems. 2021; 30(1):1.

McAllister TA, Hristov AN, Beauchemin KA, Rode LM, Cheng KJ. Enzymes in ruminant diets. In: Bedford MR, Partridge GG, eds. Enzymes in Farm Animal Nutrition. Marlborough, Wiltshire, UK: CABI Publishing; 2001. pp.273-298.

Narwal S, Kumar D, Kharub AS, Verma RPS. 11 - Barley biofortification: present status and future prospects, Editor(s): Om Prakash Gupta, Vanita Pandey, Sneh Narwal, Pradeep Sharma, Sewa Ram, Gyanendra Pratap Singh, In Woodhead Publishing Series in Food Science, Technology and Nutrition, Wheat and Barley Grain Biofortification, Woodhead Publishing, Elsevier, 2020, pp 275-294.

National Research Council (NRC). Nutrient requirements of dairy cattle. 7th ed. Washington: National Academy Press; 2001.

Oba M, Allen MS. Effects of com grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. J Dairy Sci. 2003; 86:184-194.

Palmonari A, Federiconi A, Cavallini D, Sniffen CJ, Mammi L, Turroni S, Formigoni A. Impact of molasses on ruminal volatile fatty acid production and microbiota composition in vitro. Animals, 2023; *13*(4), 728.

Pan L, Huang KH, Middlebrook T, Zhang D, Bryden WL, Li X. Rumen degradability of barley, oats, sorghum, triticalei and wheat in situ and the effect of pelleting. Agriculture. 2021; 11(7):647.

Pérez S, Baldwin PM, Gallant DJ. Structural features of starch granules I. In: James B, Roy W, eds. Starch. 3rd ed. San Diego: Academic; 2009. pp:149-192.

Saha SK, Pathak NN, Saha SK, Pathak NN. Digestion, absorption and metabolism of nutrients. In: Fundamentals of Animal Nutrition. Singapore, Springer Singapore; 2021. pp.219-246.

Slafer GA, Savin R. Comparative performance of barley and wheat acress a wide range of yielding conditions. Does barley outyield wheat consistently in low-yielding conditions? European J Agro. 2023; 143:126689.

Svihus B, Uhlen AK, Harstad OM. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Anim Feed Sci Technol. 2005; 122:303-320.

Tester RF, Karkalas J, Qi X. Starch-composition, fine structure and architecture. J Cereal Sci. 2004; 39:151-165.

Trotta RJ, Harmon DL, Matthews JC, Swansson KC. Nutritional and physiological constraints contributing to limitations in small intestinal starch digestion and glucose absorption in ruminants. Ruminants. 2021; 2(1):1-26.

Van Barneveld SL. Chemical and physical characteristics of grains related to variability in energy and amino acid availability in ruminant: A review. Aust J Agric Res. 1999; 50:651-666.

Wang S, Li C, Copeland L, Niu Q, Wang S. Starch retrogradation: A comprehensive review. Comp Rev Food Sci Food Saf. 2015; 14(5):568-585.

Wang H, Yu Z, Gao Z, *et al.* Effects of compound probiotics on growth performance, rumen fermentation, blood parameters, and health status of neonatal Holstein calves. J Dairy Sci. 2022; 105(3):2190-2200.

Wang Y, Tian Y, Christensen SJ, Blennow A, Svensson B, Moller MS. An enzymatic approach to quantify brancching on the surface of starch granules by interfacial catalysis. Food Hydrocol. 2024; 146:109162.

Weinert-Nelson JR, Kagan IA, Ely DG, Flythe MD, Davis BE. Fructan catabolism by rumen microbiota of cattle and sheep. Fermentation. 2023; 9(11):925.

Zhang G, Zihua ZA, Hamaker BR. Slow digestion property of native cereal starches. Biomacromolecules. 2006; 7:3252-3258.

ACKNOWLEDGMENTS

Author contribution: ŞE designed, search the literature and writing& drafting and revising the manuscript. ŞE: Şevket Evci

Financial Disclosure: The author declared that this study has received no financial support.

Conflict of Interests: The author declared that there is no conflict of interests.

Additional information: The author has read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to ŞE.

Review https://doi.org/10.47748/tjvr.1552322

TJVR 2024; 8 (2): 151-162

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Physiotherapy and rehabilitation in geriatric dogs and cats

Nevin Coskan[1] Pinar Can[2]

[1] *Department of Veterinary Surgery, Graduate School of Health Sciences, Ankara University, Ankara, Türkiye*
[2] *Department of Surgery, Faculty of Veterinary Medicine, Ankara University, Ankara, Türkiye.*

Correspondence: *Pinar Can (pcan@ankara.edu.tr)*

Received: 18.09.2024 Accepted: 20.10.2024

ABSTRACT

This review aims to give current approach about physical therapy and rehabilitation of the geriatric dogs and cats. The word "geriatric" refers to the medicine of the elderly and it is used in veterinary medicine for animals that have completed 75% of their lifespan. As the size of an animal increases, the age at which they become geriatric decreases, therefore; geriatric age varies amongst different species and breeds. Homeostatic changes occur with increasing age and some diseases such as obesity, tumor formation, orthopedic, cardiovascular and neurological disorders are frequently seen. Some breeds are genetically predisposed to such diseases. Thus, regular screening tests and clinical examinations are important for early diagnosis. Geriatric physiotherapy is a developing field that is noninvasive and, in some cases, can be used as a form of primary treatment or supportive treatment. Physiotherapy prevents muscle atrophy and reduces pain caused by inflammation all the while improving the patient's quality of life. There are many different methods used in physiotherapy; TENS and NMES devices, passive and active range of motion exercises, hydrotherapy, acupuncture, massage, laser therapy, ECSWT and cryotherapy. However, treatment protocols are tailored by physicians to fit each patient's needs depending on the disease and severity of the disease to be treated.

Keywords: *Physical therapy modalities, Geriatrics, Musculoskeletal diseases, Obesity, Cardiovascular diseases, Nervous system diseases*

INTRODUCTION

Terms such as elderly or aged are commonly used in human medicine, however; there were no such classifications available for animals before the 21st century. In the 21st century, as a result of the advancements in veterinary science, the term "geriatric" was also applied to animals (Davies, 1996). The American Animal Hospital Association used the term "geriatric" for dogs who have exceeded 75% of their expected lifespan in the 2019 Canine Life Stage Guidelines (Creevy et al., 2019). In 2021, The American Animal Hospital Association (AAHA) Feline Senior Life Stage Guidelines, it was mentioned that cats over 10 years of age are defined as geriatric (Quimby et al., 2021). The age for being considered a geriatric differs in different animal species, and even in the same species depending on the breed. Large breed dogs weighing >45 kg can be defined as geriatric at approximately 7 years of age, although small breed dogs weighing lower than 10 kg are geriatric in 12 years of age (Altınkaya, 2022; Quimby et al., 2021; Vogt et al., 2010). However, it is important to recognize that both the environment and genetics play a role in the aging process demonstrated in the tables (Davies, 1996).

1. Diseases Observed in Geriatric Animals

Geriatric animals are at an increased risk of acquiring diseases due to decreased strength, diminished body homeostasis, and declined

energy reserve (Ward et al., 2016). Therefore, veterinarians should examine geriatric animals more often and perform additional tests such as blood analyses, radiography and ultrasonography to detect possible disorders and delay the onset of disease. Although two different animals may have the same disorder, they might not show the same symptoms clinically (Mosier, 1989).

Veterinarians commonly fail to acquire a proper history from owners since owners believe that some signs are normal and related to aging. Thus, it is vital to apply a clinical examination along with the suitable laboratory tests in order to detect the disease (Pati et al., 2015). For this purpose, screening tests are desirable and widely recommended (performed annually for middle-aged patients and semi-annually for healthy geriatric patients) (Davies, 2012; Dhaliwal et al., 2023).

Diseases associated with the aging process include degenerative joint diseases, periodontal disorders, heart-related diseases, obesity, disorders related to the endocrine system, kidney disorders, and neoplasia. In addition, externally visible changes such as graying of the hair, alopecia, thickening of the foot pads/callus formation, and loss of elasticity of the skin would be observed (Pati et al., 2015).

Physical therapy is a treatment choice in most geriatric patients for pain reduction, improving functional movement, and quality of life (Frye et al., 2022). Therapists can also provide the optimal exercises necessary to renew neural pathways and improve joint mobility and muscle function (Carmeli, 2017; Fransen, 2004).

2. Physiotherapy in Orthopedic Diseases

With advanced age, muscle atrophy can be observed alongside structural changes in cartilage tissue. Naturally, their physiological reserves are depleted, thus; recovery after injuries will slow down or will be completely absent (Pecina et al., 2013). Due to these changes, many orthopedic diseases are observed in geriatric animals but the most remarkable observation is osteoarthritis (Beale, 2005). Dogs commonly present with osteoarthritis caused by natural aging which in some cases leads to crepitus, this phenomenon is less seen amongst cats (Dhaliwal et al., 2023). However, more than 60% of cats older than 6 years have osteoarthritis which is not easily diagnosed during a physical examination since the cats use behavioral change strategies to disguise pain instead of developing lameness (Slingerland et al., 2011). Other disorders such as cranial cruciate ligament diseases or hip dysplasia occur secondary to osteoarthritis (Mille et al., 2022).

1.1 Osteoarthritis

Osteoarthritis is a chronic progressive degenerative joint disease (Bland, 2015; Kurt et al., 2023). It is the most common and most painful disease that is seen in geriatric dogs (Anderson et al., 2018; Capon and Dycus, 2018). 20% of dogs suffer from osteoarthritis and this number increases up to 80% in dogs over 8 years of age (Capon and Dycus, 2018). Obesity and advanced age increase the severity of the disease (Altınkaya, 2022). Clinical signs observed in dogs include lameness, difficulties in jumping or climbing stairs, crepitus, joint pain, and swelling. If the condition becomes chronic, a further decrease in the range of motion is seen. Cats show similar clinical signs to dogs, but try to hide their pain due to instincts (Slingerland et al., 2011). The most common clinical sign of osteoarthritis in cats is decreased activity (Yamazaki et al., 2020). Treatment should consist of a combination of non-steroid anti-inflammatory drugs, painkillers, weight control, physiotherapy and rehabilitation, joint supplements, and/or pharmaceutical injections to the joints. In some certain cases, surgical treatment such as total joint replacement, excision arthroplasty or arthroscopy may be needed (Bland, 2015).

The goals of physical therapy in osteoarthritis are to reduce pain, enhance muscular function, and maintain the function of the joints (Mille et al., 2022). Environmental modifications depend on the individual needs of the patient. For bedridden patients, since they spend most of their time in bed, soft bedding is highly recommended to avoid ulcerations. Owners can use support slings as well to raise and ambulate the patients safely. Also, carpets or rubber mats should be placed in order to avoid injuries (Jhonson et al., 2020).

Suitable exercises should be chosen for each patient to avoid worsening of osteoarthritis. For obese animals, the first step should be weight loss since the joint load must be decreased (Capon and Dycus, 2018).

Home exercise programs can include strengthening, proprioceptive exercises, and balancing exercises. Strengthening exercises can include sit-to-stand exercises, exercises with elastic bands or carpal weights, and regular walking on

different types and inclines of terrain. Passive range of motion techniques can be used in a home environment to improve joint health (Goldberg, 2022). Massage is commonly used by physiotherapists at the beginning of therapy sessions due to its pain-relieving effect. Physiotherapists should teach owners about basic massage techniques so that they can apply these techniques at home on geriatric osteoarthritic dogs (Mille et al., 2022). Lastly, especially overweight patients, may benefit from aquatic therapy since it provides high-intensity low-impact exercise (Song and Oh, 2022).

When starting physiotherapy, muscles, and joints that are about to be treated must be warmed up in preparation, which will increase the blood flow to the area and support flexibility. Heat bags or a warm bath up to 42 °C can be used which will warm tissue 1-2 cm under the skin (Altınkaya, 2022). Another method of warming up tissue is the use of therapeutic ultrasound on a continuous mode of 1 and 3 MHz. This affects deeper tissues (5cm under the skin). Since the heating effects of therapeutic ultrasound have a short duration, they are applied right before or during the stretching process (Mille et al., 2022; Nakano et al., 2012). After warming up the tissue, stretching exercises should be performed since they will prevent stiffness after rehabilitation (Park et al., 2018). In addition, electrical stimulation methods such as NMES and TENS can be applied. NMES is mainly used in cases where patients are in too much pain or are significantly weak to the degree that they cannot perform given exercises (Mille et al., 2022). TENS is used due to its analgesic effect (Pedersen et al., 2024). After TENS application, certain exercises are suggested such as walking on a treadmill or underwater treadmill, climbing stairs, or controlled walking with a leash (Altınkaya, 2022). Underwater treadmills are valuable for physiotherapists since with the help of water, friction forces that move through the joints will decrease and the patient will move more freely (Tomlinson, 2012). However, hydrotherapy can tire out the cardiovascular system of the geriatric dogs, thus; physiotherapists should be cautious to avoid putting too much pressure on the patient. Also, obese geriatric dogs may have an increased respiratory rate during therapy, thus; respiration should be monitored (Goldberg, 2022). After each session, the patient should be allowed to cool down for 5 to 10 minutes with low-tempo walking for 3-5 minutes followed by stretching exercises (Mille et al., 2022).

1.2 Cranial Cruciate Ligament Deficiency

Cranial cruciate ligament disease is commonly seen among dogs and it is almost always degenerative (Frye et al., 2022). Common causes in geriatric dogs include mild trauma caused by daily mechanical wear and degenerative changes in the fibers. Contributing factors are obesity, sterilization, and being from a certain breed such as Rottweilers, Terriers, Mastiffs, Saint Bernards, Mastiffs, and Retrievers (Spinella et al., 2021). Canine cranial cruciate ligament disease disrupts the normal range of motion and hindlimb properties leading to lameness. Moreover, it increases the tendency and rate of progression of osteoarthritis (Comerford et al., 2011). The ideal solution for cranial cruciate ligament disease is tibial plateau leveling osteotomy surgery since direct ligament replacement is not very successful in dogs (Frye et al., 2022). During the post-operative management (up to 2 weeks), rehabilitation should be provided with icing of the incision and its surroundings, massage, gentle stretching, passive range of motion, and controlled leash walks for 5 minutes twice a day (Monk et al., 2006). Backward walking, high stepping, wobble/rocker board, and neuromuscular electrical stimulation of the quadriceps and semitendinosus muscles are performed between the 2nd and the 4th week. For 4-6 weeks post-operatively, an underwater treadmill twice a week is recommended. In weeks 6-8, an inclined land treadmill can be added to the program. Also, figure 8 walking 2 to 3 times a week can be added to the schedule since it would help the dog regain balance and the ability to use surgical legs (Frye et al., 2022).

1.3 Tendinopathies

Tendinopathies are conditions that cause pain near the tendons which occur as a result of aging or chronic trauma (Andres and Murrell, 2008; Canapp et al., 2016). They are commonly observed in retired canine athletes and geriatric dogs. Clinical symptoms include stiffness of gait, lameness, missing jumps/obstacles, and decreased extension of the stifles. Many cases do not require surgical interventions because of the availability of physiotherapy and rehabilitation (Baltzer, 2012).

When diagnosed early, rest and ice therapy are applied alongside anti-inflammatory drugs. The patient may also benefit from therapeutic

ultrasound (pulsed mode, 3.3 MHz, 2.0W/cm^2, five minutes twice weekly), laser (12 joule/cm^2 daily), and pulsed electromagnetic field therapy (Prydie and Hewitt, 2015). In order to reduce inflammation and swelling, cryotherapy should be applied to decrease the blood flow and tendon metabolic rate (Edge-Hughes, 2016). In chronic cases, shockwave therapy is applied since it leads to neovascularisation which promotes the healing process (Canapp et al., 2016; Millis and Ciuperca, 2015). In addition to these therapies, aquatic therapy is added to the schedule to help develop proprioceptive skills (Barnicoat and Wills, 2016).

1.4 Canine Hip Dysplasia

Canine hip dysplasia is a developmental anomaly that causes joint inflammation and is likely to cause excessive cartilage wear and osteoarthritis (Mikkola et al., 2019; Schachner and Lopez, 2015). It is commonly seen in large breed dogs such as Mastiffs, Boxers, Saint Bernards, Labrador Retrievers, and German Shepherds (Hou et al., 2013). In comparison to dogs, hip dysplasia is rarely seen in cats (Perry, 2016). Diagnosis is confirmed when pain and laxity occur during the external rotation and abduction of the hip joint. In geriatric patients, laxity is not easily recognized because of fibrous tissue proliferation (Güzel and Altunatmaz, 2006). Bunny hopping is a typical sign, alongside lameness, decreased physical activity, reluctance to jump, pelvic muscle atrophy, and swaying gait. In addition to the clinical signs and physical examination, radiography is also used, and common techniques are Nornberg angle examination, distraction index calculation, hip-extended radiography, and dorsal acetabular rim view examination (Butler and Gambino, 2017; Fry and Clark, 1992).

Surgery and conservative therapies are among the methods of treatment. The main goal of the treatment is to minimize the progression of osteoarthritis (Schachner and Lopez, 2015). Surgical treatment methods include triple pelvic osteotomy, juvenile symphysiodesis, femoral head and neck excision, intertrochanteric osteotomy, Shelf arthroplasty and total hip replacement (Bergh and Budsberg, 2014).

Physiotherapy for pain management includes cold therapy, massage, low and high-level heating. Icing therapy should be applied after range of motion exercising and prior to bedtime for 10 to 15 minutes (Dycus et al., 2017). Massage therapy helps reduce muscle tension, thus; relieving myofascial pain (Riley et al., 2021). While low-level heat (elevation of tissue temperature by 1–2°C) provides vasodilation and normalizes blood flow, high heat (elevation of tissue temperature by 3–4°C) helps increase the effectiveness of stretching (Corral, 2018).

Active and passive range of motion along with stretching exercises should be applied as a part of the patient's daily exercises since they provide increased flexibility, adhesion prevention, improved extensibility, increased blood and lymph flow, improved synovial production and maintained mobility (Dycus et al., 2017). Moreover, Cavaletti rail walks and sit-to-stand exercises can help improve range of motion and they can be performed during clinical therapy or as home exercises (Corral, 2018; Charalambous et al., 2022).

Figure 1. Physio-ball exercise in a geriatric dog

Geriatric patients with hip dysplasia also suffer from decreased proprioception; thus, balance exercises should be integrated into the rehabilitation program (Dycus et al., 2017).

Following these exercises, when the patient is physically stronger, perturbation exercises are performed (Saunders, 2007). In addition to balance and perturbation exercises, there are other tools with similar therapeutic effects, such as balance boards, wobble boards, exercise balls, and rolls (Figure 1). Another option is for the patient to walk on foam rubber, mattresses, or trampolines, since the surface texture is different, the patient will regain its proprioceptive abilities (Weigel and Millis, 2014). Aquatic therapy will also help with the rehabilitation process and relieving of the symptoms (Millis and Ciuperca, 2015).

Physical modalities such as therapeutic ultrasound, electrical stimulation, laser therapy, and extracorporeal shockwave therapy are performed by physicians as part of hip dysplasia therapy (Corral, 2018). Treatment protocols vary but therapy starts with 6 sessions done over 3 weeks (Dycus et al., 2017).

1.5 Patellar Luxation

Patellar luxation is common amongst dogs, especially in smaller breeds such as Terriers, Malteses, Chihuahuas, and Poodles (Harasen, 2006). In cats, it is not as common as in dogs, but some breeds such as Abyssinians, Devon Rexs, and Siameses have a higher tendency to present with a dislocation in the patella (Cerna et al., 2021). Medial luxations are more commonly observed than lateral dislocations in small breeds, while lateral luxation is more common in larger or giant breed dogs (Harasen, 2006).

The clinical signs mostly include lameness, skipping gait, and backward stretching of the leg (Harasen, 2006). There are 4 grades of luxation: grade I, grade II, grade III, and grade IV (Lara et al., 2018). In grade I, the patella is displaced but returns to its normal position with certain maneuvers. With limited exercising, the dog can tolerate luxation but is more likely to develop osteoarthritis as they age. In grade II, the patella is displaced and remains luxated until stifle extension or manual replacement takes place (Perry and Dejardin, 2021; Roush 1993). In grade III, the patella keeps luxating and even after manual replacement to its anatomical position, it spontaneously dislocates when manual pressure is removed. In grade IV, there is continuous luxation and the patella cannot be replaced manually. Thus, in grades III and IV, the patient displays persistent lameness and an abnormal gait (Roush, 1993). Depending on the case, soft tissue techniques (medial desmotomy, lateral imbrication, antirotational sutures, and release of medial musculature) and/or osteotomy procedures (tibial tuberosity transposition, trochleoplasty) can be performed for treatment (Harasen, 2006).

Cold packs (as a form of cryotherapy) can be used since they have analgesic effects and can reduce inflammation (Wiputhanuphongs et al., 2015). Due to the decreased temperature, vasoconstriction of the vessels will occur. Moreover, nerve conduction decreases, thus; muscle spasms reduce (Kwiecien and McHugh, 2021). TENS, acupuncture, laser therapy, or therapeutic ultrasound can be used as professional approaches. After the pain is under control, it is important to focus on strengthening hindlimb muscles, especially the ones opposing the dislocated side (Akaraphutiporn et al., 2024). For lateral luxation, the treatment should be focused on the sartorius and adductor muscles, while for medial luxation, the main focus is on the gluteal area and biceps femoris. For hindlimb muscle strength, 3-legged stands, 2-legged stands, inclined stands, sit-to-stand, uphill walking, side stepping, and underwater treadmills exercises are recommended (Maddox, 2017).

In some cases, when physiotherapy is not sufficient, surgery is required. Also, during the early postoperative stages, several methods of physical rehabilitation are used, such as passive range of motion, massage, and cryotherapy. While in the late recovery period, warm therapy and active exercises are applied (Perry and Dejardin, 2021).

2. Obesity and Physiotherapy

Obesity is defined as an excessive amount of fat that accumulates within white adipose tissue which results in impairment of normal body function (Moraitou et al., 2014). Currently, 65% of dogs and 63% of cats are diagnosed with being overweight and obese (Purina Institute, 2024). Some breeds of dogs (Pugs, Beagles, Retrievers, and Dachshunds) have a higher tendency to become obese (Pegram et al., 2021). Breeds of cats that are at greater risk include mixed breeds, Manxes, British Shorthairs, Norwegian Forest Cats, and Persians (Öhlund et al., 2018; Purina Institute, 2024). While age varies amongst species, most obese dogs are between 5 and 11 years old (Lund et al., 2006). While obesity causes osteoarthritis, renal diseases, skin diseases, neoplasia, oral cavity diseases, and insulin resistance in dogs, in cats it leads to dermatological problems, diabetes

mellitus, neoplasia and urolithiasis (German 2016; Loftus and Wakshlag, 2015). The most common obesity-related disease in dogs is osteoarthritis (Purina Institute, 2024).

In veterinary clinics, evaluation of obesity is achieved by body condition score (BCS). Body condition scoring is an indirect way to evaluate the body fat accumulation. BCS can be determine by visual observation and palpation of the animal's body. For dogs, the optimal BCS is between 4/9 or 5/9 and over 7/9 is obese, while for cats, the ideal score is 5/9, and over 7/9 is obese (Chun et al., 2019; Santarossa et al., 2017).

For the management of obesity in senior pets, owners should maximize activity and choose a correct diet (German, 2016). Before the arrangement of an activity program, a detailed interview about the patients' medical history is conducted with the owner since physiotherapy methods can change in case of some disorders. Activity levels are to be safely increased depending on the patients' current physical ability. Owners can also provide an activity-promoting environment. For example, owners can take their dogs out for a walk at least twice a day regardless of their age. In the case of dogs that are more lenient to water exposure, controlled low-impact swimming can be helpful as an alternative to walking (Brooks et al., 2014). In clinics; land-based exercises, joint manipulation, treadmills, underwater treadmills, and acupuncture can be applied (Chauvet et al., 2011; Fernandes et al., 2022). If an obese animal has osteoarthritis; laser therapy, massage therapy, and acupuncture can be incorporated into their physiotherapeutic program for pain management (Mille et al., 2022).

Activities for cats should be provided through playing, puzzle feeders, and challenging them to find hidden food. Since cats have short bursts of energy, owners need to provide toys that will stimulate their predatory instincts (German, 2011; Zoran, 2009). Physical rehabilitation for feline species is challenging, especially when water is involved. Thus, sessions should be brief and the environment should be quiet in order not to promote stress. Although activity through playing is the most effective way of losing weight, in case of need of physical therapy, land-based and water-based exercises are performed (Goldberg, 2016).

3. Cardiovascular Diseases and Physiotherapy

Dogs are 1,5 times more likely to develop cardiovascular disorders as they age by one year (Inoue et al., 2016). Thus, cardiovascular diseases are frequently encountered in geriatric populations and they are the 2nd most common cause of death in dogs (Kosic et al., 2017).

Geriatric patients usually have a sedentary lifestyle which increases the prevalence of obesity, and promotes cardiac diseases (Lee et al., 2022). Heart failure is one of the most common cardiovascular diseases in senior dogs and is frequently caused by dilated cardiomyopathy, valvular diseases, hypertrophic cardiomyopathy, atherosclerosis, and diabetic cardiomyopathy (Çolakoğlu et al., 2022; Turgut et al., 2017). The incidence of mitral valve disease increases as the animal ages, with a much higher prevalence in small breeds and males, while large breeds are mainly affected by dilated cardiomyopathy (Gaar-Humphreys et al., 2022; Parker and Kilroy-Glynn, 2012).

Physiotherapeutic exercising has a positive impact on cardiovascular health (Dibben et al., 2021; Taylor et al., 2022). In a study conducted on rodents, it has been proven that exercise training protects the heart from cardiac injuries such as heart failure or diabetic cardiomyopathy (Bei et al., 2021).

In humans, low-energy extracorporeal shockwave therapy was found useful for improving angiogenesis and myocardial perfusion significantly in patients with refractory angina. Although exact mechanism under the angiogenesis effect of the shockwave is still unknown, it is thought to be mediated through vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (Prasad et al., 2015). Another safe option for physiotherapy of cardiac patients is low-level laser therapy. It is noninvasive and it enhances cardiac function, myocardial contractility, myocardial reserves, coronary reserves, and aerobic reserves (Sayed et al., 2023). In addition, it helps with the treatment of ischemic regions of the heart and enhances anastomosis (Karvandi, 2021).

Since TENS affects the heart rate through the vagal nerve, it is believed that it should not be used on cardiac patients (Hsieh et al., 2021). Ganguie et al. (2017) conducted research on humans regarding the effects of TENS on congestive heart failure patients who are not able to perform aerobic exercises. However, the results have shown that the patients to which TENS was applied walked significantly more than the others without any major difference in systolic and diastolic blood

pressures (Ganguie et al., 2017). But up to now, despite the promising results of some studies, electrotherapy applications to animals with cardiac diseases are controversial (Ağırman and Aydın, 2018; Badger et al., 2017). In practice, physical therapists hesitate to use TENS on cardiac patients due to the lack of sufficient research on the effect of TENS application on cardiac patients. If these methods will be used, patients should be monitored carefully by therapists and physicians for the potential side effects during Treatment. Pacemakers are a definite contraindication for the use of electrical stimulus (Hoshiai et al., 2021; Lago et al., 2018).

Precautions must be taken during the use of cryotherapy in patients with impaired circulation and cardiac insufficiency. Also, in geriatric animals, thermal regulation or pain reception may be impaired, therefore; patients with diminished cardiovascular reserves may have a lower heat tolerance (Dragone et al., 2014). Moreover, therapeutic ultrasound directed at the heart should be avoided, as well as on patients with cardiac pacemakers since electrocardiographic changes can occur (Rennie, 2010).

4. Neurological Diseases and Physiotherapy

In geriatric animals, many neurological diseases can be observed, namely degeneration of intervertebral disks, cervical spondilomyopathies, degenerative myelopathies, spinal neoplasias, cognitive dysfunctions, and instability of lumbosacral vertebras (Bagley, 1997). The most common type of spinal disease in dogs is intervertebral disk disease (Hermansen et al., 2022). It is more commonly seen in dogs compared to cats (Amey et al., 2024; Sharp, 2012). During the neurological evaluation, the Modified Frankel Score is commonly used (Hermansen et al., 2022). Conservative management of intervertebral disk disease for grades 1 to 3 in dogs is highly successful (90%), for grade 4, the success rate is only 50%, and for grade 5, the response to conservative management is poor (Olby et al., 2022). The severity and type of disorder guide physicians to the method of treatment, some cases might require surgery and the use of anti-inflammatory drugs, while others may not, but regardless, physical therapy is recommended (Jeong et al., 2019; Nicholson, 2016; Olby et al., 2020).

In the acute phase of treatment of non-ambulatory patients, rehabilitation aims to improve the musculoskeletal system (Moore et al., 2020). Physical therapy exercises include standing exercises, range of motion exercises, pain control, toe pinch exercises, and the use of underwater treadmills (Olby et al., 2020). The goal of standing exercises is to strengthen the hip joints, strengthen the stifle joints, regain balance, and regain proprioception (Figure 2) (Brantberg et al., 2023). For this purpose, slings or Hoyer lifts can be used. Toe pinch exercises aim to stimulate flexor reflex and they can be combined with passive range of motion exercises (Drum, 2010).

During the acute phase of treatment, ambulatory patients can benefit from the exercises that are normally applied for non-ambulatory patients, however; they also require additional exercises such as sling-assisted walking, Cavaletti rails, and physio roll balancing (Drum, 2010). Attention must be paid to the placement of the foot since some patients might not be able to place their feet in the proper position. In this case, specially designed boots or white medical tape can be used to provide correct foot positioning (Sims et al., 2015). Cavalatti rails not only strengthen the hip and stifle flexors but also enhance neuromuscular coordination (Weigel and Millis, 2014). Physioroll and physioball balancing help regain coordination and proprioception (Millis et al., 2014).

Figure 2. Proprioceptive, standing and shifted weight-bearing exercise on a balance disc in a dog with intervertebral disc disease.

After the acute phase of rehabilitation, the animals' neurologic condition improves, and advanced exercises such as stair climbing, carrying/pulling weights, walking on different surfaces, hill climbing, and swimming against resistance are added to the therapy schedule (Drum, 2010). In

addition to active exercising, electrical stimulation, cryotherapy, and ultrasound have a role in neurologic rehabilitation (Spinella et al., 2022). However, they should not replace active exercising (Packer et al., 2016). During the acute phase of treatment for both ambulatory and non-ambulatory patients, TENS can be used post-operatively on incision lines and NMES can be combined with exercising to maximize muscle contraction (Drum, 2010). If there is muscle or joint contracture that delays recovery, patients can benefit from continuous ultrasound (Morishita et al., 2014). Cryotherapy is commonly used immediately in the postoperative period in order to reduce inflammation and pain (Olby et al., 2020). Lastly, acupuncture has an analgesic effect and it increases brain-derived neurotrophic factor, provides neuroprotection, reduces cell death of neurons, and reduces expression of proinflammatory mediators (Miao et al., 2023). It is recommended to start acupuncture therapy during the acute phase of a neurological disorder or early after injury/surgery (<72 hours), however; the most prominent recoveries are observed when applied within 24 hours after injury (Frank and Roynard, 2018).

Satisfactory results of physiotherapy are obtained approximately within 40 sessions. For instance, in traumatic spinal diseases, results are obtained after 20-30 sessions, and in the case of degenerative spinal diseases, it takes around 80 sessions (Henea et al.,2020).

Degenerative myelopathy is a central nervous system disease that severely affects the thoracic spinal cord segments (Bouche et al., 2023; Coates and Wininger, 2010). It is caused by a genetic mutation in the superoxide dismutase 1 gene and mainly affects geriatric dogs (Awano et al., 2009). Unfortunately, no specific medication is available for these cases. However, affected dogs can benefit from physiotherapy which improves their quality of life and survival rates (Kobatake et al., 2021). A nonrandomized study showed that extensive physical therapy largely enhances mobility and helps patients maintain mobility for a longer period which results in significantly increased survival periods (a mean of 255 days). The same results were not obtained with moderate or absent physical therapy (130 days and 55 days respectively) (Thomas et al., 2014).

CONCLUSION

There is an increasing trend to treat orthopedic and neurologic diseases as possible as conservatively. In this concept physical therapy has a pivotal role since it is non-invasive, relatively low costed, and also its promising results were shown. In geriatric small animals improved muscle strength, mobility and all in all quality of life may be achieved by physical therapy interventions. Joint flexibility and reduced pain can specifically be attained through exercise, therapeutic modalities, aquatic therapy, massage therapy, and cryotherapy. Overall, physiotherapy is a promising field due to its valuable therapeutic potential for managing age-related diseases. Physiotherapy should be recommended as a part of the standard care protocol for geriatric animals. Specific tailored programs for each individual patient can be developed considering their limitations and specific needs. Further exploration and research are ongoing to deeply understand the long-term effects and optimal frequency of physical therapy sessions.

REFERENCES

Ağırman M, Aydın HI. Effect of the conventional transcutaneous electrical nerve stimulation on heart rhythm. J PMR Sci. 2018; 21(3):122-125.

Akaraphutiporn E, Kwananocha I, Meechai C, *et al*. Comparative analysis of post-operative rehabilitation approaches for medial patellar luxation in small-breed dogs. Vet World. 2024; 17(3):550-557.

Altınkaya N. Geriatrik köpeklerde fizyoterapi ve rehabilitasyon: Güncel bakış. Adnan Menderes Üniversitesi Sağlık Bilimleri Fakültesi Dergisi. 2022; 6(3):554-561.

Amey JA, Liatis T, Cherubini GB, Decker S, Foreman MH. Outcomes of surgically and conservatively managed thoracolumbar and lumbosacral intervertebral disc herniations in cats. J Vet Intern Med. 2024; 38(1):247-257.

Anderson KL, O'Neil DG, Brodbelt DC, *et al*. Prevalance, duration and risk factors for appendicular osteoarthritis in a UK dog population under primary veterinary care. Sci Rep. 2018; 8(1):5641-5652.

Andres BM, Murrell GAC. Treatment of tendinopathy: What works, what does not, and what is on the horizon. Clin Orthop Relat Res. 2008; 466(7):1539-1554.

Awano T, Johnson GS, Wade CM, *et al*. Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis. Proc Natl Acad Sci USA. 2009; 106(8):2794-2799.

Badger J, Taylor P, Swain I. The safety of electrical stimulation in patients with pacemakers and implantable cardioverter defibrillators: A systematic review. J Rehabil Assist Technol Eng. 2017; 4:1-9.

Bagley RS. Common neurologic diseases of older animals. Vet Clin North Am Small Anim Pract. 1997; 27(6):1451-1486.

Baltzer W. Sporting dog injuries. Vet Med. 2012; 107(4):166-177.

Barnicoat F, Wills AP. Effect of water depth on limb kinematics of the domestic dog (Canis lupus familiaris) during underwater treadmill exercise. Comp Exerc Physiol. 2016; 12(4):199-207.

Beale BS. Orthopedic problems in geriatric dogs and cats. Vet Clin North Am Small Anim Pract. 2005; 35(3):655-674.

Bei Y, Wang L, Ding R, *et al.* Animal exercise studies in cardiovascular research: Current knowledge and optimal design- A position paper of the Committee on Cardiac Rehabilitation, Chinese Medical Doctors' Association. J Sport Health Sci. 2021; 10(6):660-674.

Bergh MS and Budsberg SC. A systematic review of the literature describing the efficacy of surgical treatments for canine hip dysplasia (1948–2012). Vet Surg. 2014; 43:501-506.

Bland SD. Canine osteoarthritis and treatments: A review. Vet Sci Dev. 2015; 5(2):84-89.

Bouche TV, Coates JR, Moore SA, Faissler D, Rishniw M, Olby NJ. Diagnosis and management of dogs with degenerative myelopathy: A survey of neurologists and rehabilitation professionals. J Vet Intern Med. 2023; 37(5):1815-1820.

Brantberg I, Grooten WJA, Essner A. The effect of therapeutic exercise on body weight distribution, balance, and stifle function in dogs following stifle ınjury. Animals. 2023; 14(1):92-110.

Brooks D, Churchill J, Fein K, *et al.* 2014 AAHA weight management guidelines for dogs and cats. J Am Anim Hosp Assoc. 2014; 50(1):1-11.

Butler JR, Gambino J. Canine hip dysplasia: Diagnostic imaging. Vet Clin North Am Small Anim Pract. 2017; 47(4):777-793.

Canapp SO, Canapp D, Carr BJ, Cox C, Barrett JG. Supraspinatus tendinopathy in 327 dogs: A retrospective study. Veterinary Evidence. 2016; 1(3).

Capon H, Dycus D. How to case manage the osteoarthritic dog. BSAVA Companion. 2018; 2018(5):14-19.

Carmeli E. Physical therapy for neurological conditions in geriatric populations. Front Public Health. 2017; 5:333-336.

Cerna P, Timmermans J, Komenda D, Nyvltova I, Proks P. The prevalance of feline hip dysplasia, patellar luxation and lumbosacral transitional vertebrae in pedigree cats in the Czech Republic. Animals. 2021; 11(9):2482-2490.

Charalambous D, Strasser T, Tichy A, Bockstahler B. Ground reaction forces and center of pressure within the paws when stepping over obstacles in dogs. Animals. 2022; 12(13):1702-1718.

Chauvet A, Laclair J, Elliott DA, German AJ. Incorporation of exercise, using an underwater treadmill, and active client education into a weight management program for obese dogs. Can Vet J. 2011; 52(5):491-496.

Chun JL, Bang HT, Ji SY, *et al.* A simple method to evaluate body condition score to maintain the optimal body weight in dogs. J Anim Sci Technol. 2019; 61(6):366-370.

Coates JR, Wininger FA. Canine Degenerative Myelopathy. Vet Clin North Am Small Anim Pract. 2010; 40(5):929-950.

Comerford EJ, Smith K, Hayashi K. Update on the aetiopathogenesis of canine cranial cruciate ligament disease. Vet Comp Orthop Traumatol. 2011; 24(2):91-98.

Corral C. Canine hip dysplasia: rehabilitation. The Veterinary Nurse. 2018; 9(6):304-308.

Creevy KE, Grady J, Little SE, *et al.* 2019 AAHA canine life stage guidelines. J Am Anim Hosp Assoc. 2019 Nov/Dec;55(6):267-290.

Çolakoğlu EÇ, Sevim K, Kalınbacak A, Kaya U. Retrospective evaluation of cardiopulmonary diseases in cats and dogs: 570 cases. Kocatepe Vet Derg. 2022; 15(2):182-188.

Davies M. An Introduction To Geriatric Veterinary Medicine. Canine and Feline Geriatrics. 1st. ed. Wiley Blackwell; 1996. p.4-18.

Davies M. Geriatric screening in first opinion practice – results from 45 dogs. J Small Anim Pract. 2012; 53(9):507-513.

Dhaliwal R, Boynton E, Carrera-Justiz S, *et al.* 2023 AAHA senior care guidelines for dogs and cats. J Am Anim Hosp Assoc. 2023; 59(1):1-21.

Dibben G, Faulkner J, Oldridge N, *et al.* Exercise-based cardiac rehabilitation for coronary heart disease. Cochrane Database Syst Rev. 2021; 11:1-266.

Dragone L, Heinrichs K, Levine D, Tucker T, Millis D. Superficial Thermal Modalities. In: Millis DL, Levine D, eds. Canine Rehabilitation and Physical Therapy. 2nd ed. Philadelphia: WB Saunders; 2014. p.312-327.

Drum MG. Physical rehabilitation of the canine neurologic patient. Vet Clin North Am Small Anim Pract. 2010; 40(1):181-193.

Dycus DL, Levine D, Marcellin-Little DJ. Physical rehabilitation for the management of canine hip dysplasia. Vet Clin North Am Small Anim Pract. 2017; 47(4):823-850.

Edge-Hughes L. Canine treatment and rehabilitation for orthopaedic conditions. In: McGowan C, Goff L, eds. Animal Physiotherapy Assessment, Treatment and Rehabilitation of Animals. 2nd ed. Wiley Blackwell; 2016. p.272-302.

Fernandes MMP, Cruz ACL, Nunes BPM, *et al*. Physiological effects of land and water treadmill exercise in dogs. Cienc Rural. 2022; 52(9).

Frank LR, Roynard PFP. Veterinary neurologic rehabilitation: The rationale for a comprehensive approach. Top Companion Anim Med. 2018; 33(2):49-57.

Fransen M. When is physiotherapy appropriate? Best Pract Res Clin Rheumatol. 2004; 18(4):477-489.

Fry TR, Clark DM. Canine hip dysplasia: clinical signs and physical diagnosis. Vet Clin North Am Small Anim Pract. 1992; 22(3):551-558.

Frye C, Lenfest M, Carr BJ, Miller A. Canine geriatric rehabilitation: considerations and strategies for assessment, functional scoring, and follow up. Front Vet Sci. 2022; 9:842458.

Gaar-Humphreys KR, Spanjersberg TCF, Santarelli G, *et al.* Genetic basis of dilated cardiomyopathy in dogs and its potential as a bidirectional model. Animals. 2022; 12(13):1679-1696.

Ganguie MA, Moghadam BA, Ghotbi N, Shadmehr A, Masoumi M. Immediate effects of transcutaneous electrical nerve stimulation on six-minute walking test, Borg scale questionnaire and hemodynamic responses in patients with chronic heart failure. J Phys Ther Sci. 2017; 29(12):2133-2137.

German AJ. Management of obesity in dogs and cats – maximizing success. Purina Companion Animal Nutrition Summit: Focus on Obesity and Obesity-Related Diseases. 2011; 88-91.

German AJ. Weight management in obese pets: the tailoring concept and how it can improve results. Acta Vet Scand. 2016; 58(1):57-65.

Goldberg ME. Feline Physical Rehabilitation. Today's Vet Tech. 2016; 1(6):6-17.

Goldberg ME. Osteoarthritis in canines part 2: physical rehabilitation. The Veterinary Nurse. 2022; 13(1):10-15.

Güzel Ö, Altunatmaz K. Canine Hip Dysplasia and It's Treatment Using the Triple Pelvic Osteotomy (TPO) Method. İst Üni Vet Fak Derg. 2006; 32(1):13-21.

Harasen G. Patellar Luxation. Can Vet J. 2006; 47(8):817-818.

Henea ME, Mariana G, Gradinaru AC, Solcan G. Incidence, genetic predisposition, and recovery by physiotherapy of orthopedic disorders in dogs and cats. HVM Bioflux. 2020; 12(4):172-179.

Hermansen J, Kuricova M, Liptak T. Intervertebral disc disease in dogs – the relationship between recovery and timing of surgery. Folia Vet. 2022; 66(3):54-59.

Hoshiai M, Ochiai K, Tamura Y, *et al.* Effects of whole-body neuromuscular electrical stimulation device on hemodynamics, arrhythmia, and sublingual microcirculation. Heart Vessels. 2021; 36(6):844-852.

Hou Y, Wang Y, Lu X, *et al.* Monitoring hip and elbow dysplasia achieved modest genetic improvement of 74 dog breeds over 40 years in USA. PLoS ONE. 2013; 8(10):e76390.

Hsieh CM, Lin WC, Peng HY, *et al.* Shoulder transcutaneous electric nerve stimulation decreases heart rate via potentiating vagal tone. Sci Rep. 2021; 11(1):19168.

Inoue M, Hasegawa A, Hosoi Y, Sugiura K. Association between breed, gender and age in relation to cardiovascular disorders in insured dogs in Japan. J Vet Med Sci. 2016; 78(2):347-350.

Jeong IS, Piao Z, Rahman MM, Kim S, Kim NS. Canine thoracolumbar intervertebral disk herniation and rehabilitation therapy after surgical decompression: A retrospective study. J Adv Vet Anim Res. 2019; 6(3):394-402.

Johnson KA, Lee AH, Swanson KS. Nutrition and nutraceuticals in the changing management of osteoarthritis for dogs and cats. J Am Vet Med Assoc. 2020; 256(12):1335-1341.

Karvandi M. Review of laser therapy in cardiovascular diseases. J Lasers Med Sci. 2021; 12:e52.

Kobatake Y, Nakata K, Sakai H, *et al.* The long-term clinical course of canine degenerative myelopathy and therapeutic potential of curcumin. Vet Sci. 2021; 8(9):192-201.

Kosic LS, Trailovic DR, Krstic N. Age-dependent electrocardiographic and echocardiographic changes in German Shepherd dogs. Iran J Vet Med. 2017; 18(1):43-48.

Kurt BK, Gürsel A, Şen İ. Osteoarthritis in Small Animals. İd Health Sci. 2023; 1(1):1-7.

Kwiecien S, McHugh MP. The cold truth; the role of cryotherapy in the treatment of injury and recovery from exercise. Eur J Appl Physiol. 2021; 121(8):2125-2142.

Lago AF, Oliveira AS, Souza HCD, Silva JS, Basile-Filho A, Gastaldi AC. The effects of physical therapy with neuromuscular electrical stimulation in patients with septic shock. Medicine. 2018; 97(6):e9736.

Lara JS, Alves EGL, Oliveira HP, Varon JAC, Rezende CMF. Patellar luxation and articular lesions in dogs: A retrospective: Study research article. Arq Bras Med Vet Zootec. 2018; 70(1):93-100.

Lee H, Collins D, Creevy KE, Promislow DEL. Age and physical activity levels in companion dogs: Results from the dog aging project. J Gerontol A Biol Sci Med Sci. 2022; 77(10):1986-1993.

Loftus JP, Wakshlag JJ. Canine and feline obesity: a review of pathophysiology, epidemiology and clinical management. Vet Sci. 2015; 6:49-60.

Lomas AL, Grauer GF. The renal effects of NSAIDs in dogs. J Am Anim Hosp Assoc. 2015; 51(3):197-203.

Lund EM, Armstrong PJ, Kirk CA, Klausner JS. Prevalence and risk factors for obesity in adult dogs from private US veterinary practices. Intern J Appl Res Vet Med. 2006; 4(2):177-186.

Maddox M. Luxating patellas: Pathology and treatment options. Today's Vet Tech. 2017; 2(4):38-47.

Mahaseth PK, Raghul S. Veterinary physiotherapy – a literature review. Int J Med Health Res. 2021; 6(1):288-294.

Miao C, Li X, Zhang Y. Effect of acupuncture on BDNF signaling pathways in several nervous system diseases. Front Neurol. 2023; 14:1248348.

Mikkola L, Holopainen S, Morikawa TP, *et al.* Genetic dissection of canine hip dyspalsia phenotypes and osteoarthritis reveals three novel loci. BMC Genomics. 2019; 20(1):1027-1039.

Mille MA, McClement J, Lauer S. Physiotherapeutic strategies and their current evidence for canine osteoarthritis. Vet Sci. 2022; 10(1):2-24.

Millis DL, Ciuperca IA. Evidence for canine rehabilitation and physical therapy. Vet Clin North Am Small Anim Pract. 2015; 45(1):1-27.

Millis DL, Drum M, Levine D. Therapeutic exercises: Early limb use exercises. In: Millis DL, Levine D, eds. Canine Rehabilitation and Physical Therapy. 2nd ed. Philadelphia: WB Saunders; 2014. p.495-505.

Millis DL, Levine D. The role of exercise and physical modalities in the treatment of osteoarthritis. Vet Clin North Am Small Anim Pract 1997; 27(4):913-930.

Monk ML, Preston CA, McGowan CM. Effects of early intensive postoperative physiotherapy on limb function after tibial plateau leveling osteotomy in dogs with deficiency of the cranial cruciate ligament. Am J Vet Res. 2006; 67(3):529-536.

Moore SA, Tipold A, Olby NJ, Stein V, Granger N. Current approaches to the management of acute thoracolumbar disc extrusion in dogs. Front Vet Sci. 2020; 7:1-15.

Moraitou KK, Pardali D, Soubasis N. Obesity in dogs and cats: Discovering the enemy. Hellenic J Comp Anim Med. 2014; 3(1):40-49.

Morishita K, Karasuno H, Yokoi Y, *et al.* Effects of therapeutic ultrasound on range of motion and stretch pain. J Phys Ther Sci. 2014; 26(5):711-715.

Mosier JE. Effect of aging on body systems of the dog. Vet Clin North Am Small Anim Pract. 1989; 19(1):1-12.

Nakano J, Yamabayashi C, Scott A, Reid WD. The effect of heat applied with stretch to increase range of motion: a systemic review. Phys Ther Sport. 2012; 13(3):180-188.

Nicholson H. Small animal treatment and rehabilitation for cardiorespiratory conditions. In: McGowan C, Goff L, eds. Animal Physiotherapy Assessment, Treatment and Rehabilitation of Animals. 2nd ed. Wiley Blackwell; 2016. p.247-259.

Purina Institute. Obesity in pets: Nutritional and behavioral strategies for preventing and managing excess weight. Available at: https://www.purinainstitute.com/sites/default/files/2021-10/Obesity-in-Dogs-and-Cats.pdf Accessed Aug 3, 2024.

Olby N, Costa RC, Levine JM, Stein VM. Prognostic factors in canine acute ıntervertebral disc disease. Front Vet Sci. 2020; 7:596059.

Olby NJ, Moore SA, Brisson B, *et al.* ACVIM consensus statement on diagnosis and management of acute canine thoracolumbar intervertebral disc extrusion. J Vet Intern Med. 2022; 36(5):1570-1596.

Öhlund M, Palmgren M, Holst BS. Overweight in adult cats: a cross-sectional study. Acta Vet Scand. 2018; 60(1):5-14.

Packer RMA, Seath IJ, O'Neill DG, Decker S, Volk HA. DachsLife 2015: an investigation of lifestyle associations with the risk of intervertebral disc disease in Dachshunds. Canine Genet Epidemiol. 2016; 3(8):1-15.

Park HK, Jung MK, Park E, *et al.* The effect of warm-ups with stretching on the isokinetic moments of collegiate men. J Exerc Rehabil. 2018; 14(1):78-82.

Parker HG, Kilror-Glynn P. Myxomatous mitral valve disease in dogs: Does size matter? J Vet Cardiol. 2012; 14(1):19-29.

Pati S, Panda SK, Acharya AP, Senapati S, Behera M, Behera SS. Evaluation of geriatric changes in dogs. Vet World. 2015; 8(3):273-278.

Pecina M, Ivkovic J, Cicvara-Pecina T, Ivkovic A. Orthogeriatrics – considerations in caring of older orthopaedic patient. Period Biol. 2013; 115(4):527-533.

Pedersen A, Hyytiainen HK, Rhodin M, Forterre F, Penell J, Bergh A. Effect of transcutaneous electrical nerve stimulation on gait parameters in dogs with osteoarthritis. Animals. 2024; 14(11):1626-1645.

Pegram C, Raffan E, White E, *et al*. Frequency, breed predisposition and demographic risk factors for overweight status in dogs in the UK. J Small Anim Pract. 2021; 62(7):521-530.

Perry K. Feline hip dysplasia: A challenge to recognise and treat. J Feline Med Surg. 2016; 18(3):203-218.

Perry KL, Dejardin LM. Canine medial patellar luxation. J Small Anim Pract. 2021; 62(5):315-335.

Prasad M, Ahmad WAW, Sukmawan R, *et al*. Extracorporeal shockwave myocardial therapy is efficacious in improving symptoms in patients with refractory angina pectoris – a multicenter study. Coron Artery Dis. 2015; 26(3), 194–200.

Prydie D, Hewitt I. Modalities. Practical Physiotherapy for Small Animal Practice. 1st ed. Wiley Blackwell; 2015. p.69-91.

Quimby J, Gowland S, Carney HC, DePorter T, Plummer P, Westropp J. 2021 AAHA/AAFP feline life stage guidelines. J Feline Med Surg. 2021 Mar;23(3):211-233.

Rennie S. Electrophysical agents- contraindications and precautions: an evidence-based approach to clinical decision making ın physical therapy. Physiother Can. 2010; 62(5):1-80.

Riley LM, Satchell L, Stilwell LM, Lenton NS. Effect of massage therapy on pain and quality of life in dogs: A cross sectional study. Vet Rec. 2021; 189(11):e586.

Roush JK. Canine patellar luxation. Vet Clin North Am Small Anim Pract. 1993; 23(4):855-868.

Santarossa A, Parr JM, Verbrugghe A. The importance of assessing body composition of dogs and cats and methods available for use in clinical practice. J Am Vet Med Assoc. 2017; 251(5):521-529.

Saunders DG. Therapeutic exercise. Clin Tech Small Anim Pract. 2007; 22(4):155-159.

Sayed MA, El-Sherif RM, Ismail A, Warda AEA, Mohamed AR, El-Sherif AA. Effect of low-level laser physiotherapy on left ventricular function among patients with chronic systolic heart failure. Egypt Heart J. 2023; 75(1):12-19.

Schachner ER, Lopez MJ. Diagnosis, prevention, and management of canine hip dysplasia: a review. Vet Med. 2015; 6:181-192.

Sharp B. Feline physiotherapy and rehabilitation: 2. clinical application. J Feline Med Surg. 2012; 14(9):633-645.

Sims C, Waldron R, Marcellin-Little DJ. Rehabilitation and physical therapy for the neurologic veterinary patient. Vet Clin North Am Small Anim Pract. 2015; 45(1):123-143.

Slingerland LI, Hazewinkel HA, Meij BP, Picavet P, Voorhout G. Cross-sectional study of the prevalance and clinical features of osteoarthritis in 100 cats. Vet J. 2011; 187(3):304-309.

Song JA, Oh JW. Effects of aquatic exercises for patients with osteoarthritis: systematic review with meta-analysis. Healthcare. 2022; 10(3):560-579.

Spinella G, Arcamone G, Valentini S. Cranial cruciate ligament rupture in dogs: review on biomechanics, etiopathogenetic factors and rehabilitation. Vet Sci. 2021; 8(9):186-208.

Spinella G, Bettella P, Riccio B, Okonji S. Overview of the current literature on the most common neurological diseases in dogs with a particular focus on rehabilitation. Vet Sci. 2022; 9(8):429-447.

Taylor RS, Dalal HM, McDonagh STJ. The role of cardiac rehabilitation in improving cardiovascular outcomes. Nat Rev Cardiol. 2022; 19(3):180-194.

Thomas WB, Olby N, Sharon L. Neurologic Conditions and Physical Rehabilitation of the Neurologic Patient. In: Millis DL, Levine D, eds. Canine Rehabilitation and Physical Therapy. 2nd ed. Philadelphia: WB Saunders; 2014. p.609-627.

Tomlinson R. Use of canine hydrotherapy as part of a rehabilitation programme. Today's Vet Tech. 2012; 3(10):624-629.

Turgut K, Süleymanoğlu H, Ertan M, İnce ME. Geriatric cardiology in dogs – Part 1: Classification and treatment of heart failure in geriatrics. Kafkas Univ Vet Fak Derg. 2017; 24(1):159-167.

Veenman P. Animal physiotherapy. J Bodyw Mov Ther. 2006; 10(4):317-327.

Vogt AH, Rodan I, Brown M, *et al*. AAFP-AAHA: Feline life stage guidelines. J Feline Med Surg. 2010; 12(1):43-54.

Ward JM, Youssef SA, Treuting PM. Why animals die: An introduction to the pathology of aging. Vet Pathol. 2016; 53(2):229-232.

Weigel JP, Millis D. Biomechanics of physical rehabilitation and kinematics of exercise. In: Millis DL, Levine D, eds. Canine Rehabilitation and Physical Therapy. 2nd ed. Philadelphia: WB Saunders; 2014. p.401-430.

Wiputhanuphongs A, Soontornvipart K, Janwantanakul P. Effect of physical therapy program after surgical correction of medial patellar luxation in small breed dogs. Wetchasan Sattawaphaet. 2015; 45(4):573-580.

Yamazaki A, Edamura K, Tanegashima K, *et al*. Utility of a novel activity monitor assessing physical activities and sleep quality in cats. PLoS ONE. 2020; 15(7):e0236795.

Zoran DL. Feline obesity: clinical recognition and management. Compendium. 2009; 31(6):284-293.

ACKNOWLEDGMENTS

This review is produced from the graduation thesis of Nevin Coskan entitled as "Physiotherapy and Rehabilitation in Geriatric Animals".

Author contributions: PC and NC reviewed the literature about the topic, and both are participated in drafting and revising the manuscript. PC: Pinar Can, NC: Nevin Coskan

Financial Disclosure: The authors declared that this study has received no financial support.

Conflict of Interests: The authors declared that there is no conflict of interests.

Additional information: All authors have read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to PC.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

TURKISH JOURNAL OF VETERINARY RESEARCH
E-ISSN:2602-3695

INSTRUCTIONS FOR AUTHORS

General Considerations

TJVR strictly obeys the recommendations of the ICMJE, WAME and flowcharts of COPE.

During manuscript application process "Copy Right Transfer Form" signed by all the researchers in the study should be uploaded to the system.

In studies where "Ethical Approval Document" is needed, the relevant document should be scanned in the browser and transferred to the system at the application stage.

Our journal is incorporate the ICMJE recommendations into our review and publication process.

New Manuscript Submissions

Submit manuscripts through the journal's online system. To begin, go to *Manuscript Submission Page* at the following link:

https://dergipark.org.tr/en/journal/1986/submission/step/manuscript/new

Manuscript Categories

Original Research: An Original Research article is a research paper that provides new information derived from original data and usually includes extensive statistical analysis.

Review Article: A Review Article is an in-depth analysis of the current status of a particular topic. It is not an original article with new data but represents a well-balanced summary of a timely subject with reference to the literature.

Letter to the Editor: A Letter to the Editor offers brief comments on published material and should be objective, constructive, and educational.

Case Report: A Case Report is a brief description of a single unique or unusual case. The manuscript should include a title, authors' full names, degrees, and affiliations, a brief text description/discussion without headings, up to 10 references if applicable, and figure legends; note that each figure part (ie, Figure 1A or 1B) is counted as a separate image. No more than 4 images total may be included. It should not contain tables or data analysis. Case Report are generally not peer reviewed and are published at the discretion of the editor based on space available; publication is not guaranteed.

Short Communication: A Short Communication article is a rather short paper reporting preliminary observations, new interpretations of old data, simple new techniques or devices, or points of historical interest.

Organization of The Manuscript

Manuscripts should be organized in the following order, as applicable: title page, structured abstract or summary (when applicable; letters to the editor, review articles and case reports do not have a structured abstract), full text, acknowledgments, footnotes, references, tables and figure legends. All text elements and tables should be combined in a single document, double- spaced, with line numbering.

Title Page

This first page should contain the complete title of the article, manuscript category, names and credentials of all authors, institutional affiliations of all authors, orcid (all authors of the manuscript must have a orcid number) of all authors and a short running title (≤60 characters). The corresponding author should be indicated, including a complete e-mail address and telephone number.

Structured Abstract or Summary

An abstract is required for Original Research articles. The abstract should be divided into *Objectives, Materials and Methods, Results, and Conclusions* sections. It should be between 250-350 words. Three to 6 key words should be provided at the end. The abstract should not contain figures, tables, or reference citations, trade names, or manufacturer names. Statements of statistical significance should include 'p' values or 95% confidence intervals. The abstract should not include data that are not given in the full text.

In place of an abstract, a summary is required for Letters to the editor, Review Articles, and Case Reports. Content should adhere to the guidelines for abstracts but should not be divided into sections. It should not exceed 200 words and should give a brief overview of the article. Three to six keywords should be provided at the end. Your keywords must come from the list of approved keywords (MeSH library) maintained by the Medical Subject Headings section of the U.S. National Library of Science or Türkiye Bilim Terimleri web page.

Full Text

For Original Research articles, the full text of the manuscript should be divided into the following sections: *Introduction, Materials and Methods, Results, Discussion, Conclusion*. For other articles, the text may be divided into sections if desired. Microsoft Word is preferred. All text should be double spaced, and all text elements, including tables, should be combined in a single document. Manuscripts should include continuous line numbering.

Acknowledgments

On a separate page preceding the references, contributions made by colleagues who are not authors of the paper should be acknowledged. This page should also include acknowledgments of published material reproduced with permission, previous presentations of the work at meetings, related grant support received, and disclosures of relevant commercial interests.

Footnotes

Footnotes are to be used when referencing each of the following types of information:

- Abstracts
- Conference presentations
- Online databases
- Personal communications
- Products, drugs, equipment, and other materials
- Statistical and computer software
- Theses and dissertations

Specific products, equipment, or drugs should be included in the footnotes only if they were essential to the outcome of the report or study. Products, equipment, and drugs that are commonly used materials in veterinary medicine need not be footnoted.

Footnotes should be cited in the text as superscript letters and listed alphabetically after the Acknowledgments section and before the references. If more than 26 footnotes are required, continue the sequence with double letters (eg, aa, bb, and cc). For products and equipment, provide complete information in the footnote, including manufacturer's name and location (ie, city, state, and country).

References

Each reference must be cited with first author's surname and publication year (Ceylan, 2004; Ceylan and Ekin, 2006; Ceylan et al., 2008) in the text.

In references list, when more than 6 authors are included in a reference, ***et al*** replaces the names of all authors after the first 3. Inclusive page ranges should be given where applicable. Accepted work in press includes the journal title or the publisher if it is a book. Material not accepted for publication cannot be included as a reference and should be cited parenthetically in the text as unpublished data, including all participants' initials and surnames and the year. Abbreviations of journal titles should conform to those used in the *National Library of Medicine* (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals). The style and punctuation of references should follow the formats illustrated by the following examples:

Journal Article with Up to 6 Authors

Stephane A, Luo W, Zhou X. Management of congenital cholesteatoma with otoendoscopic surgery: Case Report. Turkiye Klinikleri J Med Sci. 2010; 30(2):803-807.

Journal Article with More Than 6 Authors

Stephane A, Luo W, Zhou X, *et al*. Management of congenital cholesteatoma with otoendoscopic surgery: Case Report. Turkiye Klinikleri J Med Sci. 2010; 30(2):803-807.

Journal Article Published Online Ahead of Print

Cummisford K, Sundar S, Hagberg R, Mahmood F. Realtime three-dimensional transesophageal echocardiography and a congenital bilobar left atrial appendage [published online ahead of print June 24, 2009]. J Cardiothorac Vasc Anesth. doi:10.1053/j.jvca.2009.04.005.

Abstract of a Paper Presented at a Meeting

Shipp TD, Bromley B, Benacerraf BR. Comparison of the fundal endometrial diameters using 3-dimensional rendering for those with embedded and non-embedded intrauterine devices [abstract]. J Ultrasound Med. 2009; 28(suppl):47.

Whole Book

Solcia E, Capella C, Kloppel G. Tumors of the exocrine pancreas. Tumors of the Pancreas. 2nd ed. Washington: Armed Forces Institute of Pathology; 1997. p.145-210.

Edited Book

Underwood LE, Van Wyk JJ. Normal and aberrant growth. In: Wilson JD, Foster DW, eds. Wiliams' Textbook of Endocrinology. 1st ed. Philadelphia: WB Saunders; 1992. p.1079-1138.

Website

Animal and Plant Health Inspection Service. Bovine spongiform encephalopathy (BSE). Available at:

www.aphis.usda.gov/lpa/issues/bse/bse.html. Accessed Feb 18, 2003.

Tables

Tables must be numbered consecutively with Arabic numerals in the order in which they appear in the manuscript. Each table should appear on a separate page and should contain a title that includes the table number. No vertical lines or borders may appear in a table. Tables must contain all necessary information so that they may stand alone, independent of the text. Data given in the text should not be duplicated in tables. All tables must be cited in numerical order in the text. Tables should be included

in the text document and should not be submitted as graphics.

Figure

For initial submissions, figure legends should be provided at the end of the text document. Figures must be numbered consecutively with Arabic numerals in the order in which the figures appear in the article. All figures should be uploaded in jpeg or tiff format at least 300 dpi resolution with the name of the figure (eg, Figure 1), separately. For figures that include multiple panels, each panel should be sequentially labeled with a capital letter in the same corner of each panel. If a figure contains 2 or more rows of panels, the letter labels should be applied sequentially from left to right in the first row, then from left to right in the second row, and so on. All letters and rows used on the figures should be same size and character.

Similarity Index

Similarity index report, created by ithenticate or Turnitin software, must be uploaded during submission. Our journal expects the similarity index to be less than %25 for submitted manuscripts.

Abbreviations

Abbreviations of common clinical and technical terms listed in the *American Medical Association Manual of Style, 10th Edition*, may be used if they appear 3 or more times in the full text and abstract/summary, respectively. Each abbreviation should be defined at first use in the full text and abstract/summary, respectively. Use of other abbreviations is discouraged unless they are essential to the clarity of the manuscript.

Supplemental Materials

Additional materials that are not in themselves essential to the understanding of the article but provide an important expansion of the article contents may be submitted for publication as supplemental materials. Examples include extended descriptions of experimental methods, extended bibliographies, additional supporting data or results (eg, tables and figures), reporting checklists, copies of survey instruments or questionnaires, handouts, forms, and multimedia representations (eg, video clips) of relevant content.

Supplemental materials must be useful to readers and relevant to the article; redundant and extraneous content will not be accepted. Whether supplemental materials will be accepted for publication is solely at the discretion of the editors. Supplemental materials accepted for publication will not appear in the printed version of the journal but will be posted on the journal's website. Ideally, supplemental materials will be sent with the manuscript to external reviewers for peer review. Whether supplemental materials have or have not undergone peer review will be indicated on the landing page where the supplemental materials are posted. Supplemental materials should be prepared in compliance with the general guidelines for manuscript style. Although supplemental materials may undergo minor copy editing or formatting, they generally will not undergo the same substantive editing provided for manuscripts. Therefore, the authors are responsible for ensuring clarity and accuracy of the content.

Permission to Reproduce Published Material

Written permission from the author and publisher of any previously published figure, table, or lengthy passage of text included in the manuscript must be submitted with the paper. If any form of preliminary publication, other than an abstract of no more than 400 words, has occurred or is contemplated, a reprint or manuscript of the actual or proposed preliminary publication should accompany the manuscript.

Authority/Responsibility

The intellectual content of the paper is the responsibility of the author; the editor and the TJVR accept no responsibility for the opinions and statements of authors. While every effort is made by the editor and editorial staff to avoid any inaccurate or misleading information, they accept no liability for the consequences of wrong information. The authors agree to keep the editor and publisher fully

indemnified against any liability or claim that may arise from the publication of incorrect or misleading data.

Ethics in Experimentation

If the work involves the use of human and animal subjects, the author should ensure that the work described has been carried out in accordance with the internationally accepted rules and codes of conduct. The authors should clearly indicate in the manuscript that such guidelines have been followed and the study has been approved by the appropriate ethical committee along with name of committee and reference code.

Editing

Accepted manuscripts will be copyedited to eliminate grammatical and typographical errors and to ensure conciseness and clarity. The corresponding author will receive proofs of the copyedited article before publication.

Permission Requests

Manuscripts are considered for publication with the understanding that they are submitted solely to the Turkish Journal of Veterinary Research and are not under consideration elsewhere.

CONTACTING THE JOURNAL

The Turkish Journal of Veterinary Research Editorial Office may be contacted through the following:

Prof. Dr. Ebubekir CEYLAN

Ankara Üniversitesi Veteriner Fakültesi

İç Hastalıkları AD Dışkapı, 06110 Ankara, Türkiye

E-mail: *ebubekirceylan@gmail.com*, *infotjvr@gmail.com*

Manuscripts are considered for publication with the understanding that they are submitted solely to the Turkish Journal of Veterinary Research and are not under consideration elsewhere.

TURKISH JOURNAL OF VETERINARY RESEARCH
E-ISSN:2602-3695

Article Copyright Transfer Agreement

We, the undersigned researchers, certify that; the article we have sent; is original, wasn't sent to or disapproved of potential publication by any other journal, has not initially published, and we bear the responsibility concerning the Scientific content and Ethical values related to the article, and transfer any kind and form of copyright related to the Article to Turkish Journal Veterinary Research (TJVR) since it is published in the journal, and accept that we will not make any changes wholly or partly in the article and chose ……..……..…… named author as the authorized researcher.

Title of the article: ……..……..……

……..……..……..……

……..……..……..……

……..……..……..……

	Authors Name	Date	Signature
1.			
2.			
3.			
4.			
5.			
6.			

Authorized Researcher

Title, Name-Surname :

Full Address :

e- mail :

Tel, Fax :

Date and Signature :