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İstanbul ve Çevresinde Tüketilen Sütlerde Pestisit Kontaminasyonlarının Belirlenmesi*

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Öz: Süt ve süt ürünleri; sosyoekonomik ve uluslararası ticaret açısından kritik bir öneme sahiptir. Süt tüketimi, pestisitlerin yağ dokuda yoğunlaşma ve süte geçme özellikleri nedeniyle düzenli olarak maruziyet açısından ciddi halk sağlığı endişesi yaratmaktadır. Son zamanlarda artan kimyasal kontaminasyon endişeleri, tüketicilerin organik süte yönelik taleplerinde artışa neden olmuştur. Bu çalışma kapsamında, piyasada çeşitli marketlerde satışa sunulan, farklı ticari markalara ait 10 organik (pastörize), 20 klasik (UHT ve pastörize) yöntemle üretilmiş olmak üzere toplam 30 ısıl işlem görmüş süt örneği 177 pestisit açısından gaz kromatografisi/kütle spektroskopisi (GC-MS/MS) kullanılarak analiz edilmiştir. Analiz edilen süt örneklerinin hiçbirinde Türk Gıda Kodeksi (TGK)'nın ilgili yönetmeliğindeki maksimum kalıntı limitlerinin (0.01 mg/kg) üzerinde bir pestisit kontaminasyonuna rastlanmamıştır. Bununla birlikte çalışma kapsamına alınan lindane, heptachlor, fenamiphos ve aldrin pestisitlerine ait TGK limit değerlerinin, Codex Alimentarius (2019) limit değerlerine göre oldukça yüksek olduğu, benzer şekilde lindane, heptachlor, fenamiphos, aldrin, fipronil, endrin, chlordane, hexachlorobenzene pestistlerine ait değerlerin ise Avrupa Birliği Komisyonu (EC, 2010) tarafından belirlenen limitlerin çok üzerinde kaldığı gözlenmiştir. Elde edilen sonuçlar, TGK'nin ilgili yönetmeliğindeki bazı pestisitlere ait maksimum kalıntı limit değerlerinin güncellenmesi gerektiğini, benzer şekilde ulusal referans laboratuvarların da alt yapı ve metotlarını bu limit değerlere göre düzenlemesi gerektiğini ortaya koymuştur. Pestisit maruziyetleri açısından halk sağlığının korunabilmesi için iyi tarım uygulamalarının ve pestisit takip sistemlerinin geliştirilmesi gerekmektedir. Farklı üretim metotlarının kontaminasyon düzeylerine olan etkilerinin daha iyi anlaşılabilmesi için konuya ilişkin daha kapsamlı çalışmaların planlanmasına ihtiyaç duyulmaktadır.
Anahtar kelimeler: Halk sağlığı, kontaminasyon, pestisit, süt

Pesticide Contaminations in Organic Milk Samples in and Around İstanbul

Abstract: Milk and milk products have critical importance in terms of socio-economic and international trade. Considering the ability of pesticides to concentrate in fat tissue and pass into milk, milk consumption cause serious public health concerns as a source of regular pesticide exposure. Recently, increasing chemical contamination concerns have led to an increase in consumer demand for organic milk. Within the scope of this study, a total of 30 heat-treated milk samples of different commercial brands, 10 organic (pasteurized) and 20 conventionally (UHT and pasteurized) produced milk samples sold in markets, were analyzed using gas chromatography/mass spectrometry (GC-MS/MS) for 177 pesticides. No pesticide contamination was found in any of the analyzed milk samples above the maximum residue limits (0.01 mg/kg) of related Turkish Food Codex (TGK) regulations. However, it was determined that the TGK limit values of some pesticides (lindane, heptachlor, fenamiphos, aldrin) included in the study were quite high compared to the limit values of Codex Alimentarius (2019). Similarly, the values of some pesticides (lindane heptachlor, fenamiphos, aldrin, fipronil, endrin, chlordane, hexachlorobenzene) in TGK were observed to be well above the limits determined by the European Commission (EC, 2010). The results revealed the need of the maximum residue limit (MRL) values of some pesticides in the related TGK regulations to be updated, and the infrastructure and methods of national reference laboratories to be revised accordingly. Good agricultural practices and pesticide tracking systems need to be implemented to protect public health in terms of pesticide exposures. More comprehensive studies are needed in order to better understand the effects of different production methods on pesticide contamination levels in milk and milk products.
Keywords: Contamination, milk, pesticide, public health

Giriş

Tarım alanlarında 1940'ların başından beri yoğun bir şekilde kullanılan pestisitler organik klorlu, organofosfat, karbomat, senteteik pretiroit, trazin, dioksin ve klorofenoksi pestisitler olmak üzere çeşitli gruplara ayrılmakta bunlar arasında organik klorlu pestisitler en agresif grup olarak bilinmektedir. Organik klorlu pestisitlerin insan ve çevre sağlığı için ciddi sorunlara yol açtığının belirlenmesi sonucu 1960'lı yılların sonunda pestisit kullanımına uluslararası bir yasaklama

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çağrısı gündeme gelmiştir. Bu yasaklama çağrısına gelişmiş ülkeler olumlu yanıt verirken, gelişmekte olan ülkelerde; kolay bulunması, ucuz olması ve çok etkili olması dolayısıyla söz konusu kimyasal ajanların kullanımına devam edilmiştir (Keswani ve ark., 2022).

Pestisitler tüm dünyada; insekt, rodent ve fungusları kontrol altına alarak gıda üretimini artırmak amacıyla çok yaygın bir şekilde kullanılmaktadır. Codex Alimentarius'un (CA) pestisit kalıntı komitesi (CCPR), süt ve süt ürünlerindeki pestisit kalıntı limitlerini 0.4 ila 2000µg/kg olarak bildirmektedir (CA, 2019). Bununla birlikte süt ve süt ürünlerindeki kalıntı düzeyleri proses esnasında azalıp artabilmekte ve maruziyet düzeyleri, hangi süt ürününün tüketildiğine bağlı olarak önemli ölçüde değişiklik gösterebilmektedir (Duan ve ark., 2018).

Pestisitler, insanlarda endokrin ve mitokondriyal fonksiyon bozukluklarına neden olmalarının yanı sıra insan ve hayvanlarda üreme sistemini de etkileyen karsinojenik ve mutajenik bileşiklerdir. Bazı çalışmalar pestisit maruziyetinin obezite ve diyabete neden olduğunu da belirtmektedir (Keswani ve ark., 2022). Pestisitlerin kimyasal yapısı ve lipofilik karakterleri, söz konusu ajanların yağ dokuda birikmesine dolayısıyla biyolojik sistemlerdeki dekompozisyon mekanizmalarına yüksek direnç göstermelerine neden olmaktadır. Bu kimyasallar; çok yavaş bozulmaları, uzun ömürlü ve kalıcı olmaları sebebiyle insan eliyle üretilen en dayanıklı kimyasallar arasında sayılmaktadır (Hasan ve ark., 2022).

Çocuklarda metabolizmanın ve immun sistemin tam olarak gelişmemiş olması ve çocukların süt ve süt ürünlerini en çok tüketen yaş grubu olmaları dolayısıyla pestisit kontaminantlarına yetişkinlerden daha duyarlı oldukları bildirilmektedir (Tolentino ve ark., 2014; Santos ve ark., 2015; Shakerian ve ark., 2020).

Pestisitlerin düşük doğum ağırlıklı çocuk doğumuna motor ve nörolojik gelişim geriliklerine neden olduğu ve kanser riskini artırdığı bildirilmektedir. Pestisitlerin genel olarak akut toksisite durumlarında neden oldukları semptomlar; halsizlik, kusma, nöbet ve diğer sinirsel semptomlar, karaciğer ve kromozomal hasar olarak sıralanmaktadır. Bazı pestisitler (Organofosforlular), merkezi sinir sisteminde asetilkolinesteraz enzim aktivitesini baskılayarak sinir iletilerine engel olmakla birlikte organofosfat, karbamat ve piretiroit gibi multiple pestisit maruziyetleri depresyon, anksiyete, obsesif kompulsif bozukluklar gibi daha derin ve endişe verici rahatsızlıklara neden olabilmektedir (Yuan ve ark., 2022). Bununla birlikte uzun süreli düşük düzeydeki multiple pestisit maruziyetinin ne gibi sağlık sorunları yaratacağına ilişkin çalışmalar oldukça sınırlı sayıdadır (Gomes ve ark., 2020).

Kontrolsüz pestisit kullanımı sonucu hayvan yemi, hava, toprak ve su yoğun bir şekilde pestisitlerle kontamine olabilmekte, sütü tüketilen hayvanların kaçınılmaz maruziyeti ile pestisitler hayvanın sütüne geçmektedir (Yuan ve ark., 2022). Bilinçli veya bilinçsizce pestisite maruz kalan hayvanlara ait sütlerin ciddi konsantrasyonlarda aktif pestisit barındırabileceği kaydedilmektedir (Fischer ve ark., 2011).

Gıdalardaki maksimum kalıntı veya tolerans limitleri gıdanın alınma sıklığına, miktarına, kimyasalın toksisitesine ve potansiyel maruz kalma yollarına bağlı olarak belirlenmektedir (US Environmental Protection Agency, 2018). Bu sınırlar yetişkin ve çocuklar arasındaki beslenme farklılıklarını ve ihtiyaçlarını dikkate alsa da pestisit maruziyet yolları ve ilgili riskler hakkındaki bilgiler çok sınırlı kalmaktadır. Günlük hayatta sıklıkla maruz kalınan pestisit kombinasyonlarının uzun süreli etkilerine ilişkin bilgiler oldukça sınırlıdır (Sheldon ve ark., 2012; Nicolopoulou-Stamati ve ark., 2016). Bunun yanı sıra tüketicilerin içme sütü yoluyla pestisitlere maruz kalma riskleri ve bu maruziyetin inorganik ve klasik yöntemlerle üretilen sütlerdeki düzeyine ilişkin veriler yetersiz olduğundan süt tüketimine ilişkin pestisit maruziyet düzeyleri ve halk sağlığı riskleri de tam olarak yorumlanamamaktadır.

Bu çalışmanın amacı, İstanbul ve çevrelerinde organik ve klasik yöntemlerle üretilen sütlerde pestisit kalıntı düzeylerini belirleyerek sütte pestisit düzeylerine ilişkin literatür verilerine katkıda bulunmak ve bölgedeki içme sütlerinin halk sağlığı açısından barındırdığı pestisit risk potansiyelleri hakkında güncel veriler ortaya koymaktır.

Gereç ve Yöntem

Çalışma kapsamında İstanbul'da ticari olarak satışa sunulan farklı firmalara ait 10 organik (pastörize) ve 20 klasik (UHT ve pastörize) süt olmak üzere toplam 30 süt örneği (her biri 1 L) Ekim-Aralık 2021 aralığında toplanmıştır. Marketlerden toplanan süt örnekleri soğuk zincir altında Bursa Gıda ve Yem Kontrol Merkez Araştırma Enstitüsü Müdürlüğü'ne bağlı laboratuvara getirilmiş ve analiz yapılana kadar -18°C'de saklanmıştır.

Gereç ve Yöntem

Ekstraksiyon prosedürü

Örnekler homojen hale getirildikten sonra 50 ml'lik santrifüj tüpüne 15'er ml alınarak üzerine %1 asetik asit içeren 15 ml asetonitril eklenmiş ve çalkalanmıştır. Daha sonra, üzerine 6 g susuz $MgSO_4$ ve 1.5 $C_2H_3NaO_2$ (Quechers kit 1) eklenerek 1 dk boyunca tekrar çalkalanmıştır. Elde edilen karışım 5000 rpm' de 1dk santrifüjlenerek ön ekstraksiyon aşaması tamamlanmıştır. Dispersif katı-faz ekstraksiyon için; 400 mg PSA, 400 mg C_{18} ve 1200 mg susuz $MgSO_4$ içeren 15 mL'lik santrifüj tüpüne (Quechers kit 2), 5000 rpm' de 1 dk santrifüjlenerek üstte toplanan

asetonitril fazından 8 mL eklenmiştir. Yaklaşık 30 saniye elle karıştırıldıktan sonra 5000 rpm'de 1 dk santrifüj işlemine tabi tutulmuş, elde edilen ekstraktan GC-MS/MS analizi için 0.5 ml kullanılmıştır. Gaz ve kromatografi ile analiz edilen pestisit sonuçları GC/MS/MS analizine göre, örnekteki kalıntı miktarı, analitik standardın konsantrasyonuna karşılık gelen pik alanlarına göre çizilen kalibrasyon eğrisine göre hesaplanmıştır.

Bulgular

Çalışmada toplam 30 içme sütü örneği 177 farklı pestisit açısından analiz edilmiş ve örneklerin hiçbirinde Türk Gıda Kodeksi'nde (TGK) belirtilen maksimum pestisit kalıntı limitlerinin (0.01mg/kg) üzerinde bir pestisit kontaminasyonuna rastlanmamıştır.

Bununla birlikte çalışma kapsamına alınan pestisitlerden bazılarına (lindane, heptachlor, fenamiphos, aldrin) ait TGK limit değerlerinin, CA (2019) limit değerlerine göre oldukça yüksek olduğu, benzer şekilde bazı pestisitlere (lindane, heptachlor, fenamiphos, aldrin, fipronil, endrin, chlordane ve hexachlorobenzene) ait değerlerin ise AB (EC, 2010) tarafından belirlenen limitlerin çok üzerinde olduğu belirlenmiştir. Bahsi geçen pestisitlere ilişkin CA (CA, 2019), AB (EC,2010) ve TGK limit değerleri Tablo 1'de özetlenmiştir.

azaltmak için de kullanılabilmektedir (FAO, 2021). Pestisit kullanımı ve hasat arasındaki zorunlu bekleme sürelerine riayet edilmemesi durumunda bu kimyasalların gıdalarda yüksek miktarlarda bulunabileceği, (Gomes ve ark., 2020) aynı durumun sütçü sığırlara uygulanan ilacın vücuttan atılım sürelerine riayet edilmediği durumlarda da gözlendiği belirtilmektedir (Fischer ve ark., 2011).

Pestisitlere maruziyet genellikle oral, parenteral veya deri yolu ile olmakta, etken emildikten ve metabolize olduktan sonra süte geçmektedir. Pestisitlerin çoğu yağda çözünür özellikte olduğundan süt, pestisit kalıntılarının dağılabilmesi için ideal bir matrikstir. Pestisitlere kronik maruziyet sinir sistemi toksisitesi endokrin disregulasyonu ve birçok kanser türü ile ilişkilendirilmektedir.

Gıda güvenliğini sağlamak için CA ve AB gibi bazı uluslararası ajanslar sütte maksimum kalıntı düzeyleri belirleme çalışmaları yaparak sıkı regülasyonlar ortaya koymuşlardır. Türkiye'de 1985 yılından itibaren organik klorlu pestisit kullanımı yasaklanmıştır (Acara, 2006)

Bu çalışmada analiz edilen sütlerden hiçbirinde TGK'nın ilgili limit değerlerinin üzerinde bir pestisit kalıntısına rastlanmamıştır. Benzer şekilde Güvenç (2008) tarafından planlanan bir çalışmada, Samsun

Tablo 1. TGK ile uyuşmayan pestisit maksimum limit değerleri (mg/kg)

Pestisitler	TGK	CA	EU
Lindane	0.01	0.001	0.01
Heptachlor	0.01	0.006	0.004
Chlordane	-	-	0.002
Hexachlorobenzene	0.01	-	0.005
Fenamiphos	0.01	0.005	0.005
Aldrin	0.01	0.006	0.006
Endrin	0.01	0.005	0.001
Fipronil	0.01	0.02	0.005

Codex Alimentarius MRL (2019), EU (2010), TGK (2016).

Tartışma ve Sonuç

Süt ve süt ürünleri yeterli ve dengeli beslenmenin yapı taşlarından biri olarak görülmektedir. Türkiye'de ise kişi başı içme sütü tüketiminin Avrupa ve Amerika' tüketim ortalamalarının oldukça gerisindedir. Türkiye'de üretilen içme sütlerinin %88.6'ünün UHT süt olduğu belirtilmekle birlikte son zamanlarda daha maliyetli olan organik süt taleplerinde görülen artışın, tüketicinin kimyasal kontaminantlara maruz kalma konusundaki endişelerini yansıttığı düşünülmektedir (USK, 2021).

Pestisitler özellikle tarım amaçlı olmak üzere üretimi artırmak ve artan gıda ihtiyacını karşılamak amacıyla çok yoğun bir şekilde kullanılmaktadır (Sharma ve ark., 2019). Pestisitler gıda üretimi, işlenmesi, depolanması, nakil ve dağıtım aşamalarında kayıpları

ve çevrelerinden toplanan 100 çiğ süt örneğinde pestisit kalıntısına rastlanmadığı bildirilmiştir. Öte yandan Türkiye'de Bulut ve ark. (2010) tarafından yapılan bir çalışmada toplam 50 inek sütü örneğinin %80'i; HCH, heptachlor, chlordane, endosulfan, endrin ve methoxychlor ile kontamine bulunmuş, örneklerden % 2'sinin endrin ve %24'ünün endosulfan kontaminasyon düzeylerinin CA (2019) değerlerinin üzerinde olduğu rapor edilmiştir. Çalışma kapsamında incelenen 50 koyun sütü örneğinden %90'nının endrin veya endosulfan ile kontamine bulunduğu belirtilmiştir.

Sana ve ark. (2021) tarafından yapılan bir çalışmada inek ve manda sütlerinden sıklıkla HCH, DDT ve Heptaklor kalıntılarına rastlandığı bildirilmiştir. Uganda'da (Kampire ve ark., 2011) ve Etiyopya'da (Deti ve ark., 2014) sıtma vakalarının çok yoğun bir şekilde görüldüğü bölgelerdeki çiğ sütlerde, Dünya Sağlık

Örgütü ile AB tarafından tanımlanan maksimum kalıntı limit (MRL) düzeylerinin (40 µg/kg) (EC, 2010) yaklaşık sekiz kat fazla (328.5 µg/kg) DDT bulunduğu bildirilmiştir. Bunun da bu bölgelerde sıtma vektörünün kontrolü için sıklıkla ve yoğun bir şekilde insektisit olarak yüksek miktarda DDT kullanımına bağlanmıştır (Haylamicheal ve Dalvie 2009). Gutierrez ve ark. (2013) tarafından Meksika'da çiğ sütlerde yapılan bir çalışmada sırasıyla heptaklorepoksit, endosulfan ve HCH kontaminasyonlarına rastlandığı, hiçbirinin kodekste yer alan limitleri aşmadığı belirtilmiş ve bu düşük konsantrasyonlardaki kalıntıların varlığı, OC pestisitlerin 30 yıldan beri yasaklı olmasına bağlanmıştır. Rusu ve ark. (2016) tarafından Romanya'da çiğ ve pastörize süt örnekleriyle yapılan bir çalışmada tüm örneklerde HCH varlığı belirlenirken DDT varlığına rastlanmadığı bildirilmiştir. Öte yandan Tsakiris ve ark. (2015) tarafından Yunanistan'da yürütülen bir çalışmada pastörize sütlerin %97.4'ünde DDT'ye ait en az bir izomer veya metabolit bulunduğu kaydedilmiş, her iki çalışmada elde edilen verilerin AB limitlerini aşmadığı vurgulanmıştır. Farklı bölgelerden rapor edilen çalışma sonuçları, çalışma bölgelerinde kullanılan pestisitin miktarına ve çeşidine bağlı olarak değişkenlik göstermektedir (Regol ve ark., 2019).

Brezilya'da Santos ve ark. (2015) tarafından yapılan bir çalışmada 2010-2012 yılları arasında ticari süt örneklerinde organik klorlu pestisit varlığı belirlenmiş, bunlardan %82.1'nin gama-HCH olduğu belirtilmiştir. Shahzadi (2013) tarafından yapılan bir çalışmada farklı hayvan türlerine ait süt örneklerinin %50'sinin başta deltametrin ve karbofuran olmak üzere çeşitli pestisit türleriyle kontamine olduğu, en fazla kontaminasyon yüküne koyun sütü örneklerinde rastlandığı belirtilmiştir.

Sütte pestisit kalıntı düzeylerini belirlemek için duyarlı ve kesin sonuç veren analitik metodların kullanılması gerekmektedir (Ramezani ve ark., 2022). Farklı ülkelerde planlanan prevalans çalışmalarından elde edilen farklı veriler, kullanılan farklı ekstraksiyon ve analiz yöntemleriyle ilişkilendirilebilir. Genellikle süt protein ve yağ içeriği açısından zengin olduğundan, sütte söz konusu kimyasalların analizi yanıltıcı sonuçlar ortaya koyabilmektedir. Dolayısıyla analiz öncesinde bu yanıltıcı içerikleri süt matriksinden uzaklaştırmak için çeşitli ön uygulama ve temizlik basamaklarının uygulanması tavsiye edilmektedir (Manav ve ark., 2019). Yapılan bir çalışmada organik klorlu pestisitlerin süt ve süt yağından ayrılarak tayini amacıyla yeni bir sıvı/sıvı ekstraksiyon yöntemi geliştirilmiş, örnekler GC-MS/MS ile analiz edilmiş tespit sınırları 0.36 ve 1.11µg/kg aralığında bulunmuştur. Araştırmacılar 60'ı UHT, 27'si pastörize süt örneğinden oluşan toplam 87 ısı işlem görmüş süt örneğinde herhangi bir pestisit kalıntısına rastlamadıklarını bildirmişleridir (Karataş ve Coşkun, 2018).

Sütte pestisit kontaminasyonlarına ilişkin farklı çalışmalardan bildirilen farklı sonuçlar, süte uygulanan ısı işlemine göre de değişiklik arz edebilmektedir. Nitekim pastörize sütlerde pestisit degradasyon oranının %8 ila 18 arasında değişiklik gösterdiği, kaynatma işlemiyle birlikte bu oranın %27-46'ya, sterilizasyon işlemi ile de %40-63'e kadar ulaştığı bildirilmektedir (Schopf ve ark, 2022). Benzer şekilde Heck ve ark. (2007) tarafından yapılan bir çalışmada çiğ sütte toplam DDT düzeylerinin pastörize ve UHT sütlere göre anlamlı derecede yüksek olduğu bildirilmektedir.

Isı işleminin pestisit kalıntılarını azalttığı belirtilse de, ısı işlemi sonrası pestisitlere ait spesifik izomer konsantrasyonları ve bunlara ilişkin sağlık riskleri de tartışılmaktadır. Nitekim pastörize sütlerde rapor edilen pestisit kontaminasyonları, bazı ajanların ısıl işleme dirençli olduklarını da ortaya koymaktadır. Öte yandan pestisit kalıntılarının, lipofilik yapılarından dolayı, yağlı süt ve süt ürünlerinde (tereyağı, krema ve peynir) yoğunlaştığı, bununla birlikte özellikle organofosforlu bileşiklerin proteinlere bağlanma kapasitesinin de bulunduğu belirtilmektedir (Chandra ve ark., 2020).

Yapılan bir çalışmada ise, yer altı atık alanlarının eski ve yasaklanmış pestisit kalıntılarını uzun süre muhafaza ettiği ve ikincil bir yer altı kontaminasyonu ile sonuçlandığı vurgulanmaktadır. Nitekim Witczak ve ark. (2019) tarafından 2009-2013 yılları arasında Polonya'da organik keçi sütlerinde organik klorlu pestistlerin araştırıldığı bir araştırmada örneklerde 1982 yılından beri kullanımının yasak olmasına rağmen 4.85 mg/kg' a kadar gama-HCH varlığının tespit edildiği kaydedilmiştir. Yine organik sütlerde yapılan bir çalışmada, heksaklorobenzen, p,p'- DDT ve onun metaboliti olan p,p'-DDE' ye yasaklı olmalarına rağmen bütün örneklerde rastlandığı belirtilmiş, tüm dünyada organik çiftliklerde pestisit kullanımına izin verilmese de hala tespit ediliyor olmaları bazı pestisitlerin yarılanma ömrünün uzun olmasına, dolayısıyla etkenlerin organik sütlere kadar ulaşmasına bağlanmaktadır (Welsh ve ark., 2019).

Genel olarak; lipofilik olmalarından dolayı organoklorlu pestisitlere maruziyet açısından; süt yağ oranları yüksek olan hayvan türlerine ait sütler (manda, koyun) ve yağlı süt ürünleri (tereyağı, krema ve peynir) daha riskli, ısı işlemi görmüş sütler ise daha güvenli bulunmaktadır. Süt ve süt ürünlerinin işlenmesi sonucu ortaya çıkan yeni toksik ürünlerin halk sağlığı açısından teşkil ettiği riskleri değerlendirebilmek için daha çok çalışmaya ihtiyaç duyulmaktadır.

Her ne kadar bu çalışmada analiz edilen organik veya klasik UHT ve pastörize sütlerde TGK değerlerini aşan herhangi bir pestisit kontaminasyonuna rastlanmamış olsa da literatür verileri süt ve süt ürünlerinde pestisit varlığını ortaya koymaktadır. Pestisit maruziyetlerinin değerlendirilmesi ve azaltılması açısından; pestisit satışlarının denetim altına alınması, çiftçilerin

eğitimi, pestisit kalıntı izleme programlarının uygulanması ve iyi tarım uygulamalarının hayata geçirilmesi kritik öneme sahiptir.

Bu çalışmadan elde edilen sonuçlar, TGK'nin ilgili yönetmeliğindeki bazı pestisitlere ait maksimum kalıntı limit değerlerinin güncellenmesi gerektiğini, benzer şekilde ulusal referans laboratuvarlarının da alt yapı ve metotlarını bu limit değerlere göre düzenlemesi gerektiğini ortaya koymuştur.

Kaynaklar

Acara A. Türkiye'nin kalıcı organik kirletici maddelere (POP'ler) ilişkin Stockholm sözleşmesi için taslak ulusal uygulama planı. Unido-POP'ler Projesi. Proje No. GF/TUR/03/008, 2006; s. 237.

Bulut S, Akkaya L, Kov V, Konuk M. Organochlorine pesticide residues in butter and kaymak in Afyonkarahisar, Turkey. J Anim Vet Adv 2010; 9(1-4): 2797-801.

Codex Alimentarius (CA) 2019 Codex pesticides residue online database in/on milk. https://www.fao.org/fao-who-codexalimentarius/codextexts/dbs/pestres/commodities detail/en/?lang=enandc_id=187. Accessed Date: 14.11.2021.

Chandra P, Enespa Singh R, Arora PK. Microbial lipases and their industrial applications: A comprehensive review. Microb Cell Factories 2020; 19: 1-42.

Deti H, Hymete A, Bekhit AA, Mohamed AMI, Bekhit AE-DA. Persistent organochlorine pesticides residues in cow and goat milks collected from different regions of Ethiopia. Chemosphere 2014; 106: 70-4.

Duan J, Cheng Z, Bi J, Xu Y. Residue behavior of organochlorine pesticides during the production process of yogurt and cheese. Food Chem 2018; 245: 119-24.

European Commission (EC) 2010 Pesticides EU MRLs regulationno. 396/2005. Active Substances Directive 91/414/EEC. https://ec.europa.eu/food/plants/pesticides/maximum-residue-levels_en. Accessed Date: 14.11.2021.

Fischer WJ, Schilter SB, Tritscher AM, Stadler RR. Contaminants of milk and dairy products: Contamination resulting from farm and dairy practices. Encyclopedia of Dairy Sci Second Edition 2011; 2: 887-97.

Food and Agriculture Organization (FAO) 2021 Dairy market review: Emerging trends and outlook. https://www.fao.org/3/cb7982en/ cb7982en.pdf. Accessed Date: 13.12.2022.

Gomes HO, Menezes JMC, Costa JGM, Coutinho HDM, Teixeira RNP, Nascimento RF. A socio-environmental perspective on pesticide use and food production. Ecotox Environ Safe 2020; 197: 110-627.

Gutierrez R, Ortiz R, Vega S,Schettino B, Ramirez ML,Perez JJ. Residueslevels of organochlorinepesticide in cow's milk from industrial farms in Hidalgo, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, Mexico. J Environ Sci Health 2013; 48(11): 935-40.

Güvenç D. Samsun yöresinden toplanan çiğ süt örneklerinde bazı pestisit kalıntılarının araştırılması üzerine bir çalışma. Doktora tezi, Ondokuz Mayıs Üniv Sağ Bil Ens, Samsun 2008; s. 64.

Hasan GMMA, Das AK, Satter MA. Multi residue analysis of organochlorine pesticides in fish, milk, egg and their feedby GC-MS/MS and their impact assessment on consumers health in Bangladesh. NFS Journal 2022; 27: 28-35.

Haylamicheal ID, Dalvie MA. Disposal of obsolete pesticides, the case of Ethiopia. Environ Int 2009; 35(3): 667-73.

Heck, MC, dos Santos JS, Junior SB, Costabeber I, Emanuelli T. Estimation of children exposure to organochlorine compounds through milk in Rio Grande do Sul, Brazil. Food Chem 2007; 102(1): 288-94.

Kampire E, Kiremire BT, Nyanzi SA, Kishimba M. Organochlorine pesticide in fresh and pasteurized cow's milk from Kampala markets. Chemosphere 2011; 84(7): 923-7.

Karataş M, Coşkun H. UHT ve pastörize sütlerde organik klorlu pestisitlerin tayini. Gıda 2018; 43(5): 733-44.

Keswani C, Dilnashin H, Birla H, Roy P, Tyagi RK, Singh D, Rajput VG, Minkina T, Singh SP. Global foot prints of organochlorine pesticides: A pan-global survey. Environ Geochem Health 2022; 44 (1): 149-77.

Manav ÖG, Dinç-Zor Ş,Alpdoğan G. Optimization of a modified QuEChERS method by means of experimental design formulti residue determination of pesticides in milk and dairy products by GC–MS. Microchem J 2019; 144: 124-9.

Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens L. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front Public Health 2016; 4: 148.

Ramezani S, Mahdavi V,Gordan H, Rezadoost H, Conti GO, Khaneghah AM. Determination of multi-class pesticidesresi- Critical Reviews in Food Sci-

ence and Nutrition 15 dues of cow and human milk samples from Iran using UHPLC-MS/ MS and GC-ECD: A probabilistic health risk assessment. Environ Res 2022; 208: 112730.

Rêgol CV, DosSantos GNV, Ribeiro JS, Lopes RB, DosSantos SB, De Sousa A, Mendes RDA, Taketomi ATF, Vasconcelos AA. Residues of organochlorinated pesticides en lechecommercial: Una revisión sistemática. Acta Agron 2019; 68(2): 99-107.

Rusu L, Harja M, Suteu D, Dabija A,Favier L. Pesticide residues contamination of milk and dairy products. A case study: Bacaudistrictarea. Romania. J Environ Prot Ecol 2016; 17(3): 1229-41.

Sana S, Qadir A, Mumtaz M, Evans NP, Ahmad SR. Spatial trends and human health risks of organochlorinated pesticides from bovine milk; a case study from a developing country, Pakistan. Chemosphere 2021; 276: 130110-30.

Santos SJ, Schwanz TG, Coelho AN, Heck-Marques MC, Mexia MM, Emanuelli T, Costabeber J. Estimated Daily intake of organochlorine pesticides from dairy products in Brazil. Food Control 2015; 53: 23-8

Schopf MF, Pierezan MD, Rocha R, Pimentel TC, Esmerino EA, Marsico ET, Verruck S. Pesticide residues in milk and dairy products: An overview of processing degradation and trends in mitigating approaches. Crit Rev Food Sci Nutr 2023; 63(33): 12610-24.

Shahzadi N, Imran M, Saewar M, Hashmi AS, Wasim M. Identification of pesticides residues in differents amples of milk. J Agroaliment Processes Technol 2013; 19(2): 167-72.

Shakerian A, Karimi B, Rahimi E. Presence of pesticides in milk and infant formulas produced in Pegah dairy plants. Egypt J Vet Sci 2020; 51(3): 303-9.

Sharma AV, Kumar B Shahzad M Tanveer GPS, Sidhu N, Handa SK, Kohli P, Yadav AS, Bali RD, Parihar RD, Dar OI, Singh K, Jasrotia S, Bakshi P, Ramakrishnan M, Kumar S, Bhardwaj R, Thukral AK. Worldwide pesticide usage and its impacts on ecosystem. SN Appl Sci 2019;1(11): 1-16.

Sheldon I. North–South trade and standards: what can general equilibrium analysis tell us? World Trade Rev 2012;11(3): 376-89.

Türk Gıda Kodeksi (TGK). Pestisitlerin Maksimum Kalıntı Limitleri Yönetmeliği. 2016. https://www.resmigazete.gov.tr/eskiler/2016/11/20161125M1-1.htm. Accessed Date: 01.12.2018.

Tolentino RG, León SV, Bermúdez BS, Flores GP, Vega MLR, Vázquez CR, Vázquez MR, Francisca MV.Organochlorine pesticides in ınfant milk formula marketed in the south of Mexico city. Food Nutr Sci 2014; 5(13):1290-8.

Tsakiris IN, Goumenou M, Tzatzarakis MN, Alegakis AK, Tsitsimpikou C, Ozcagli E, Vynias D, Tsatsakis A. Risk assessment for children exposed to DDT residues in various milk types from the Greek market. Food ChemToxicol 2015; 75: 156-65.

US Environmental Protection Agency. 2018. Why We Use Pesticides. https://www.epa.gov/safepestcontrol/whywe-use-pesticides?. Accessed Date: 01.12.2018.

USK, 2021. 2020 Süt Raporu, Dünya ve Türkiye'de Süt Sektörü İstatistikleri. Ulusal Süt Konseyi, Ankara.

Welsh JA, Braun H, Brown N, Um C, Ehret K, Figueroa J, Barr DB. Production-related contaminants (pesticides, antibiotics and hormones) in organic and conventionally produced milk samples sold in the USA. Public Health Nutr 2019; 22(16): 2972-80.

Witczak A, Malek AM. The comparison of probiotic monocultures influence on organochlorine pesticides changes in fermented beverages from cow and goat milk during cold storage. Mljekarstvo 2019; 69: 172-82.

Yuan S, Yang F, Yu H, Xie Y, Guo Y, Yao W. Degradation mechanism and toxicity assessment of chlorpyrifos in milk by combined ultrasound and ultraviolet treatment. Food Chem 2022; 383: 132550.

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Köpek Scapula ve Humerus 3D Baskılarının Üretiminin ve Eğitimdeki Etkinliğinin Araştırılması*

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Öz: Bu çalışmanın amacı, 3D lazer tarayıcı ve yazıcı kullanarak köpek iskeletine ait bazı kemiklerin üretilmesi ve 3D kemiklerin eğitimdeki etkinliğinin araştırılmasıdır. Bu amaçla bir köpek iskeletine ait scapula ve humerus 3D lazer tarayıcı ile taranmış, 3D yazıcı kullanılarak 3D kemikler üretilmiştir. Sonrasında 3D kemiklerin eğitimdeki etkinliğinin araştırılması için öğrenci grupları üzerinde pratik eğitim ve ardından sınav yapılmıştır. Sonuç olarak 3D kemiklerin, en az gerçek kemikler kadar eğitimde başarılı olduğu ortaya konulmuştur.
Anahtar kelimeler: Anatomi, 3D, 3D modelleme, iskelet, köpek

Investigation of the Production of Dog Scapula and Humerus 3D Prints and Their Effectiveness in Education

Abstract: The aim of this study is to produce some bones of the dog skeleton using a 3D laser scanner and a printer, and to investigate the effectiveness of 3D bones in education. For this purpose, the scapula and humerus of a dog skeleton were scanned with a 3D laser scanner, and 3D bones were produced using a 3D printer. Afterwards, practical training and then an examination were conducted on student groups to investigate the effectiveness of 3D bones in education. As a result, it has been shown that 3D bones are at least as successful in training as real bones.
Keywords: Anatomy, 3D, 3D modeling, dog, skeleton

Giriş

3D tarama teknolojisi; endüstri alanında kullanılmakta olup bilgisayar teknolojilerinin sürekli gelişmesi sayesinde insan vücudunu hızlı bir sürede ve yüksek çözünürlükle tarayabilir ve bilgisayara aktarabilir hale gelmiştir (Yüksel ve Bulut, 2019). Taranmak istenen objenin 3D modelini elde edebilmek için farklı açılarda birden fazla tarama gerekebilmektedir (Karasaka ve Beg, 2021). Lazer tarayıcıların çalışma prensibi, optik aynalar sayesinde, lazer ışınının yatay ve düşey yönlerde yönlendirilmesi ile modelin taramasına dayanır.

3D yazdırma teknolojisi ve 3D yazıcılar, mimarlık, tıp ve daha birçok alanda sıklıkla kullanılmakta ve eğitim alanında da giderek popülerliği artmaktadır. 3D yazıcıların en büyük avantajları ise maliyet ve zamandan tasarruf sağlamasının yanında çevre dostu olmasıdır (Kuzu Demir ve ark., 2016). 3D baskı teknolojisi, biyoteknoloji (insan dokusunun yenilenmesi), medikal sektör (protez), gıda (pasta), mimarlık (ev), endüstriyel tasarım (araba) gibi birçok farklı dalda kullanım alanı bulmaktadır (Aydın ve Küçük, 2014). Teknoloji, birçok alanda olduğu gibi, eğitimde de öğrencilerin derse olan ilgisini artırmak için önemli bir role sahiptir (Güler ve Erdem, 2014). Eğitim başlığı altında 3D yazıcılar stratejik bir öneme sahiptir. Yaratıcılığı artırmasından dolayı teknik ve mekanik derslerde kullanımı yaygındır (Kökhan ve Özcan, 2018). 3D teknolojileri ayrıca diş hekimliğinde (Bulut, 2020), sağlık eğitiminde, klinik öncesi eğitim ve uzmanlık eğitiminde kullanılmaktadır (Sezer ve Şahin, 2016).

Kompleks anatomik detaylar bilgisayar ekranındaki 3D görsellerden tam olarak anlaşılmayabilmektedir. Çoğunlukla zorlu patolojik ve anatomik koşullarda, 3D baskı materyalleri öğrenmeyi kolaylaştırdığı ve geliştirdiği için cerrahi uzmanlık eğitiminde tercih edilmektedir (Sezer ve Şahin, 2016). 3D baskı materyalleri, klinik öncesi sağlık eğitiminde, özellikle anatominin daha kolay anlaşılmasında tercih edilir (Rengier ve ark., 2010). 3D dijital objelerin elde edilmesi için iki ana yöntem bulunmaktadır. Birincisi, istenilen objenin 3D çizim ve animasyon programları ile sıfırdan oluşturulması, ikincisi ise, tarama yöntemleri kullanılarak cismin kopyalanması tekniğidir. Tarama işlemi için üç farklı cihaz ve teknik kullanılır: Bilgisayarlı Tomografi (BT), Magnetik Resonans (MR) ve Lazer tarayıcı.

3D baskı, nadir örneklerin çoğaltılması ve büyük sınıflara anatomik modellerin sağlanması için de bir çözüm olabilir. Bu nedenle 3 boyutlu tarama ve yaz-

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dırma, kullanıcıları içerik oluşturmaya teşvik ettiğinden dolayı anatomi eğitiminde önemli bir ilerlemedir (Thomas ve ark., 2016).

3D baskı kemik modellerinin öğrenciler tarafından kabul edilebilirliğinin yüksek olduğu ve bu nedenle veteriner anatomi eğitiminde 3D baskı modellerinin güvenilir bir alternatif olduğu bildirilmektedir (Li ve ark., 2018).

Bu çalışmanın amacı; 3D lazer tarayıcı ve yazıcı kullanarak köpek scapula ve humerus kemiklerinin elde edilmesi, sonrasında ise bu ürünlerin eğitimdeki etkinliğinin araştırılmasıdır. Bu çalışmanın hipotezi; 3D yazdırma teknolojilerini kullanarak Veteriner Hekimlikte daha hızlı, modern ve etik osteolojik eğitim materyalleri ile yapılan uygulama eğitiminin en az gerçek materyaller ile yapılan uygulama eğitimi kadar başarılı olduğu şeklindedir.

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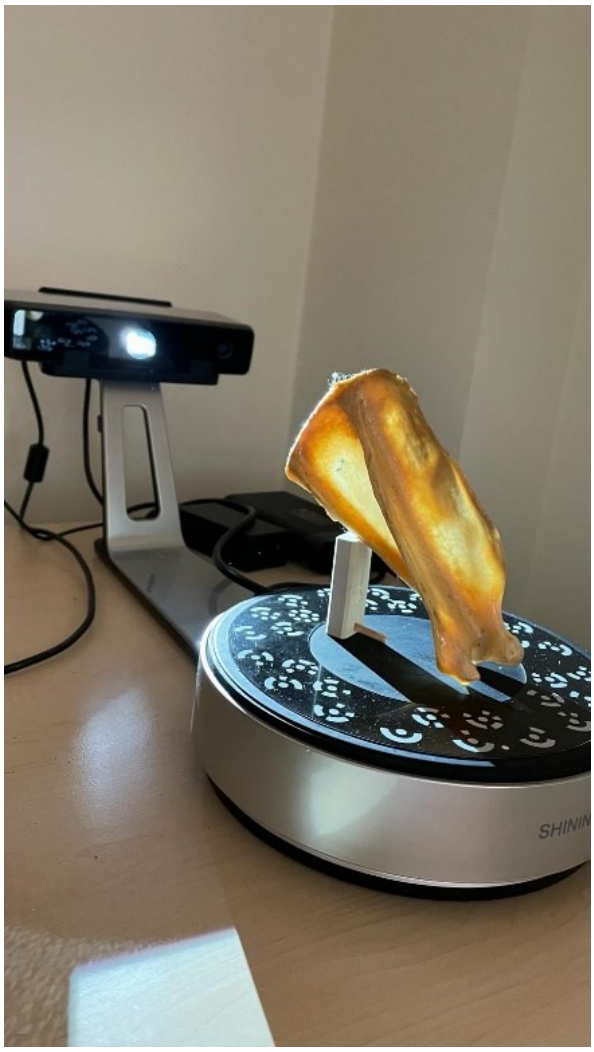
Birleştirilmiş köpek iskeletindeki sağ ve sol tarafa ait scapula ve humerus kemikleri özenli bir şekilde iskeletten ayrıldı ve temizlenerek taramaya hazır hale getirildi.

Tarama

Bu çalışmada, Creality 3D scanner ve Shining EinScan SP 3D tarayıcı modeli kullanıldı. Uygun ortam koşulları sağlandı (ışık vb.). Köpek iskeletinde bulunan iki scapula ve iki humerus olmak üzere toplam dört kemik tarandı. Her kemik doğrudan tarayıcı tablasına yerleştirilmedi. Tarayıcının okunmasını kolaylaştırmak amacıyla, kemiğe uygun konum ve doğru açı bulunabilmesi için, kemikler özel aparatlar ile desteklendi. Her bir yüz taranıp, farklı bir yüze geçildiğinde mesh yapıldı. Böylece bir bütün kemik modeli elde edildi. Bütün anatomik yapı ve ayrıntıların tarandığından emin olunana kadar aynı kemikler birkaç kez tarandı (Şekil 1). Elde edilen model ile orijinal kemikte bulunan anatomik ayrıntılar var/yok şeklinde kıyaslandı. Her bir ayrıntının tarandığından emin olunduğunda STL (Standard Triangle Language) dosyasına kemiklerin isimleri belirtilerek kaydedildi.



Şekil 1. Tarama işlemi.

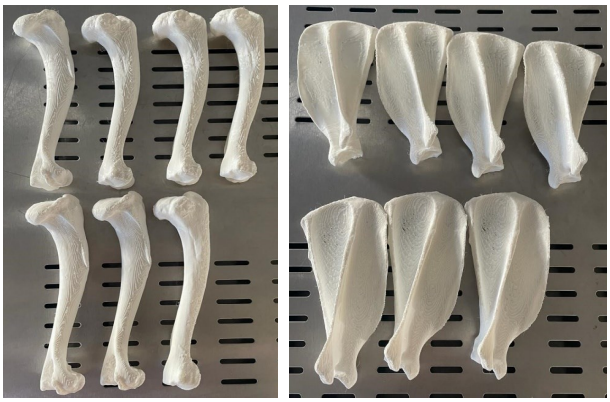
Yazdırma

Çalışmada, Creality CR- 6 SE (Ender CR-6 Second Edition, Type: FDM) yazıcı modeli kullanıldı. Dilimleme ve g-code oluşturma işlemleri için Ultimaker Cura (V 5.1.1) programı kullanıldı. Modellerin orijinal boyutlarında herhangi bir değişiklik ve ölçeklendirme yapılmadan 1:1 oranında modelleme yapılmıştır. STL dosyası şeklinde kayıtlı olan kemik modelleri yazıcı modelleyicisinde yeterli hız ve yeterli detayda model verecek şekilde, kemiklerin anatomik özelliklerine göre modellendi. Birçok farklı deneme yaparak yazıcının bu boyutlarda en ideal kemikleri yazabilmesi için, optimum parametreler aşağıdaki tabloda (Tablo 1) belirtildiği şekilde tespit edildi.

Tablo 1. Yazıcı parametreleri

Katman Yüksekliği	0.2
Dolgu Yoğunluğu	20.0
Yazdırma Hızı	50
Destek Yapısı	Normal
Destek Ara Yüzü Şekli	Izgara
Yazdırma Sıcaklığı	200.0

Bu çalışmanın ikinci bölümü olan ölçme ve değerlendirme aşamasında yapılacak sınavda, cartilago scapula yapısı mevcut olmayan scapula ve humerus kemiği kullanılacağı için, laboratuvar ortamında bulunan masa ve öğrenci sayısına uygun olacak şekilde 3D yapay humerus ve cartilago'su olmayan scapula yazdırıldı (sağ/sol) (Şekil 2).



Şekil 2. Sınav materyaline uygun scapula (sol/sağ) ve humerus (sağ) modeli. (lateral görünüm).

Sınav

Sınava tabii tutulan öğrenciler, Erciyes Üniverstesi Veteriner Hekimliği Fakültesini yeni kazanmış ve Anatomi dersini ilk kez alan öğrencilerdir. Öğrencilere çalışmaya başlamadan önce bilgilendirme yapıldı ve onam formları imzalatıldı. Toplam birinci sınıfa kayıtlı 102 öğrenci bu çalışmaya katıldı. Sınav materyali olarak kullanılacak scapula ve humerus kemikleri teorik derste, her iki şubeye de gruplara ayrılmadan anlatıldı. Bu öğrenciler üniversiteye giriş başarı puanlarına göre dengeli olarak iki gruba ayrıldı. Bu gruplardan birisi kontrol grubu, diğeri deney grubu olarak isimlendirildi. Birbirinden bağımsız girişlere sahip laboratuvarda deney ve kontrol grubu öğrencilerin birbirlerini ve çalışma materyallerini görmemesi özellikle sağlandı. Kontrol grubunda bulunan öğrenciler, gerçek kemik olan scapula ve humerus ile çalıştı. Deney grubu olan öğrenciler, 3D scapula ve humerus ile çalıştı. Bu sırada kemikler üzerindeki çıkıntılar kontrollü bir şekilde yardımcı eğitmenler tarafından, bir saatlik pratik ders süresi boyunca her iki gruptaki öğrencilere de anlatıldı.

Bu sürenin sonunda, zilli sınava geçildi (Şekil 3). Sınav için kedi, köpek ve tavşan kemiklerinden oluşan sadece gerçek kemikler kullanıldı. Gerçek kemikler ile çalışan (kontrol grubu) ve 3D kemikler ile çalışan öğrencilere (deney grubu), iki farklı kemikten toplamda 10 soru soruldu. Scapula ve humerus kemikleri için sorular şu şekilde dizayn edilmiştir.

Soru no	Humerus için sorular	Scapula için sorular
1	Bu kemiğin adı nedir?	Bu kemiğin adı nedir?
2	Bu kemik hangi hayvana aittir?	Bu kemik hangi hayvana aittir?
3	Bu kemiğin yönü nedir (sağ/sol)?	Bu kemiğin yönü nedir (sağ/sol)?
5	Kemik üzerinde kırmızı renk ile çevrelenmiş deliğin adı nedir?	Kemik üzerinde kırmızı renk ile boyanmış yapının adı nedir?
5	Kemik üzerinde mavi renk ile boyanmış yapının adı nedir?	Kemik üzerinde mavi renk ile boyanmış yapının adı nedir?

Şekil 3. Sınav ortamı.

Anket

Anket sürecinde; her iki gruptaki öğrenciler de karışık olarak laboratuvara alındı ve çalışma masalarında hem gerçek kemikler, hem de 3D kemikler konularak uygulama yapmaları sağlandı. Sonrasında öğrencilere anketler dağıtılarak, yanıtlamaları için süre tanındı. Anketlerde çalışma konusu ile ilgili soruların yanısıra, araştırma hakkında bilgi içeren kısa bir bölüm, uygulama sonrası kazanımlarla ilgili görüşler ve bilgilendirilmiş gönüllü onam formları da yer almaktadır.

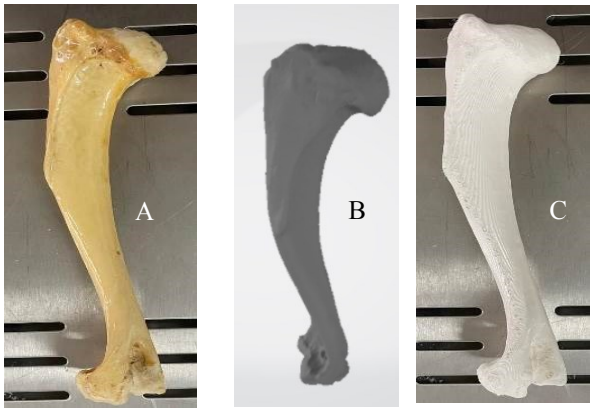
Veriler SPSS for Windows 22.0 istatistik paket programı aracılığıyla çözümlendi. Kategorik değişkenler sayı ve yüzde dağılımlarıyla sunuldu. Sürekli değişkenlerin ortalama ve standart sapması hesaplandı. Değişkenlerin normal dağılıma uygunluğu Kolmogorov-Smirnov testi ile yapılmış ve verilerin normal dağılım göstermediği belirlenmiştir (P<0.05). İstatistiksel çözümlemede; Pearson Ki-kare Testi, Fisher'in Kesin Testi, Mann-Whitney U Testi ve Spearman korelasyon analizi kullanıldı. İstatistiksel anlamlılık düzeyi P<0.05 olarak kabul edildi.

Bulgular

Köpek iskeletine ait sağ ve sol scapula ve humerus olmak üzere toplam dört kemik başarıyla tarandı. Köpek iskeletine ait sağ ve sol scapula ve humerus olmak üzere toplan dört kemik başarıyla yazdırıldı. Kemikler üzerinde, yazıcının filament izlerinin olduğu gözlendi. Bu izlerin, kemiklerin normal anatomik yapılarını etkilemediği, bu tip yüzey tırtıkları oluşan kemikler için, törpüleme işlemlerinin olumlu sonuçlar verdiği gözlendi.

Köpek iskeletine ait gerçek kemikler, 3D dijital kemikler ve 3D kemikler kendi içlerinde birbirleriyle kıyaslandığında, anatomik yapılar bakımından birbirlerine benzerlik oranlarının çok yüksek olduğu görüldü (Şekil 4, 5). Bu benzerliğin kıyaslaması için Nomina Anatomica'da bulunan yapıların tek tek incelenerek her üç materyalde de bu yapıların varlığı belirlendi. Bu yöntemle inceleme yapıldığında birbirlerine benzerlik oranlarının %100 olduğu belirlendi. Öğrencilere uygulanan sınavların sonuçlarına göre ve yapılan anketlerin de sonuçları değerlendirildiğinde, bu benzerlik oranının eğitim kalitesini etkilemeyecek kadar başarılı olduğu tespit edildi.

Şekil 4. Scapula iskeleti, dijital görünüm ve 3D yazıcı modeli.: A. Gerçek kemik, B. 3D dijital kemik, C. 3D kemik.



Şekil 5. Humerus iskeleti, dijital görünüm ve 3D yazıcı modeli. A. Gerçek kemik, B. 3D dijital kemik, C. 3D kemik.

Öğrencilerin sınav kağıtları tek bir eğitmen tarafından değerlendirildi ve sınavdan alınan notların (Tablo 2, Şekil 6) ortalaması deney ve kontrol grubunda ayrı ayrı hesaplandığında şu sonuç elde edildi:

$\overline{D}$: 61.96078 (Deney grubu)

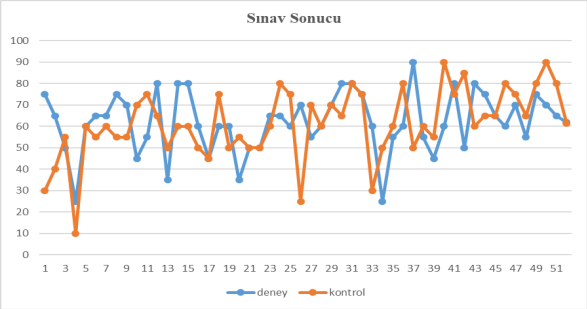
$\overline{K}$: 61.37255 (Kontrol grubu)

Buradan edinilen sonuca göre, deney grubunun elde ettiği başarı, kontrol grubunun elde ettiği başarıdan daha fazladır. İki grup arasında sayısal olarak çok büyük fark olmaması, 3D materyalleri ile yapılan uygulama eğitiminin en az gerçek materyaller ile yapılan uygulama eğitimi kadar başarılı olduğu hipotezimizin doğruluğunu kanıtlamaktadır.

Öğrencilere uygulanan anketlerin sonuçları Tablo 3'te gösterilmiştir. Çalışmaya katılan öğrencilerden, 3D kemiklerin en az gerçek kemikler kadar öğretici olduğu konusunda, "Tamamen Katılıyorum" ve "Katılıyorum" seçimini yapan öğrenciler %57'lik bir çoğunluk oluşturmaktadır. Ayrıca bu soruya "Tamamen Katılıyorum" diyen öğrencilerin sayısı deney grubunda kontrol grubuna göre, anlamlı olarak daha fazla bulunmuştur (P<0.05).

Tablo 2. Deney ve kontrol grubunda elde edilen sınav sonucu

Alınan Not	10	25	30	35	40	45	50	55	60	65	70	75	80	85	90	**Toplam**
Kişi Sayısı	1	3	2	2	1	4	11	11	19	12	8	11	13	1	3	102



Şekil 6. Deney ve kontrol grubunun başarı grafiği.

Bu çalışmaya katılan öğrencilerin yaklaşık olarak % 60'ı 3D kemiklerin hijyen konusunda kendilerini rahat hissettirdiğini bildirmişlerdir. Ankette yer alan diğer tanımlayıcı sorulara verilen cevaplarda, iki grup arasında anlamlı bir fark görülmemiştir (P>0.05, Tablo 3).

Tablo 3. Anket soruları ve sonuçları.

		Deney grubu (n=51)	**Kontrol grubu (n=51)**	**P***
		n (%)	**n (%)**	
Köpek 3D kemikler, uygulama eğitiminde en az gerçek kemikler kadar öğreticiydi.	Tamamen Katılıyorum	8 (15.7)	1 (2.0)	**<0.001**
	Katılıyorum	25 (49.0)	24 (47.1)	
	Kararsızım	4 (7.8)	12 (23.5)	
	Katılmıyorum	12 (23.5)	4 (7.8)	
	Kesinlikle Katılmıyorum	2 (3.9)	10 (19.6)	
Gerçek kemikler olmasa da aynı konuyu sadece köpek 3D kemikleriyle de öğrenebilirim.	Tamamen Katılıyorum	7 (13.7)	4 (7.8)	0.217
	Katılıyorum	14 (27.5)	12 (23.5)	
	Kararsızım	16 (31.4)	15 (29.4)	
	Katılmıyorum	11(21.6)	9 (17.6)	
	KesinlikleKatılmıyorum	3 (5.9)	11 (21.6)	
Köpek 3D kemiklerin üzerindeki detaylar gayet başarılıydı.	Tamamen Katılıyorum	5 (9.8)	3 (5.9)	0.236
	Katılıyorum	17 (33.3)	19 (37.3)	
	Kararsızım	14 (27.5)	11 (21.6)	
	Katılmıyorum	12 (23.5)	8 (15.7)	
	Kesinlikle Katılmıyorum	3 (5.9)	10 (19.6)	
Köpek 3D kemiklerin plastikten yapılmış olması hijyen konusunda beni rahatlattı.	Tamamen Katılıyorum	11 (21.6)	11 (21.6)	0.990
	Katılıyorum	20 (39.2)	20 (37.3)	
	Kararsızım	8 (15.7)	7 (13.7)	
	Katılmıyorum	9 (17.6)	11 (21.6)	
	KesinlikleKatılmıyorum	3 (5.9)	3 (5.9)	
Köpek 3D kemik uygulama materyallerinin bir canlıya ait olmadığını bilmek psikolojik olarak rahat hissettirdi.	Tamamen Katılıyorum	7 (13.7)	5 (9.8)	0.584
	Katılıyorum	11 (21.6)	9 (17.6)	
	Kararsızım	13 (25.5)	12 (23.5)	
	Katılmıyorum	11 (21.6)	18 (35.3)	
	KesinlikleKatılmıyorum	9 (17.6)	7 (13.7)	

Tartışma ve Sonuç

Literatürde birçok kaynakta belirtildiği gibi MR ve BT tarama yöntemleri sağlık alanında, özellikle de canlının iç yapılarının görüntülenmesi amacıyla kullanılan fakat ulaşılabilirlik ve maliyet açısından uygun olmayan tekniklerdir (Aydoğdu ve ark., 2017; Bahar ve ark., 2019; Yüksel, 2019). Bu çalışmada, lazer tarama metodunun seçilmesi, kemiklerin sadece dış yapılarının kemik kopyalama açısından yeterli olması nedeniyledir. Sadece köpek kemiklerinin üretilmesi, iç yapılarının bu çalışma için gerekli olmaması, yüzey tarama işlemini yeterli kılmıştır.

Daha önce Sarıtaş ve ark. (2019) tarafından yapılan çalışmada, nesnenin her açıdan görünüşünü elde edebilmek için, birçok açıdan birden fazla tarama yapılmasına ihtiyaç duyulduğu belirtilmiştir (Sarıtaş ve ark., 2019). Bu durum bu çalışmada da ortaya

çıkmıştır**.** Tarayıcının sahip olduğu "auto align" özelliği sayesinde, nesnenin döner tabla üzerine farklı pozisyonlarda konulduğunda, kendisine referans olarak belirlediği noktalardan eşleştirme yaparak taranmamış yüzeylerin tamamlanmasını sağlamaktadır.

FDM tipi yazıcılarda kullanılan PLA flamentleri ile üretilen materyaller sert olabilmektedir (Yavuz ve Yılmaz, 2021). Bu sertlik aslında kemik üretimi için olmasını istediğimiz bir özelliktir. Sertlik aynı zamanda kemiklere sağlamlık da katmıştır**.** Sert olmasına rağmen bir kemik kadar kırılgan olmadığı düşünülmektedir.

3D yazıcıların ofis ortamında, rahatlıkla kullanılabilir olduğu bilinmektedir (Çelik ve ark., 2013). Çalışmamızda da, ofis ortamında tarama ve yazdırma işlemleri kolaylıkla yapılmıştır. Anatomi laboratuvarlarının çalışma ortamları düşünüldüğünde, 3D kemiklerin üretim süreçleri ve ürünler açısından hem sağlık hem de canlı materyal kullanımındaki etik problemler nedeniyle tercih edilebilir olduğu düşünülmektedir.

Literatürde, kadavra ve deney hayvanları üzerindeki uygulamalar yerine 3D plastik modeller ve bilgisayar teknolojileri gibi araçların kullanımının, etik açıdan daha tercih edilebilir olmasından bahsedilmektedir (Balcombe, 2001). Bu çalışmada ise, öğrencilere yapılan ankette **,** "Köpek 3D kemik uygulama materyallerinin bir canlıya ait olmadığını bilmek psikolojik olarak rahat hissettirdi" ifadesine verilen olumlu görüş, yaklaşık %32 olmuştur. Bu oranın düşük olmasının sebebinin, öğrencilerin hekimlik eğitimine yeni başlamış olması ve bu konunun önemini henüz bilmiyor olduklarından kaynaklandığı düşünülmektedir.

Thomas ve ark. (2016) yaptığı çalışmada kullandığı kurbağa ve köpek balığı iskeletinin 3D dijital ve plastik olarak elde etmiş ve bunların birbirleri arasındaki benzerlik oranlarını, bir tablo şeklinde bazı anatomik yapıların varlığı ve yokluğu üzerine değerlendirmeler yaparak belirlemiştir. Yüzdelik olarak bir benzerlik değeri verilmeyen bu çalışmada kurbağa iskeleti için 48 yapıdan 44'ünün dijital iskelette var olduğunu bildirmektedir. Bu çalışmada kullanılan kurbağa kemikleri, mevcut çalışmada kullandığımız scapula ve humerus kemiklerine göre daha küçük ve daha fazla anatomik detaya sahiptir. Bu nedenle Thomas ve ark. (2016**)**'nın kullandığı benzerlik değerlendirme yöntemi mevcut çalışmada kullanılmamıştır.

Li ve ark. (2018) tarafından yapılan çalışmada yetişkin bir sığıra ait femur, costae ve altıncı cervical vertebra kemikleri lazer tarayıcı ile taranmış ve 3D yazıcıdan plastik kemikler de üretilmiştir. Sonrasında hem dijital model hem de plastik kemik, gerçek kemikler ile kıyaslanmıştır. Uygulamada bu plastik kemikleri kullanan öğrencilere de anket yapılmıştır. Eğitimdeki başarısının ölçümlenmediği bu çalışmada yapılan anketlerin sonuçlarının mevcut çalışmadaki ile benzer olduğu görülmüştür. Mevcut çalışmada farklı olarak öğrencilere sınav yapılmış ve eğitimdeki başarısı ölçülmeye çalışılmıştır.

Yapılan bir çalışma sonucunda, plastik modellerin diğer eğitim araçlarına seçenek olabileceği, fakat kullanılabilirliği hakkında, öğrencilerin "kararsız" oldukları ifade edilmiştir (Gültiken, 2012). Bu çalışmada yapılan ankette, **"**Gerçek kemikler olmasa da, aynı konuyu sadece köpek 3D kemikleriyle de öğrenebilirim" ifadesine verilen olumlu yanıt %36 iken, kararsızlar %30, olumsuz yanıt verenler ise %34 olmuştur. Bu sonucun da, literatürde yapılan çalışmanın sonucuna benzer şekilde öğrencilerin tek alternatif olarak düşünmelerinin "kararsız" olduğu görülmektedir. Buna rağmen, yapılan sınav sonucunun, iki grup arasında da eşit olması, öğreticilik bakımından alternatif olarak kullanılabilirliğini ortaya koymaktadır. Bir başka çalışmada (Özen ve ark., 2009; Özen ve Özen, 2010), öğrencilerin hayvan kullanımının şart olduğunu beyan etmiş olmaları, bu çalışmamızdaki sonuçlar ile karşılaştırıldığında, çalışmamıza katılan öğrencilerin daha ılımlı olduğunu göstermektedir**.**

Literatürde, kalbin yüzey anatomisinin öğretilmesinde, 3D materyalin etkinliğinin ortaya konulduğu çalışmada, 3D modellerinin öğrencinin başarısında herhangi bir dezavantaj oluşturmadığı sonucuna varılmıştır (Lim ve ark., 2016). Bu çalışmada da, literatür ile paralel olarak iki grup arasındaki başarı durumunun birbirinden farklı olmaması, 3D materyallerin öğreticilik açısından negatif bir durum oluşturmadığını göstermektedir**.**

Çalışma yapılan iki öğrenci grubunun, sınav başarısı arasında sayısal olarak çok büyük fark olmaması, hipotezimiz olan, 3D kemikler ile yapılan uygulama eğitiminin, en az gerçek kemikler ile yapılan uygulama eğitimi kadar başarılı olduğunu göstermiştir.

Bu çalışmaya katılan öğrencilerin yaklaşık olarak % 60'ının, 3D kemiklerin hijyen konusunda kendilerini rahat hissettirmiş olması, 3D kemiklerin biyogüvenlik açısından kullanıcı tercihi bir materyal olduğunu göstermektedir.

3D kemiklerin eğitimdeki etkinliğinin kanıtlanmış olduğu bu çalışma süresince birebir deneyimlemiş olduğumuz avantajlar şunlardır:

Hayvan materyali olmadığı için, etik açıdan ve psikolojik açıdan hiçbir probleminin olmaması,

Organik olmaması ve bundan dolayı hijyenik ve sağlığa zararsız olması,

Elde edilme yönteminin, geleneksel metotlara göre çok daha kolay olması,

Özel saklama koşulları gerektirmemesi, her ortamda rahatlıkla çalışılabilir olması,

Elde edilmiş olan dijital kemiklerin, arşivlenerek istenilen zamanlarda, istenilen miktarlarda ve istenilen boyutlarda 3D kemikler elde edilebilir olması.

Bu çalışmada üretilen materyallerin sınırlı kaldığı alan kemiklerin içyapılarıdır. Lazer yüzey tarayıcı ile tarama yapıldığı için sadece kemiklerin yüzeyleri taranmış, içyapıları değerlendirilmemiştir. Bu materyaller ile kemiğin içyapısı konusunda bilgi sahibi olmak mümkün değildir.

Sonuç olarak; 3D teknolojiler kullanılarak elde edilmiş olan kemiklerin eğitimdeki etkinliği açıkça ortaya konulmuştur. Ayrıca kullanıcıların kısa süreli ve sınırlı miktarda materyal ile çalışmış olmalarına rağmen, 3D kemiklerin tercih edilebilir olduğunu beyan etmeleri, bu teknolojinin, anatomi laboratuvarlarındaki eksikleri gidermede çok faydalı olacağını göstermektedir.

İlerleyen çalışmalarda, üretilmiş olan 3D kemiklere dayanıklılık-çekme-germe testlerinin de yapılarak, gerçek kemikler ile aralarındaki farklılık ve benzerlikleri ortaya konulabilir. Ayrıca yapılacak yeni çalışmalarda, 3D kemiklerin ağırlıklarının tartılarak, gerçek kemikler ile aralarındaki farklılığın ortaya konulması, 3D kemiklerin hafif olması durumunda, ağırlığın artırılması için dolgu miktarında yapılacak değişiklikler ile bu parametrelerin belirlenmesi sağlanabilir. Sadece kemiklerin dış yüzeylerinin taranarak, dış anatomik yapılarının oluşturulduğu bu teknikte 3D kemiklere uygulanacak intramedullar pin, levha, vida gibi cerrahi girişimlerde, gerçek kemiklere kıyaslandığında mevcut durumlarını ortaya koyacak çalışmalar yapılabilir.

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Kaynaklar

Aydın L, Küçük S. Üç boyutlu yazıcıyla ayak bileği ortezinin tasarımı ve geliştirilmesi. Tıp Teknolojileri Ulusal Kongresi, Ekim, 15-19, 2014; Nevşehir-Türkiye.

Aydoğdu A, Aydoğdu Y, Yakıncı ZD. Temel radyolojik inceleme yöntemlerini tanıma. İÜ Sağlık Hizmetleri Meslek Yüksekokulu Dergisi 2017; 5(2): 44-53.

Bahar S, Karaoğlan İ, Özdemir V, Nayman A, Karaoğlu N, Turgut N, Aydoğdu S. 3 Boyutlu interaktif veteriner osteoloji atlasının hazırlanması; At, Sığır, Koyun ve Köpek. TÜBİTAK 2019; 1160385.

Balcombe J. Dissection: The Scientific Case for Alternatives. J Appl Anim Welf Sci 2001; 4(2): 117-26.

Bulut AC. Düşük maliyetli, üç boyutlu bir yazıcı kullanılarak oluşturulan diş modellerinin değerlendirilmesi. Kırıkkale Üniv Tıp Fak Derg 2020; 22(3): 461 -9.

Çelik İ, Karakoç F, Çakır MC, Duysak A. Hızlı protipleme teknolojileri ve uygulama alanları. DPÜ Fen Bilimleri Enstitüsü Dergisi 2013; 31: 53-70.

Güler O, Erdem OA. Mesleki eğitimde etkileşimli 3D eğitimin uygulaması ve stereoskopik 3b teknolojisi kullanımı. Bil Tek Derg 2014; 7(3): 1-11.

Gültiken ME. Plastik model kullanımı veteriner anatomi eğitiminde alternatif olabilir mi? Animal Health Prod Hyg 2012; 1: 53-8.

Karasaka L, Beg AAR, Yersel lazer tarama yöntemi ile farklı geometrik özelliklerin modellenmesi. J Geomat 2021; 6(1); 54-60.

Kökhan S, Özcan U. 3D yazıcılarının eğitimde kullanımı. BEST Dergi 2018; 2(1): 81-5.

Kuzu Demir EB, Çaka C, Tuğtekin U, Demir K, İslamoğlu H, Kuzu A. Üç boyutlu yazdırma teknolojilerinin eğitim alanında kullanımı: Türkiye'deki uygulamalar. Ege Eğitim Dergisi 2016; (17)2: 481-503.

Li F, Liu C, Song X, Huan Y, Gao S, Jiang Z. Production of accurate skeletal models of domestic animals using three-dimensional scanning and printing technology. Anat Sci Educ 2018; 11(1): 73-80.

Lim KHA, Loo ZY, Goldie SJ, Adams JW, McMenamin PG. Use of 3d printed models in medical education: a randomized control trial comparing 3D prints versus cadaveric materials for learning external cardiac anatomy. Anat Sci Educ 2016; 9: 213-21.

Özen A, Özen R, Yaşar A, Armutak A, Bayrak S, Gezman A, Şeker İ. Türk veteriner hekimliği öğrencilerinin ve eğitimcilerin hayvanların ahlaki konumları ve türlerin derecelendirilmesine ilişkin tutumları. Kafkas Univ Vet Fak Derg 2009; 15(1): 111-8.

Özen R, Özen A. Erciyes Üniversitesi öğrencilerinin araştırmalarda hayvan kullanımına yaklaşımları. Kafkas Univ Vet Fak Derg 2010; 16(3): 477-81.

Rengier F, Mehndiratta A, Tengg-Kobligk H. von, Zechmann CM, Unterhinninghofen R, Kauczor HU, Giesel FL. 3D printing based on imaging data: Review of medical applications. Int J CARS 2010; 5: 335-41.

Sarıtaş S, Kibar MK, Yakar M, Akkuş C, Aydın M. 3B tarayıcı tasarımı ve protip üretimi. Forth International Congress on 3D Printing (Additive Manufacturing) Technologies and Digital Industry, April, 11-14, 2019; Antalya-Türkiye.

Sezer H, Şahin H. 3D baskı materyalinin eğitimde

kullanımı: QUA VADİS? Tıp Eğitim Dünyası 2016; (46): 5-13.

Thomas DB, Hiscox, JD, Dixon BJ, Potgieter J. 3D scanning and printing skeletal tissues for anatomy education. J Anat 2016; 229 (3): 473-81.

Yavuz E, Yılmaz S. Diş hekimliğinde yeni ve hızla ilerleyen üretim teknolojisi: 3 boyutlu yazıcılar. Akd Tıp D 2021; 7(2): 197-205.

Yüksel H, Bulut MO. Üç Boyutlu tarama sistemleri. J Text Eng 2019; 26(116): 406-14.

Yüksel Z. Manyetik rezonans görüntüleme fizik temelleri ve sistem bileşenleri. BSJ Eng Sci 2019; 2 (2): 57-65.

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Çeşitli Hayvan Türlerine Ait Çiğ Sütlerde *Staphylococcus aureus* ve Stafilokokal Enterotoksinlerin Varlığının Araştırılması

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Atıf yapmak için: Güngör C, Gündoğ DA, Özkaya Y, Onmaz NE. Çeşitli hayvan türlerine ait çiğ sütlerde *Staphylococcus aureus* ve stafilokokal enterotoksinlerin varlığının araştırılması. Erciyes Univ Vet Fak Derg 2024; 21(2):86-91

Öz: *Staphylococcus aureus* ve Stafilokokal Enterotoksinler (SE), hastane ve toplum kaynaklı hastalıklarla ilişkili ciddi bir halk sağlığı sorunudur. Özellikle süt hayvanlarından çiğ süte geçen *S. aureus*, insanlarda gıda zehirlenmelerine yol açmaktadır. Bu çalışmada, Kayseri bölgesinde satışa sunulan 500 çiğ süt örneğindeki (inek sütü=200; koyun sütü=200; manda sütü= 100) *S. aureus* ve SE'lerin prevalans ve çeşitliliği PCR ve ELISA yöntemleri ile araştırıldı. Analiz edilen süt örneklerinin 380'inden (%76) koagülaz pozitif *S. aureus* (KPS) izole edildi. PCR testi ile KPS izolatlarının 136'sı (% 35.7) *S. aureus* olarak identifiye edildi. Bu izolatların 52'si koyun, 48'i inek ve 36'sı manda sütlerine ait idi. Belirlenen 136 izolatın m-PCR metodu ile incelenmesi sonucunda, 16 izolatın SE genlerinden en az birini içerdiği gözlendi. Bu genlerin 6'sının *sea,* 1'inin *seb*, 4'ünün *sec* ve 5'inin *sed* geni olduğu belirlendi. ELISA testi sonucu, çiğ sütlerdeki enterotoksin dağılımları ise; *SEA*: 2 inek, 1 koyun ve 1 manda; *SEC*: 2 manda ve 1 inek; *SED*: 1 inek ve 2 koyun şeklinde idi. Sonuç olarak bu çalışma, Kayseri bölgesinde satışa sunulan çiğ sütlerde *S. aureus* ve SE'lerin varlığını ortaya koyarak, sütte gıda güvenliği ve halk sağlığı için iyi üretim uygulamaları (Good manufacturing practices; GMP), personel hijyeni ve eğitimi, çiftlik ve ekipmanların sanitasyonu, meme hijyeni ve sağlığı ve tehlike analizleri ve kritik kontrol noktaları (Hazard Analysis and Critical Control Points; HACCP) uygulamaları ile kontaminasyon riskinin azaltılmasının önemini göstermektedir.
Anahtar kelimeler: Çiğ süt, halk sağlığı, *S. aureus,* stafilokokkal enterotoksinler

Investigation of the Presence of *Staphylococcus aureus* and Staphylococcal Enterotoxins in Raw Milk of Various Animal Species

Abstract: *Staphylococcus aureus* and Staphylococcal Enterotoxins (SE) are a serious public health issue associated with hospital- and community-acquired diseases. *S. aureus*, especially transmitted from dairy animals to raw milk, causes food poisoning in humans. In this study, the prevalence and disruption of *S. aureus* and SEs in 500 raw milk samples (cow milk= 200; sheep milk= 200; buffalo milk= 100), offered for sale in the Kayseri region, were investigated by PCR and ELISA methods. Coagulase positive *S. aureus* (CoPS) was isolated from 380 (76%) of the analysed milk samples. According to the PCR test, 136 (35.7%) of the CoPS isolates were identified as *S. aureus*, 52 of these isolates belonged to sheep, 48 to cow and 36 to buffalo milk. Among the 136 isolates, 16 of them found to contain at least one of the SE genes with the m-PCR method. It was determined that 6 of these genes were *sea*, 1 was *seb*, 4 was *sec* and 5 was *sed*. According to the ELISA test, enterotoxin distributions in raw milks are *SEA*: 2 cow's, 1 sheep and 1 buffalo; *SEC*: 2 buffalo's and 1 cow; *SED*: 1 cow and 2 sheep. As a result, this study revealed the presence of *S. aureus* and SEs in raw milk sold in the Kayseri region and shows the importance of reducing the risk of contamination through good manufacturing practices (GMP), personnel hygiene and training, sanitation of farms and equipment, udder hygiene and health and Hazard Analysis and Critical Control Points (HACCP) applications for food safety and public health in milk.
Key words: Public health, raw milk, *S. aureus*, staphylococcal enterotoxins

Giriş

Gıdalar, patojen zoonoz mikroorganizmaların taşınmasında bilinen en önemli kaynaklardandır. Bu patojenlerin yol açtığı gıda kaynaklı enfeksiyonlar, önemli bir halk sağlığı riski oluşturarak her yıl global ölçekte önemli ekonomik kayıplara sebep olmaktadır (Garcia ve ark., 2020). İnsan diyetinde önemli bir yeri olan süt, zengin besin içeriği ve nötral pH'sı sebebiyle faydalı ve patojen mikroorganizmaların üremesi ve taşınmasında önemli bir gıdadır (Sudhanthiramani ve ark., 2015). Bu bağlamda çiğ sütler, *Brucella* spp., *Campylobacter* spp., *Escherichia coli*, *Listeria monocytogenes*, *Mycobacterium* spp. ve *Salmonella* spp., gibi patojenleri ve bakteriyel toksinleri taşıyarak gıda kaynaklı hastalıklara sebep olmaktadır (Dhanashekar ve ark., 2012). Bu patojenlerin arasında, sağlıklı insan ve hayvanların mukoza ve deri florasında bulunan gram-pozitif *Staphylococcus aureus,*

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salgıladıkları Stafilokokkal Enterotoksinler (SE) ile dünya genelinde gıda kaynaklı gastroenteritisin ana sebeplerinden biri olarak kabul edilmektedir (Oliveira ve ark., 2018). Stafilokokkal gıda zehirlenmelerinden sıklıkla insan kaynaklı *S. aureus* suşları sorumlu tutulsa da çiğ süt, çiğ süt peynirleri, çiğ veya pişmiş et gibi hayvansal orijinli suşlar da enterotoksijenik *S. aureus*'lar için önemli bir kaynak olarak kabul edilmektedir (Zhang ve ark., 2022). Gıdaların toplanma, işleme, taşınma, depolama, pişirme veya servis ve muhafaza aşamalarında *Staphylococcus* türleri ile kontamine olması veya toksin üretmelerini destekleyecek ortamların oluşması Stafilokokkal gıda zehirlenmelerine yol açmaktadır (Hennekinne ve ark., 2012). Bu toksinler arasında klasik SE'ler olarak kabul edilen *SEA*, *SEB*, *SEC*, *SED* ve SEE, et ve süt ürünleri gibi protein açısından zengin gıdalarda, uygun üreme koşullarında (10-46°C ve pH 5-9), enterotoksijenik *S. aureus* suşlarının yüksek yoğunluklarda çoğalmasından sonra üretilir (Sankomkai ve ark., 2020). Bunlar arasında, gıda zehirlenmeleri olgularında en çok *SEA* enterotoksini görülmekle birlikte bunu sırasıyla *SED* ve *SEB* izlemektedir (Liu ve ark., 2022). SE proteinleri, ısı, dondurma, kurutma ve düşük pH gibi kriterlere yüksek direnç göstermekte ve gastrointestinal sistemde bulunan proteaz etkili enzimlere karşı kolayca hidrolize olmamaları onları gıda güvenliği ve halk sağlığı açısından önemli bir risk haline getirmektedir (Hennekinne ve ark., 2012; Liu ve ark., 2022). SE'lerin tüketiminden sonra, gastrointestinal konjesyon ve ödem, elektrolit metabolizma bozuklukları, ishal, abdominal ağrı, vagus sinirinin uyarımıyla kusma gibi semptomlar gözlenirken, daha şiddetli vakalarda tüm vücut doku ve organlarında purulent enfeksiyon, pnömoni, sepsis ve toksik şok sendromu şekillenebilmektedir (Hennekinne ve ark., 2012). SE'lerin minimum enfektif dozu 1 µg'dır ve gıdalardaki *S. aureus* sayısı 10^5 cfu/ml veya g'nin üzerinde olduğunda görünür hale gelmektedir. Ancak 100-200 ng SE'nin alınması duyarlı hastalarda Stafilokokal gıda zehirlenmesine neden olabilmektedir (Ahmed ve ark., 2019). Gıdalardaki SE varlığının tespitinde, immunoassay, immunodifüzyon, radyoimmun, lateks aglütinasyon ve çift jel difüzyon yöntemleri kullanılmaktadır (Féraudet Tarisse ve ark., 2021). Enterotoksin genlerinin varlığı ise Polimeraz Zincir Reaksiyonu (Polimerase Chain Reaction, PCR) yöntemi veya İlmiğe Dayalı İzotermal Amplifikasyon (Loop Mediated Isothermal Amplification, LAMP) yöntemleri ile tespit edilmektedir (Goto ve ark., 2007; Yin ve ark., 2016). Bu çalışmada, Kayseri bölgesindeki farklı hayvan türlerine ait çiğ sütlerde enterotoksijenik *S. aureus* varlığının araştırılması, çiğ sütlerin klasik SE ile kontaminasyon durumlarının belirlenmesi ve elde edilen izolatlarda SE genlerin tespit edilmesi ve dolayısıyla çiğ sütün halk sağlığı açısından güvenilirliğinin değerlendirilmesi amaçlandı.

Gereç ve Yöntem

Süt örnekleri

Çalışma kapsamında, Mart 2015- Ağustos 2016 tarihleri arasında Kayseri ilinde satışa sunulan farklı hayvan türlerine ait toplam 500 adet çiğ süt (200 koyun sütü, 100 manda sütü ve 200 inek sütü) toplandı. Süt örnekleri steril poşetler içerisine her biri 500 mL olacak şekilde aseptik şartlarda alınarak soğuk zincirde laboratuvara getirildi ve 1-2 saat içinde *S. aureus* varlığı açısından analiz edildi.

Süt örneklerinde S. aureus izolasyonu

Süt örneklerinden *S. aureus* izolasyonu daha önce ISO 6888-1 standart prosedüründe (ISO, 1999) tanımlandığı gibi gerçekleştirildi. Kısaca her bir süt örneğinin 25 mL'si 225 mL steril tamponlanmış peptonlu su ile (Oxoid CM0509) ile homojenize edildikten sonra on kat seri (10^{-1}-10^{-4}) dilüsyonları hazırlandı. Her bir dilüsyon %5 yumurta sarısı ve tellürit (Merck, Almanya) içeren Braid-Parker Agar Besiyeri (BPM; Oxoid, İngiltere) üzerine yayma plak yöntemi ile ekilerek 37°C'de 24 saat inkübe edildi. İnkübasyon süresi sonrasında, gri ve siyah renkli berrak bölgeyle çevrelenmiş karakteristik koloniler stafilokok şüpheli olarak değerlendirildi. BPM'de üreyen şüpheli kolonilerden beşi seçilerek kanlı agara (Merck,Almanya) pasajlandı ve 37°C'de 24 saat inkübe edildi. Kanlı agarda büyüyen koloniler Gram boyama, koagülaz, katalaz ve oksidaz testlerine tabii tutuldu. Gram pozitif, katalaz pozitif, oksidazı negatif ve koagulaz pozitif olan koloniler PCR ile test edildi.

DNA ekstraksiyonu

Fenotipik testler ile tespit edilen koagulaz pozitif Stafilokok izolatlarının (KPS) genomik DNA'sı (gDNA), izolatların Brain Heart Infusion Broth'ta (Merck, Almanya) 37°C'de18 saat boyunca inkübasyonu sonucu elde edilen taze kültürlerinden InstaGene™ Matrix kiti (BIO-RAD, ABD) kullanılarak üretici firma talimatlarına göre ekstrakte edildi.

İzolatlarda nuc geninin belirlenmesi

Çalışma kapsamında süt örneklerinden elde edilen KPS pozitif izolatlarının DNA'sı, *S. aureus* 'un ısıya dirençli nükleaz genini kodlayan *nuc*(A) geninin belirlenmesi amacı ile Tablo 1'de belirtilen spesifik primerler kullanılarak PCR analizine tabii tutuldu. PCR reaksiyon karışımı toplam hacmi 50 µL olacak şekilde; 1 x PCR tamponu (Thermo, ABD), 200 µM dNTP karışımı (Thermo, ABD), 1.5 mM $MgCl_2$ (Thermo, ABD), 2 U Taq polimeraz, 30 pmol her bir primer çifti (NUC-F166 ve NUC-R565) ve 5 µL şablon DNA'dan oluştu. PCR koşulları: 94°C 5 dk İlk denatürasyonu takiben, 30 siklustan oluşan 94°C'de 1 dk denatürasyon, 56° C'de 1 dk primer bağlanması, 68°C'de 1 dk uzama ve daha sonra tek siklustan oluşan 72°C'de 7dk son

uzama aşamalarında gerçekleştirildi (Cremonesi ve ark., 2005).

S. aureus pozitif izolatlarda klasik S. aureus enterotoksin genlerinin belirlenmesi

İzolatlarda klasik SE genlerinin (*sea, seb, sec, sed ve see*) varlığı Ertaş ve ark. (2010) tarafından kullanılan metoda göre multipleks PCR (m-PCR) ile belirlendi. Kısaca, PCR reaksiyon karışımı, 2.5 uL şablon DNA, 1 x PCR tamponu, 1 U Taq polimeraz (Vivantis), 0.2 uM dNTP Karışımları (Vivantis), 4 mM $MgCl_2$ (Vivantis) ve Tablo 1'de belirtilen analiz edilen toksinler için spesifik primerlerin (SA-U, SA-A, SA-B, SA-C/ ENT-C, SA-D) her birinden 25 pmol olacak şekilde total 25 µL hacimde hazırlandı. PCR amplifikasyonu, 5 dakika boyunca 94°C'lik bir başlangıç denatürasyonunun ardından, 94 °C'de 30 sn, 50°C 'de 30 sn ve 72°C'de 30 sn'den oluşan 35 siklus ve 72 °C'de 2 dk. tek siklustan oluşan son uzatma aşamalarından oluştu.

Tablo 1. Çalışmada kullanılan primer dizilimleri ve baz büyüklükleri

Hedef gen	Primer adı	Primer dizilimi (5'-3')	Baz büyüklüğü (bp)
nuc	nuc-F	AGTTCAGCAAATGCATCACA	400
	nuc-R	TAGCCAAGCCTTGACGAACT	
-	SA-U	TGTATGTATGGAGGTGTAAC	-
sea	SA-A	ATTAACCGAAGGTTCTGT	270
seb	SA-B	ATAGTGACGAGTTAGGTA	165
sec	ENT-C	AATTGTGTTTCTTTTATTTTCATAA	102
sed	SA-D	TTCGGGAAAATCACCCTTAA	306
see	SA-E	GCCAAAGCTGTCTGAG	213

Süt örneklerinde klasik S. aureus ve SE'lerin varlığının ELISA testi ile belirlenmesi

Çalışma kapsamında toplanan süt örneklerinde *S. aureus* enterotoksinlerinin (SET A, B, C, D, E) varlığı ticari bir kit (Ridascreen® SET A, B, C, D, E, r-biopharm, Almanya) kullanılarak üretici firma talimatlarına göre Enzim- Linked Immunosorbent Assay (ELISA) metodu ile test edildi. Kısaca, çiğ süt örneklerı soğutmalı santrifüjde 3500 g'de 10°C'de, 10 dk süreyle santrifüj edildikten sonra üstteki krema tabakası uzaklaştırıldı. Daha sonra süt örnekleri distile su ile 1:20 oranında sulandırıldıktan sonra filtre edildi. Elde edilen filtratın 100 µL'si ELISA testinde kullanıldı. ELISA testi için, kit içeresinde bulunan mikroplakadaki A'dan G'ye kadar olan kuyucuklara 100'er µL çiğ süt örneklerinden elde edilen filtrat, H kuyucuğuna ise 100 µL pozitif kontrol ilave edildi. Daha sonra mikroplakalar 37°C'de bir saat inkübe edildikten sonra otomatik yıkayıcıda yıkama solüsyonu kullanılarak 6 kez yıkandı. Sonrasında her bir kuyucuğa 100'er µL konjugat 1 ilave edilerek 37 °C'de 1 saat inkübasyonu takiben kuyucuklar 6 kez yıkandı. Ardından kuyucuklara 100'er µL konjugat 2 eklendi ve 30 dk bekletildikten sonra tekrar 6 defa yıkama işlemi yapıldı. Bu işlemden sonra kuyucuklara 50'şer µL substrat/ kromojen ilave edildi ve nazik bir şekilde karıştırılarak 37°C'de karanlık ortamda 15 dk inkübe edildikten sonra her bir kuyucuğa 100 µL stop solüsyonu ilave edilerek ELISA otomatik okuyucuda (Thermo Scientific, Multiskan Spectrum, ABD) 450 nm dalga boyunda örneklerin absorbansları okutuldu. ELISA testi sonuçları Rida® Soft Win programı kullanılarak değerlendirildi.

Bulgular

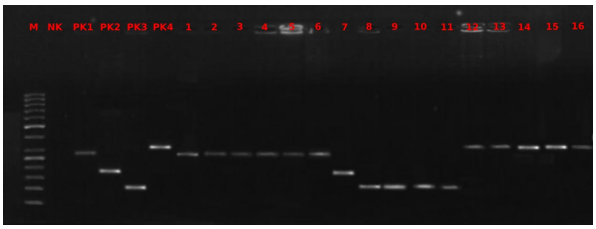
Analiz edilen süt örneklerinin 380 (%76)'i KPS pozitif olarak belirlendi, KPS izolatlarının130 (%34.2)'u inek sütünden, 160 (%42.1)'ı koyun sütünden ve 90 (% 23.6)'ı manda sütünden izole edildi. Süt örneklerindeki KPS sayıları $1x10^2$-$6.2x10^8$ kob/mL arasında idi. İzole edilen KPS izolatlarının örneklere göre dağılımı ve sayıları Tablo 2'de belirtilmiştir.

Tablo 2. Çalışmada izole edilen Koagulaz Pozitif Stafilokokların örneklere göre dağılımı

Analiz edilen süt örnekleri	Analiz edilen örnek sayısı	İzole edilen KPS sayısı (%)	Örneklerdeki KPS sayısı (kob/mL)		
			$\leq 10^2$	$1x10^2$-$1x10^4$	$\geq 10^5$
İnek sütü	200	130 (65)	40 (30.8)	60 (46.2)	30 (23.1)
Koyun sütü	200	160 (80)	75 (46.9)	65 (40.6)	20 (12.5)
Manda Sütü	100	90 (90)	35 (38.9)	38 (42.2)	17 (18.9)
Toplam	500	380 (76)	150 (38.5)	163 (42.9)	67 (17.6)

Elde edilen KPS izolatlarının 136'sında (%35.7) *nuc* geni tespit edildi ve *S. aureus* olarak identifiye edildi. Bu izolatların 52 (%38.2)'si koyun sütünden, 48 (% 35.2)'i inek sütünden ve 36 (%26.4)'sı manda sütüne aitti. Örnek bazında değerlendirildiğinde; koyun inek ve manda sütlerinin sırasıyla %26, %24 ve %36'sının *S. aureus* ile kontamine olduğu saptandı.

S. aureus pozitif izolatların 10 (%7.3)'unda enterotoksin (SE) sentezleme yeteneği belirlendi. Bu SE'lerin 4 (%2.9)'ü *SEA*, 3 (%2.2)'ü *SEC* ve 3 (%2.2)'ü *SED* idi. Tip A enterotoksin 2 inek (%4.1), 1 (%1.9) koyun ve 1 (%2.7) manda sütünde, *SEC*; 2 (%5.5) manda ve 1 (%2) inek sütünde, *SED* ise 1 (%2) inek sütü, 2 (% 3.8) koyun sütünde belirlendi. mPCR sonuçları ise analiz edilen 136 *S. aureus* izolatının 16 (% 11.7)'sının SE geninden en az birini taşıdığını gösterdi. Bu izolatların, 6 (%37.5)'si *sea*, 1 (%6.25)'i *seb*, 4 (%25)'ü *sec* ve 5 (%31.25)'i *sed* geni içeriyordu (Şekil 1).



Şekil 1. M: Merdiven (50-1000 bp), **NK**: Negatif Kontrol (Distile Su), **PK1:** Pozitif kontrol (Enterotoksin A: ATCC 29231), **PK2:** Pozitif Kontrol (Enterotoksin B: NCTC 10654), **PK3:** Pozitif Kontrol (Enterotoksin C: NCTC 10655), **PK4:** Pozitif kontrol (Enterotoksin D: NCTC 10652), **1-6:** *S. aureus* enterotoksin A (270 bp), **7:** *S. aureus* enterotoksin B (165 bp), **8-11:** *S. aureus* enterotoksin C (102 bp), **12-16:** *S. aureus* enterotoksin D (306 bp).

Analiz edilen inek, koyun ve manda sütüne ait izolatların sırasıyla 3 (%6.2)'ü, 2 (%3.8)'si ve 1 (%2.7)'i *sea* geni taşıyordu. *seb* geni 1 (%2) inek izolatlarında, *sec* geni; 2 (%5.5) manda, 1(%2) inek ve bir (% 1.9) koyun sütünde, *sed* geni ise 2 (%4) inek sütü, 2 (%3.8) koyun sütü ve 1 (%2.7) manda sütünde saptandı (Tablo 3).

Tartışma ve Sonuç

Süt insan ve yeni doğan hayvanlar için önemli bir besin kaynağı olmakla birlikte aynı zamanda mikroorganizmaların büyümesi ve çoğalması içinde uygun bir ortamdır (Sudhanthiramani ve ark., 2015). Uygun koşullarda üretilmeyen ve pastörize edilmeyen sütlerin tüketilmesi sonucu oluşabilecek sağlık problemlerine rağmen, doğal işlenmemiş gıdalara artan yönelim, çiğ süte olan talebi de arttırmıştır (Yıbar ve Küçük, 2019). Süt ve süt ürünleri üzerine yapılan çalışmalarda, Stafilokokların çiğ sütte bulunan yaygın fırsatçı patojenler olduğu ve ürettikleri enterotoksinler nedeni ile önemli gıda zehirlenmelerine neden olabileceği bildirilmiştir (Yildirim ve ark., 2019; Kou ve ark. 2021). Bu çalışmada, Kayseri ilinde satışa sunulan farklı hayvan türlerine ait 500 çiğ süt örneğinden 380'ninin KPS olduğu ve bunlardan %60,5'inin kontaminasyon düzeyinin $1x10^2$ ila $\geq 10^5$ kob/mL arasında olduğu belirlendi. Bu sonuçlar Polonya, Portekiz, İtalya, Yeni Zelanda, Norveç, Hindistan ve Çin gibi birçok ülkede yapılan çalışmalarda belirlenen prevalans (yaklaşık %40-70) ile uyumlu idi (Bianchi ve ark., 2014; Sudhanthiramani ve ark., 2015; 2016; Liu ve ark., 2017; Ahmed ve ark., 2019; Kou ve ark., 2021; Oliveira ve ark., 2022). Stafilokokların doğada yaygın olarak bulunması ve mastitisin ana nedenlerinden biri olması, çiğ sütteki bu yüksek prevalansa neden olabilir (Kaya ve ark., 2015; Ahmed ve ark., 2019). Hayvanlarda meme sağlığı, sağım esnasındaki hijyen, düşük depolama sıcaklığı gibi kontrol önlemleri ile enterotoksijenik *S. aureus*'ların üremesi engellenebilir (Kaya ve ark., 2015; Ahmed ve ark., 2019; Oliveira ve ark., 2022).

Bu çalışmada tespit edilen KPS izolatlarının %35.7'si *S. aureus* olarak identifiye edildi. Çalışma kapsamında incelenen izolatlar da en yüksek *S. aureus* prevalansı %38.2 ile koyun sütünde bulunurken bunu %35.2 ve %26.4 ile sırasıyla inek ve manda sütü takip etti. Benzer şekilde Sudhanthiramani ve ark. (2015) tarafından Hindistan'da ve Keyvan ve ark. (2020) tarafından Türkiye'de, çiğ sütte yapılan çalış-

Tablo 3. Stafilokokal enterotoksinlerin ve enterotoksin genlerinin analiz edilen izolatlara göre dağılımı

Örnekler	*S. aureus* pozitif örnek sayısı (%)	SE belirlenen Örnek sayısı (%)				SE genlerinin Dağılımı (%)			
		SEA	***SEB***	***SEC***	***SED***	***sea***	***seb***	***sec***	***sed***
İnek sütü (n=200)	48 (24)	2 (4.1)	ND	1 (2)	1 (2)	3 (6.2)	1 (2)	1 (2)	2 (4)
Koyun sütü (n=200)	52 (26)	1 (1.9)	ND	ND	2 (3.8)	2 (3.8)	-	1 (1.9)	2 (3.8)
Manda Sütü (n=100)	36 (36)	1 (2.7)	ND	2 (5.5)	ND	1 (2.7)	-	2 (5.5)	1 (2.7)
Toplam (n=500)	136 (27.2)	4 (2.9)	ND	3 (2.2)	3 (2.2)	6 (16.6)	1 (0.7)	4 (2.9)	5 (3.6)

ND: Belirlenemedi

malarda *S. aureus* varlığı sırasıyla %39 ve %38.3 olarak bildirilmiştir. Bu çalışma sonuçlarından farklı olarak, %12.5'lik bir prevalansı ile Zeinhom ve Abed (2020) %14'lük bir prevalans ile Ertas ve Gönülalan (2010) ve yine %14'lük bir prevalans ile Omwenga ve ark. (2019) süt örneklerinde nispeten daha düşük *S. aureus* prevalansı, Bianchi ve ark. (2014) ve Kou ve ark. (2021) ise daha yüksek *S. aureus* prevalansı (sırasıyla %40 ve %43) bildirmişlerdir.

Zhang ve ark. (2022) tarafından gerçekleştirilen küresel bir meta-analiz çalışmasına göre, *S. aureus*'un prevalansı dünya genelinde inek, manda ve koyun sütünde sırasıyla %35, %33.4 ve %18.5 olarak rapor edilmiştir. Bu bulgular, çalışmamızdaki prevalans sonuçlarıyla uyumlu idi.

Ülkemizde çiğ sütlerde ilgili etkene ilişkin yasal bir sınır olmamasına rağmen (TGK, 2000), *S. aureus*'un gıdalarda $>10^5$ kob/mL kontaminasyon düzeyine ulaştığında enterotoksin üretim olasılığını arttırarak gıda zehirlenmelerine neden olabileceği bildirilmiştir (Rahimi ve ark., 2012; Ahmed ve ark., 2019). Çalışma kapsamında incelenen *S. aureus* izolatlarının % 7.3'ü klasik SE sentezleme yeteneğine sahipti. Bu izolatların 4'ü *SEA*, 3'ü *SEC* ve 3'ü *SED* sentezliyordu. Bu sonuçlar Keyvan ve ark. (2020) tarafından yapılan bir çalışma sonuçlarına göre yüksek iken, daha önce farklı ülkelerde yapılan çalışmalarda rapor edilen enterotoksin prevalansından (Asiimwe ve ark., 2017; Ahmed ve ark., 2019) oldukça düşük idi. Bu çalışmalar ile uyumlu olarak en yaygın tespit edilen enterotoksin *SEA* idi. Stafilokokal gıda zehirlenmeleri vakalarının %95'inden klasik enterotoksinler sorumludur. Özellikle *SEA* ve *SED*, zehirlenmelerden sorumlu baskın enterotoksinlerdir (Ahmed ve ark., 2019). Enterotoksijenik *S. aureus* prevalansındaki farklılıkların nedenleri daha önce bildirildiği gibi farklı gıdaların ve suşların, farklı enterotoksinler taşıması ve coğrafi koşullar olabilir (Morandi ve ark., 2007; Asiimwe ve ark., 2017). Ayrıca bu çalışmada belirlenen klasik enterotoksin prevalansı ile uyumlu olarak, *S. aureus* izolatlarının %11.7'sinin bu toksinlerin üretiminden sorumlu genleri içeriyordu. Bu genlerin dağılımı sırası ile 6'sı *sea*, 5'i *sed*, 4'ü *sec* ve 1'i *seb* şeklinde idi. Yapılan literatür taramasına göre enterotoksin genlerinin yaygınlığı, Morandi ve ark. (2007), Omwenga ve ark. (2019) ve Oliveira ve ark. (2022) tarafından bildirilen sonuçlar (sırasıyla %67, %74.1 ve %46.8) ile çalışmamız sonuçlarına göre oldukça yüksek iken, Keyvan ve ark. (2020)'nın sonuçları (%16) çalışmamıza benzer idi. Daha önce Normanno ve ark.'nın (2005) bildirdiği gibi çalışmamızda da *S. aureus*'un enterotoksijenik suşlarında en sık gözlenen enterotoksin geninin klasik *sea* geni olduğu görülmüştür. Benzer şekilde Morandi ve ark. (2007) yaptıkları çalışmada, *sea* ve *sed* genlerinin diğer genlere oranla daha yaygın olduğunu rapor etmişlerdir. Bu çalışmada klasik SE'leri kodlayan genlerin bir kısmının tespit edilmesi, bakterinin uygun sıcaklık ve koşullarda bu enterotoksinleri çiğ sütte üreterek gıda zehirlenmelerine neden olabileceğini düşündürmektedir (Omwenga ve ark., 2019).

Sonuç olarak; çiğ sütlerde enterotoksijenik *S. aureus*'un varlığı, tüketicinin çiğ süte olan ilginin artması ve bu sütlerden üretilen ürünlerin tüketilmesi ile halk sağlığı açısından ciddi bir tehdit oluşturabilir. Ayrıca ülkemizde çiğ sütlerde *S. aureus* ve enterotoksinleri ile ilgili yasal bir izleme programının olmayışı bu patojenin besin zinciri yoluyla yayılma riskini arttırmaktadır. Bu nedenle, Stafilokokal gıda zehirlenmeleri ve ekonomik kayıpların önüne geçilebilmesi amacıyla, çiğ sütün toplama, taşıma, depolama ve satış aşamalarında denetimlerinin yapılması için yasal düzenlemelerin geliştirilmesi, uygulanması gerekmektedir. Ayrıca süt işletmelerinde iyi hijyen uygulamalarının takip edilmesi ve personele sağım ve kişisel hijyen konusunda kapsamlı eğitimlerin planlanarak periyodik olarak verilmesi bu patojenin besin zincirinde çoğalarak yayılmasını sınırlandırabilir.

Kaynaklar

Ahmed AAH, Maharik NMS, Valero A, Kamal SM. Incidence of enterotoxigenic *Staphylococcus aureus* in milk and Egyptian artisanal dairy products. Food Control 2019; 104: 20-7.

Asiimwe BB, Baldan R, Trovato A. Prevalence and molecular characteristics of *Staphylococcus aureus*, including methicillin resistant strains, isolated from bulk can milk and raw milk products in pastoral communities of South-West Uganda. BMC Infect Dis 2017; 17: 422.

Bianchi DM, Gallina S, Bellio A, Chiesa F, Civera T, Decastelli L. Enterotoxin gene profiles of *Staphylococcus aureus* isolated from milk and dairy products in Italy. Lett Appl Microbiol 2014; 58: 190-6.

Cremonesi P, Luzzana M, Brasca M, Morandi S, Lodi R, Vimercati C, Castiglioni B. Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from milk and dairy products. Mol Cell Probes 2005; 19(5), 299-305.

Dhanashekar R, Akkinepalli S, Nellutla A. Milk-borne infections. An analysis of their potential effect on the milk industry. Germs 2012; 2(3): 101.

Ertaş N, Gönülalan Z. Kayseri ilinde satılan çiğ sütlerde *Staphylococcus aureus* ve enterotoksinlerinin varlığı üzerine araştırmalar. Fırat Univ Sağlık Bilim Vet Derg 2010; 24(1): 11-5.

Féraudet Tarisse C, Goulard-Huet C, Nia Y, Devilliers K, Marcé D, Dambrune C, Simon S. Highly sensitive and specific detection of staphylococcal enterotoxins SEA, SEG, SEH, and SEI by immunoassay.

Toxins 2021; 13(2): 130.

Garcia SN, Osburn BI, Jay-Russell MT. One health for food safety, food security, and sustainable food production. Front Sustain Food Syst 2020; 4: 1.

Goto M, Hayashidani H, Takatori K, Hara-Kudo Y. Rapid detection of enterotoxigenic *Staphylococcus aureus* harbouring genes for four classical enterotoxins, SEA, SEB, SEC and SED, by loop-mediated isothermal amplification assay. Lett Appl Microbiol 2007; 45(1): 100-7.

Hennekinne JA, De Buyser ML, Dragacci S. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiol Rev 2012; 36(4): 815-36.

Kaya H, Ertaş Onmaz N, Gönülalan Z, Al S. Kayseri ilinde tüketime sunulan tavuk etlerinde *Staphylococcus aureus* ve enterotoksin varlığının araştırılması. Erciyes Üniv Vet Fak Derg 2015; 12(2): 93-8.

Keyvan E, Yurdakul O, Şen E. Staphylococcal enterotoxins and enterotoxigenic *Staphylococcus aureus* in raw milk: A screening study. Kocatepe Vet J 2020; 13(2): 104-9.

Kou X, Cai H, Huang S, Ni Y, Luo B, Qian H. Prevalence and characteristics of *Staphylococcus aureus* ısolated from retail raw milk in Northern Xinjiang, China. Front Microbiol 2021; 12: 2187.

Liu H, Li S, Meng L, Dong L, Zhao S, Lan X. Prevalence, antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from dairy herds in northern China. J Dairy Sci 2017; 100: 8796-803.

Liu C, Shen Y, Yang M, Chi K, Guo N. Hazard of Staphylococcal enterotoxins in food and promising strategies for natural products against virulence. J Agric Food Chem 2022; 70(8): 2450-65.

Morandi S, Brasca M, Lodi R, Cremonesi P, Castiglioni B. Detection of classical enterotoxins and identification of enterotoxin genes in *Staphylococcus aureus* from milk and dairy products. Vet Microbiol 2007; 124(1-2): 66-72.

Normanno G, Firinu A, Virgilio S, Mula G, Dambrosio A, Poggiu A, Celano GV. Coagulase-positive Staphylococci and *Staphylococcus aureus* in food products marketed in Italy. Int J Food Microbiol 2005; 98(1): 73-9.

Oliveira D, Borges A, Simões M. *Staphylococcus aureus* toxins and their molecular activity in infectious diseases. Toxins 2018; 10(6): 252.

Oliveira R, Pinho E, Almeida G, Azevedo NF, Almeida C. Prevalence and diversity of *Staphylococcus aureus* and staphylococcal enterotoxins in raw milk from Northern Portugal. Front Microbiol 2022; 13: 703.

Omwenga I, Aboge GO, Mitema ES, Obiero G, Ngaywa C, Ngwili N, Bett B. *Staphylococcus aureus* enterotoxin genes detected in milk from various livestock species in northern pastoral region of Kenya. Food Control 2019; 103: 126-32.

Rahimi E, Momtaz H, Shakerian A, Kavyani HR. The detection of classical enterotoxins of *Staphylococcus aureus* in raw cow milk using the ELISA method. Turkish J Vet Anim Sci 2012; 36(3): 319-22.

Sankomkai W, Boonyanugomol W, Kraisriwattana K, Nutchanon J, Boonsam K, Kaewbutra S, Wongboot W. Characterisation of classical enterotoxins, virulence activity, and antibiotic susceptibility of *Staphylococcus aureus* isolated from Thai fermented pork sausages, clinical samples, and healthy carriers in northeastern Thailand. J Vet Res 2020; 64(2): 289.

Sudhanthiramani S, Swetha CS, Bharathy S. Prevalence of antibiotic resistant *Staphylococcus aureus* from raw milk samples collected from the local vendors in the region of Tirupathi, India. Vet World 2015; 8(4): 478-81.

TGK (Türk Gıda Kodeksi) 2000. Mikrobiyolojik Kriterler Tebliği. Tarım ve Köy İşleri Bakanlığı, 23960, Tebliğ No: 2001-19, Ankara, Türkiye.

Yıbar A, Küçük SC. Çiğ süt ve pastörize süt tüketiminin halk sağlığı üzerine etkileri. Food and Health 2019; 5(3): 197-204.

Yildirim T, Sadati F, Kocaman B, Siriken B. *Staphylococcus aureus* and Staphylococcal enterotoxin detection in raw milk and cheese origin coagulase positive isolates. IJSL 2019; 1(1): 30-41.

Yin HY, Fang TJ, Wen HW. Combined multiplex loop-mediated isothermal amplification with lateral flow assay to detect sea and seb genes of enterotoxic *Staphylococcus aureus*. Lett Appl Microbiol 2016; 63(1): 16-24.

Zeinhom M, Abed A. Prevalence, characterization, and control of *Staphylococcus aureus* isolated from raw milk and Egyptian soft cheese. J Vet Med Res 2020; 27(2): 152-60.

Zhang J, Wang J, Jin J, Li X, Zhang H, Shi X, Zhao C. Prevalence, antibiotic resistance, and enterotoxin genes of *Staphylococcus aureus* isolated from milk and dairy products worldwide: A systematic review and meta-analysis. Food Res Int 2022; 162: 111969.

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Determination of the Ideas and Expectations of the Students Taking the Laboratory Animal Breeding Course towards their Clinical Skills Acquisition and their Achievements and Anxiety Levels at the end of the Application

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Abstract: This study aimed to determine the thoughts and expectations of the students who took laboratory animal breeding courses about clinical skill acquisition and their achievements and anxiety levels at the end of the application with a survey study. One hundred thirtyeight students participated in the survey. The questionnaire method was applied to the propositions and questions created using the State-Trait Anxiety Scale and the literature. A statistically significant difference (P<0.05) was found between the students' total scores of opinions and expectations towards the practice before and after the practice course. The total score of opinion and expectation towards the application after the application lesson was found to be higher (P<0.01) than the total score of opinion and expectation towards the application before the application lesson. State anxiety (P<0.05) and trait anxiety total score (P<0.01) were lower after the application lesson than before the application lesson. It was concluded that the applied education made a difference in the students' clinical skill acquisition and positively affected their mood.
Keywords: Clinic, emotion, laboratory animal, practice, student

Laboratuvar hayvanı yetiştiriciliği dersi alan öğrencilerin klinik beceri kazanımlarına yönelik düşünce ve beklentileri ile uygulama sonunda kazanımları ve kaygı düzeylerinin belirlenmesi

Öz: Bu çalışmada, laboratuvar hayvanı yetiştiriciliği dersi alan öğrencilerin klinik beceri kazanımına yönelik düşünce ve beklentileri ile uygulama sonundaki başarıları ve kaygı düzeylerinin anket çalışması ile belirlenmesi amaçlanmıştır. Ankete yüz otuzsekiz öğrenci katılmıştır. Durumluk-Sürekli Kaygı Ölçeği ve literatür kullanılarak oluşturulan önerme ve sorulara anket yöntemi uygulanmıştır. Öğrencilerin uygulama dersi öncesi ve sonrasında uygulamaya ilişkin görüş ve beklentileri toplam puanları arasında istatistiksel olarak anlamlı bir fark (P<0.05) bulunmuştur. Uygulama dersi sonrası uygulamaya yönelik görüş ve beklenti toplam puanı, uygulama dersi öncesi uygulamaya yönelik görüş ve beklenti toplam puanından yüksek bulunmuştur (P<0.01). Uygulama dersi sonrasında durumluk kaygı (P<0.05) ve sürekli kaygı toplam puanı (P<0.01) öncesine göre daha düşük bulunmuştur. Uygulanan eğitimin öğrencilerin klinik beceri kazanımlarında fark yarattığı ve duygu durumlarını olumlu yönde etkilediği sonucuna varılmıştır.
Anahtar kelimeler: Duygu, klinik, laboratuvar hayvanı, öğrenci, uygulama

Introduction

Clinical practice and theoretical knowledge constitute an essential part of veterinary medicine education and are inseparable. Veterinarian candidates are offered the opportunity to reinforce the theoretical knowledge they have acquired through the courses in the education programs with clinical skills (Özen and Özen, 2006). Clinical skill is the name given to all of the procedures and interventions that a clinician veterinarian will do in practice to her patients throughout her/his professional life. Usage areas of laboratory animals are; basic science research, veterinary and human medicine research, production and breeding, reliability and validity testing, and education. The purpose of use in the field of education is to develop basic and unique clinical skills for those who work or will work in areas where clinical skills are needed, as well as ensuring that researchers understand the physiology, anatomy, and manipulation of these animals and become competent in scientific studies with animal experiments. However, no study was found that followed and determined the development of clinical skills of veterinary medicine students studying. In studies (Çiftçili et al., 2006; Tosun et al., 2008; Sabancıoğulları et al., 2012) on the development of practice skills of medical and nursing profession candidates, it has been determined that the practice program contributes to the increase of professional com-

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petence of the students, making them feel safe and ready for the profession. Clinical skill practice can create anxiety in clinician veterinarian candidates for the procedures and interventions to be performed in practice. Anxiety is a feeling of worry about a non-objective problem. In studies conducted with university students studying in different fields in our country, it has been determined that the high level of anxiety in students is affected by many variables related to the field of education as well as individual and environmental factors (Bayar et al., 2009; Deveci et al., 2012). Anxiety about the chosen department and profession in biology and medicine students was associated with high anxiety levels (Çakmak ve Hevedanlı, 2005; Canbaz et al., 2007). Considering the anxiety level of the students before the clinical practice may be a guiding finding for the educator in terms of developing a sense of relaxation and confidence in the students during the practice. Lack of adequate training and self-control in this area can threaten animal health in clinical and surgical treatments and practices and affect the results of scientific research and animal welfare. In order to prevent this potential negatif effect, different applications are made, and training using live animals in these applications have been the subject of discussions for a long time (Hansen and Boss, 2002; Daly et al., 2014). In a study of animal welfare, Platto et al. (2022) reported that the attitudes towards animal welfare of students who had previously participated in a laboratory study using an animal were positively affected. Daly et al. (2014) reported that more than 90% of students in the field of health should have an education using live animals in the curriculum. Another advantage of using laboratory animals in student education is that different animal species' physiological and anatomical characteristics (Daly et al., 2014) provide study diversity. In this study, it was aimed to determine the anxiety levels of students who took a laboratory animal breeding course, their achievements in basic surgical techniques at the end of clinical practice, and the relationship between their feelings, thoughts, and expectations for clinical practice and their anxiety level.

Materials and Methods

This cross-sectional study was conducted at Erciyes University, Faculty of Veterinary Medicine. The participants of the study were second-year students taking the Laboratory Animal Breeding course at Erciyes University Faculty of Veterinary Medicine in the 2016-2017 and 2017-2018 academic years. The study aimed to reach the entire participant population (n=138) without making a sample selection. Before starting the research, permission was obtained from The Erciyes University Clinical Research Ethics Committee (Decision no: 2017/ 81; Date: 03.02.2017). The research data were obtained by the questionnaire applied and collected with the State-Trait Anxiety Scale and Form A: Student Introduction and Pre-Application Questionnaire Form, and Form B: Post-Application Questionnaire, which were created by the researchers by using the literature.

Clinical practice method

All the students participating in the study were shown the techniques of animal holding, blood collection, injection, drug administration, oral gavage, anesthesia, incision, suturing, abdominal dissection, and necropsy used in clinical practices. As animal material, 40 mice (BALB/c), 40 rats (Wistar albino), and 20 rabbits (New Zealand) were used.

Tools used

Form A: Student Introduction and Pre-Application Questionnaire Form. This questionnaire consists of 8 questions with more than one proposition/question content. The questionnaire includes questions to determine the students' sociodemographic characteristics, their views on the department they are studying, their feelings and thoughts about clinical practice, and their views and expectations about clinical practice. Before the practice lesson, the positive views and expectations of the students were determined with a question consisting of 8 positive propositions. Responses to each suggestion were evaluated using a five-point Likert method (1: totally agree- 5: strongly disagree). In the analysis, "strongly agree" and "agree" were grouped as "agree", "disagree" and "strongly disagree" as "disagree".

Form B: Post Implementation Questionnaire Form. This questionnaire consists of 3 questions with more than one proposition/question content. The questionnaire includes questions to determine the student's feelings during the clinical practice and their views on their achievements after it. After the practical lesson, the students' positive views and expectations about the application were determined with a question consisting of 8 positive propositions. Responses to each suggestion were evaluated using a five-point Likert (1: strongly agree - 5: strongly disagree) method.

State-Trait anxiety inventory: The state-trait anxiety scale developed by Spielberg et al. (1983) was used in the study. The scale was used to determine how people felt at that moment with some expressions they used to describe their feelings. The Turkish language validity and reliability study of the scale used in the study was carried out according to the statement of Öner and Le Compte (1983). This inventory includes two separate scales containing 40 items in total. The State Anxiety Inventory is concerned with identifying the emotions of the individuals participating in the research at a certain time and in specific conditions and responding according to these emotions. The Trait Anxiety Scale is intended to describe the feelings of individuals in the general process.

Both scales contain two types of statements with twenty items. A high scale score indicates a high anxiety level (Öner and Le Compte, 1983). The data were analyzed using the SPSS for Windows 22.0 (IBM 2013) statistical package program. Categorical variables are presented with number and percentage distributions. The mean and standard deviation of continuous variables were calculated. The conformity of the variables to the normal distribution was made with the Kolmogorov-Smirnov test, and it was determined that the data showed normal distribution. In statistical analysis, t test for independent groups, (Homogeneity of variances was evaluated with Levene test), and Pearson correlation analysis were used. The statistical significance level was accepted as $P<0.05$.

Results

The percentage values (%) of the opinions and thoughts of the students about the department they study are shown in Table 1.

The male students constitute 65.2% of the participants and the females correspond to 34.8%. The mean age was 20.17±2.18. Most of the students (96.4%) who participated in the research stated that they preferred the department for their goals and were satisfied with their studies. Most students (94.9%) stated that they consider it appropriate to do veterinary medicine, and 96.4% of them think of doing this profession after graduation (Table 1). The relationship between pre-clinical students' feelings and thoughts (Mean±SD) about clinical practice and their anxiety level is shown in Table 2.

About two-thirds of the students (68.1%) stated that they felt ready for preclinical practice, 83.3% stated that their theoretical knowledge was insufficient for clinical practice, and 94.2% were not afraid of contacting animals. When the relationship between the emotions and thoughts of the students about the clinical practice and their anxiety levels were examined before the clinical practice, it was determined that the state anxiety score was significantly higher only in the

Table 1. The percentage values (%) of the opinions and thoughts of the students about the department they study

Opinions	Yes n (%)	No n (%)
Did you choose your department willingly?	133 (96.4)	5 (3.6)
Are you satisfied with studying in your department?	133 (96.4)	5 (3.6)
Do you feel suitable for this department?	131 (94.9)	7 (5.1)
Have you received counseling/advice on your career choice?	62 (44.9)	76 (55.1)
Is there anyone in your family or close circle who is a veterinarian?	52 (37.7)	86 (62.3)
Do you intend to pursue this profession after graduation?	133 (96.4)	5 (3.6)

Table 2. The relationship between pre-clinical students' feelings and thoughts (Mean±SD) about clinical practice and their anxiety level

Feelings and thoughts			SATS	TATS
		n (%)	Mean±SD	Mean±SD
Do you feel ready for clinical practice?	**Yes**	94 (68.1)	38.04±8.56	54.12±3.72
	No	44 (31.9)	40.50±13.73	54.20±5.39
Do you think your theoretical knowledge is sufficient for clinical practice?	**Yes**	23 (16.7)	35.43±7.86	55.39±3.40
	No	115 (83.3)	39.50±10.85	53.90±4.44
Are you afraid of handling/contacting animals?	**Yes**	8 (5.8)	51.87±21.30*	52.37±1.92
	No	130 (94.2)	38.02±9.01	54.26±4.39
Is there an application that you are afraid of doing?	**Yes**	40 (29.0)	40.87±13.11	53.52±3.82
	No	98 (71.0)	37.98±9.18	54.40±4.48
Are you afraid of having a negative experience during the application?	**Yes**	69 (50.0)	40.02±11.59	54.34±4.66
	No	69 (50.0)	37.62±9.21	53.95±3.94

***P<0.05, T-test in independent groups. SATS: State anxiety total score, TATS: Trait anxiety total score**

Table 3. The change of the percentage values (%) in students' feelings about clinical practice before and after the practice

Feeling about clinical practice	Before n (%)	After n (%)
Fear	17 (12.3)	15 (10.9)
Exictement	98 (71.0)	80 (58.0)
Anxiety	51 (37.0)	40 (29.0)
Comfort	31 (22.5)	49 (35.5)
Reluctance	6 (4.3)	9 (6.5)
Mixed feelings	33 (23.9)	36 (26.1)

students who were afraid of dealing/contacting with animals (P<0.05, Table 2). The change of the percentage values (%) in students' feelings about clinical practice before and after the practice are shown in Table 3.

The percentage values (%) of student's views and expectations about the practice before the practice lesson are shown in Table 4. It was observed that the feelings of fear, excitement, and anxiety that existed before the application decreased, and the proportion of students with feelings of reluctance, comfort, and mixed feelings increased. When the positive opinions and expectations of the students about the practice were evaluated before the practice lesson (8 items), it was found that approximately three-quarters of the students had positive expectations; 81.2% of them agreed that this application would make it easier for them to adapt to clinical science courses, 89.2% of them agree that this application will provide clinical practice skills, and 85.5% of them agree that this application will create awareness about the use of personal protective equipment (Table 4).

The total score of the student's opinions and expectations towards the application before the practice lesson was higher than that of the students' opinions and expectations after the application lesson (P<0.01). It was observed that the students' state anxiety and trait anxiety total scores were lower after the practice course than before the practice course (respectively: P<0.05, P<0.01) (Table 5).

A low level of positive correlation was found between the student's total score of opinions and expectations about the practice before the practice lesson and the state anxiety level before the practice (r= 0.25, P<0.01). After the practice course, there was a low level of positive correlation between the total score of opinion and expectation towards the application and the level of state anxiety (r= 0.41, P<0.01), and a low level of negative correlation between the total score of trait anxiety (r= -0.23, P< 0.01) was found (Data not shown).

Table 4. The percentage values (%) of student's views and expectations about the practice before the practice lesson

Propositions	Agree (%)	Undecided (%)	Disagree (%)
This practice will facilitate my adaptation to clinical science courses	112 (81.1)	22 (15.9)	4 (2.8)
This practice will enable me to gain clinical practice skills	123 (89.2)	10 (7.2)	5 (3.6)
This practice will contribute to the development of my basic intervention skills	123 (89.2)	10 (7.2)	5 (3.6)
This practice will enable me to feel my responsibilities as a physician	117 (84.8)	14 (10.1)	7 (5.1)
I have a lot to learn from this practice	107 (77.6)	26 (18.8)	5 (3.6)
This application will create my awareness of the use of individual protective equipment.	118 (85.5)	17 (12.3)	3 (2.1)
This application will give me the habit of using individual protective equipment.	114 (82.6)	21 (15.2)	3 (2.1)
This practice will encourage me to learn more	117 (84.8)	17 (12.3)	4 (2.8)

Changes (Mean±SD) in the student's views and expectations about the practice before and after the practice lesson, and the state and trait anxiety levels are shown in Table 5.

Table 5. Changes (Mean±SD) in the student's views and expectations about the practice before and after the practice lesson, and the state and trait anxiety levels

Factors	Mean±SD	P*
Opinion and expectation score before the practice lesson	14.78±5.40	**<0.01**
Opinion and expectation score after the application lesson	15.05±4.99	
State anxiety total score before the practice lesson	38.82±10.50	**<0.05**
Post-practice state anxiety total score	37.03±8.67	
Trait anxiety total score before the practice lesson	54.15±4.31	**<0.01**
Trait anxiety total score after the practice lesson	40.76±8.04	

*** T test in dependent groups**

Discussion and Conclusion

Dewhurst et al. (1994) had conducted a study on learning intestinal epithelial absorption efficiency with 2nd-year students studying physiology. In the study, they compared the effects of using rat intestines on the learning levels of students with modeling in the computer environment. It has been emphasized that computer systems are more economical, protect animal welfare and create less stress for students. Dewhurst et al. (1994) and Costa et al. (2019) had reported that the e-learning platform could positively affect students' practical learning and effectively reduce their pre-study anxiety in a study comparing e-learning with face-to-face classroom applications in laboratory animal science education. However, although these alternative learning applications help close a crucial theoretical gap in a way that protects animal welfare, they will not be enough to meet all the requirements in the development of clinical dexterity, which is essential in both research and medicine. The results of our research reveal the advantages of practicing with animal models to consolidate the theoretical knowledge given in the lessons and to overlap with the dynamics of practice, to create the competence of the practitioners and the ability to work away from the risk of emotional approach. To confirm this determination, Guenther and Miller (2011) had reported in their research that students prefer the use of live animals in their education to virtual methods, and with this method, students can interact more. Elcoro and Trundle (2013) reported that students found the learning method using live animals more fun and exciting in an experiment they conducted using virtual and live rats. They reported that with this method, the concept of responsibility towards laboratory animals was better understood, the results obtained were more generalizable, and the level of fear before the application was reduced. Although there are answers that virtual applications can replace the use of live animals in the perception of animal behavior, 54.17% of the students participating in the research did not agree with the fact that studies with virtual mice would give generalizable results to human behavior and it was emphasized that the use of live animals in other manipulative processes could be more advantageous. The definition of "active learning that encourages participation", which is characterized in Cherney (2011) research, is also emphasized in the study of Elcoro and Trundle (2013). Again, Elcoro and Trundle (2013) state that it is an active learning way for students to have direct contact with objects relevant to the subject in the laboratory environment. It is seen that these results are in harmony with the data obtained from the changes in the opinions and expectations of the students determined in our research about the practice before and after the practice lesson and the changes in their state and trait anxiety levels. Similarly, the findings on the "reduction of fear level" seen in that research (Elcoro and Trundle, 2013) again support our results. In their study, Liddell et al. (2002) found that students receiving education in the field of health will increase their self-confidence with practices aimed at increasing procedural skills in the early period, which may increase skill-based effectiveness in the long term. In our study, the fact that students' fear, anxiety, and excitement levels were lower and comfort levels were higher after the intervention is in line with these explanations. Likewise, the data on students' opinions and expectations about the practice before and after the practicum course and the change in continuity anxiety levels also supported this approach. De Masi et al. (2016) reported that in their training study to improve the surgical skills of medical students using live animals, the student's practical skills improved at the end of the applications, and the feeling of self-confidence required during the operation changed positively. In our study, students' opinions and expectations about the practice before and after the practice course, the change in state and trait anxiety levels, and their gains from the practices align with this study's results. Schoeb et al. (2016) reported that students' theoretical knowledge and self-confidence levels were significantly affected by the practice in a realistic surgical education study organized to evaluate preclinical surgical skill development. The results obtained are supportive when we look at the change in status before and after the application in our research. The increase in the value of opinion and expectation and the decrease in the value of trait anxiety status after the practice lesson seem to be identical to the relevant literature (Schoeb et al., 2016). In a study (Redondo et al., 2010) in which the use of live animals in animal science education was investigated in terms of students' attitudes and welfare perception, it was reported that the teaching-learning process was positively affected after training with live rabbits and that this method was able to reach a level higher than the previous level of knowledge. In our study, it has seen that the students' "I agree" responses to the propositions "I have a lot to learn from this application" and "This application will encourage me to learn more" were at a high level before the application and that this expectation was realized positively and at a statistically significant level after the application. Küçükaslan et al. (2019) had reported that written print materials were more effective with the use of animals in the learning skills of veterinary faculty students. They concluded that in the training using plastic modeling, there was difficulty in grasping the systems in which the internal organs were located, and that the training in general with the animal use method was more successful than plastic models and internet-based training.

In this study, it has seen that the application made

with the students taking laboratory animal husbandry course positively affected the opinions and expectations of the students and that the application reduced the state and trait anxiety levels, which may make the education that the students will receive in this field more useful. It was concluded that training in the field of laboratory animal science that includes interventional procedures using live animals could increase clinical skill gains and positively affect emotional states and that this training can be beneficial in adapting to professional life more successfully. On the other hand, it may be considered that the availability of interventional practice training, which is the subject of our research, may be helpful to ensure standardization in the curriculum of courses in the field of laboratory animal science.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

References

Bayar K, Çadır G, Bayar B. Determination thought and anxiety levels of nursing students intended for clinical practice. TAF Prev Med Bull 2009; 8(1): 37-42.

Canbaz S, Sünter AT, Aker S, Pekşen Y. Bir tıp fakültesi son sınıf öğrencilerinin kaygı düzeyi ve etkileyen faktörler. Genel Tıp Derg 2007; 17(1): 15-9.

Cherney ID. Active learning. Miller RL, Kowalewski BM, Beins BC, Keith KD, Peden BF. eds. In: Promoting Students Engagement Volume 1: Programs, Techniques, and Opportunities Society for the Teaching of Psychology, Div. 2. USA: American Psychological Association 2011; pp. 150-6.

Costa A, Costa A, Anna I, Olsson S. Students' acceptance of e-learning approaches in laboratory animal science training. Lab Anim 2019; 54(5): 487-97.

Çakmak Ö, Hevedanlı M. The examination of concern levels of biology student's class by various variables. ESOSDER 2005; 4(14): 115-27.

Çiftçili S, Uzuner A, Ünalan P, Akman M. Marmara Üniversitesi Tıp Fakültesi klinik beceri laboratuvarı uygulamaları. TED 2006; 21(21): 1-10.

Daly SC, Wilson NA, Rinewalt DE, Bines SD, Luu MB. A subjective assessment of medical student perceptions on animal models in medical education. J Surg Educ 2014; 71(1): 61-4.

De Masi SC, Katsuta E, Takabe K. Live animals for preclinical medical student surgical training. Edorium J Surg 2016; 3(2): 24-31.

Deveci SE, Çalmaz, A, Açık Y. The relationship between anxiety level and health, social and demographical factors in the students of a newly established university in Eastern Anatolia. Dicle Med J 2012; 39(2): 189-96.

Dewhurst DG, Hardcastle J, Hardcastle PT, Stuart E. Comparison of a computer simulation program and a traditional laboratory practical class for teaching the principles of intestinal absorption. Am J Physiol 1994; 267(6): 95-104.

Elcoro M, Trundle M. Student preferences for live versus virtual rats in a learning course. JoSoTL 2013; 7(1): 1-13.

Guenther C, Miller RL. Factors that promote engagement. Miller RL, Kowalewski BM, Beins BC, Keith KD, Peden BF. eds. In: Promoting Students Engagement Volume 1: Programs, Techniques, and Opportunities Society for the Teaching of Psychology, Div. 2. USA: American Psychological Association, 2011; pp.10-7.

Hansen LA, Boss GR. Use of live animals in the curricula of U.S. medical schools: Survey results from 2002. Acad Med 2001; 77(11): 1147-9.

IBM. Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. 2013. Armonk, NY: IBM Corp.

Küçükaslan Ö, Erdoğan S, Bulu İ. Turkish undergraduate veterinary students' attitudes to use of animals and other teaching alternatives for learning anatomy. J Vet Med Educ 2019; 46(1): 116-27.

Liddell MJ, Davidson SK, Taub H, Whitecross LE. Evaluation of procedural skills training in an undergraduate curriculum. Med Educ 2002; 36(11): 1035-41.

Öner N, Le Compte A. Süreksiz Durumluk/Sürekli Kaygı Envanteri El Kitabı, Birinci Baskı, İstanbul, Türkiye, Boğaziçi Üniversitesi Yayınları. 1983.

Özen R, Özen A. Veterinary education in Turkey. J Vet Med Educ 2006; 33 (2): 187-96.

Platto S, Serres A, Jingyi A. Chinese College Students' attitudes towards animal welfare. Animals 2022; 12(2): 156.

Redondo PG, Caravaca FP, Castel JM, Mena Y, Delgado-Pertinez M. Using live animals for teaching in

animal sciences: Students' attitudes to their leraning process and animal welfare concern. J Anim Vet Adv 2010; 9(1): 173-9.

Sabancıoğulları S, Doğan S, Kelleci M, Avcı D. Hemşirelik son sınıf öğrencilerinin internlik programına ilişkin görüşlerinin belirlenmesi. DEUHFED 2012; 5(1): 16-22.

Schoeb DS, Brennecke E, Andert A, Grommes J, Von Trotha KT. Assessment of a course of realistic surgical training during medical education as a tool for pre-residential surgical training. BMC Med Educ 2016; 16(45): 1-7.

Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA. Manual for The State-Trait Anxiety Inventory. USA, Consulting Psychologists Press, 1983.

Tosun N, Oflaz F, Akyüz A, Kaya T, Yava A. Hemşirelik Yüksek Okulu öğrencilerinin intörn eğitim programından beklentileri ile program sonunda kazanım ve önerilerinin değerlendirilmesi. Gülhane Tıp Derg 2008; 50(3): 164-71.

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Effects of Different Starch Sources Used at High Levels in Cattle on Ruminal Fermentation Properties and Some Blood Parameters*

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Abstract: In this study, it was aimed to determine the effects of different starch sources on ruminal fermentation and *in situ* digestibility characteristics and some blood parameters in cows. In the study, three different total mixed rations (TMR) with similar energy, protein and starch contents were prepared and these TMR's formed the groups of the experiment. The main starch sources of the TMR's were from the barley, wheat, and corn grains, respectively. The study was carried out as two consecutive trails using 3 non-lactating Holstein female cattle with rumen cannulate within a 3 × 3 Latin square trial design. These TMRs were fed at *ad libitum* and then nutrient intakes, ruminal fermentation (pH, acetic, propionic, butyric, and lactic acids), some serum (urea, glucose, total protein, albumin, triglyceride) and blood gas parameters (pH, pCO_2, pO_2, HCO_3^-, Na^+, K^+, Ca^{++}, Cl^-, anion gap, lactate) were determined. Also, *in situ* dry matter and starch degradability were carried out in these animals. Nutrient intakes of cows fed different TMRs were similar ($P>0.05$), except neutral detergent fiber (NDF) intake ($P<0.05$). Both ruminal fermentation, serum and blood gas parameters did not change among treatment groups ($P>0.05$). As a result, it was determined that there were no serious changes in the ruminal fluid, serum, and blood gas parameter values of the subjects due to the content difference of the trial TMR's. On the other hand, it was determined that *in situ* dry matter (DM) and starch degradability of barley and wheat were significantly different among cereal grains, ruminal DM and starch degradability of corn followed a slower, stable, and gradual increase.
Keywords: Biochemistry, blood gas, degradability, rumen, starch

Sığırlarda Yüksek Düzeyde Kullanılan Farklı Nişasta Kaynaklarının Ruminal Fermentasyon Özellikleri ve Bazı Kan Parametreleri Üzerine Etkileri

Öz: Sunulan çalışmada, farklı nişasta kaynaklarının sığırlarda ruminal fermantasyon ve *in situ* sindirilebilirlik özellikleri ile bazı kan parametreleri üzerine etkilerinin belirlenmesi amaçlanmıştır. Araştırmada benzer enerji, protein ve nişasta içeriklerine sahip üç farklı karma rasyon hazırlanmış ve bunlar denemenin gruplarını oluşturmuştur. Karma rasyonların ana nişasta kaynakları sırasıyla arpa, buğday ve mısır tanesi kökenlidir. Çalışma, 3 x 3 Latin kare deneme tasarımında, rumen kanüllü, laktasyonda olmayan 3 Holştayn dişi sığır kullanılarak ardışık iki deneme halinde gerçekleştirilmiştir. Bu karma rasyonlarla *ad libitum* besleme yapılmış ve ardından hayvanların besin alımları, ruminal fermantasyon (pH, asetik, propiyonik, bütirik ve laktik asitler), bazı serum (üre, glikoz, toplam protein, albümin, trigliserit), kan gazı (pH, pCO_2, pO_2, HCO_3^-, Na^+, K^+, Ca^{++}, Cl^-, anyon gap, laktat) parametreleri. Ayrıca nişasta kaynağı yemlerin kuru madde ve nişasta sindirilebilirliği de incelenmiştir. Farklı karma yemlerle beslenen sığırların nötral-deterjan lif (NDF) alımı ($P<0.05$) dışındaki diğer besin madde tüketimi parametreleri benzer bulunmuştur ($P>0.05$). Ruminal fermantasyon, serum ve kan gazı parametreleri deneme grupları arasında değişim göstermemiştir ($P>0.05$). Sonuç olarak deneme rasyonlarının içerik farklılığından dolayı deneklerin rumen sıvısı, serum ve kan gazı parametre değerlerinde ciddi bir değişiklik olmadığı belirlenmiştir. Diğer yandan, arpa ve buğdayın kuru madde ve nişasta sindirilebilirliğinin tahıl taneleri arasında önemli derecede farklı olduğu, mısırın rumen kuru madde ve nişasta sindirilebilirliğinin daha yavaş, istikrarlı ve kademeli bir artış gösterdiği belirlenmiştir.
Anahtar Kelimeler: Biyokimya, kan gazı, nişasta, rumen, sindirilebilirlik

Introduction

As it is known, one of the basic nutrient groups required by all living species is carbohydrates. Herbivorous and omnivorous animals basically obtain their

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carbohydrate needs from the plants they consume. Carbohydrates found in plants are classified as structural (cellulose, hemicellulose, etc.) and non-structural (starch, glucose, etc. sugars) carbohydrates. Starch has a special importance in ruminant nutrition because it is both the main metabolic energy source and one of the main activators of the ruminal fermentation mechanism (Giuberti et al., 2014). Starch can undergo ruminal and/or intestinal digestion much faster than structural carbohydrates (Huntington et al., 2006). In parallel with the rapid ruminal digestion of starch, an increase in the rate of ruminal microbial fermentation is observed, and subsequently the synthesis of organic fatty acids (and especially propionic acid) accelerates (Ferraretto, 2017). If the amount of starch in the diet is increased to a certain level, gradual increases are observed in performance of the animals, while the probability of encountering ruminal acidosis cases increases when these limits are exceeded (Boerman et al., 2015; Abdela, 2016). Studies have shown that the rate of starch digestion and the probability of acidosis formation related to it vary depending on the starch source (cereal grain) type and feed processing techniques. It has been determined that while the degradation rates of barley and wheat are generally close to each other and faster (29-34%/hour), and corn, rice, potato and sorghum starches are also close to each other but much slower (2-6/hour) (Monteils et al., 2002; Wang et al., 2009; Mosavi et al., 2012).

In the diets of high-producing ruminants, high starch-containing feedstuffs must be included to the diet to meet the animal's energy needs. However, over 28% starch in dairy cow diet causes a decrease in milk fat and the risk of subclinical acidosis. One way to prevent such problems, especially in dairy cows, is to add feed sources high in starch to the diet with low ruminal starch degradation.

When the chemical structure of starch is examined, it is seen that it basically consists of two different glucose polymers called amylose and amylopectin, and it is a molecule with a granular structure in the part called endosperm of the cereal seeds (Allen and Piantoni, 2014). However, it has been determined that starch molecules are not in a standard and stable structure and have some physical and chemical structure differences, depending on the source (plant type) from which they are obtained. Therefore, the differences in the endosperm structure of the seeds according to the plant type, the difference in starch amylose/amylopectin ratio, the granule structure size, and the processing of feed by various physical-chemical methods etc. also significantly change the ruminal/intestinal digestibility values of starch structures (Gomez et al., 2016, Qi and Tester, 2016). The granule size ranges from less than 1 µm to more than 100 µm, depending on the plant species from which the starch is obtained (Fuentes et al., 2019), this value is in the range of 1-20 µm in corn starch, while it is in the range of 1-110 µm in potato starch, and accordingly, the ruminal digestibility of potato starch is more difficult than corn starch (Monteils et al., 2002; Singh et al., 2016). It has been determined that wheat, barley, and oat starches can be digested more easily than corn due to the difference in seed endosperm structure, and that digestibility increases by processing the feeds such as grinding, gelatinization and conservation (Allen and Piantoni, 2014).

Corn, wheat, and barley grains are the most commonly used feed materials in ruminant diets. When the chemical compositions of corn, wheat and barley grains added to the diets were examined, it was found that they contained an average of 76.0%, 70.3% and 64.3% starch, respectively. It has been determined that the ruminal total digestibility values of these starches in dairy cows can vary between 72-89.9%, 88.1-88.3% and 80.7-84.6%, respectively, depending on the different feed processing techniques (Gomez et al., 2016) and feeding managements.

Today, many studies have been conducted to explain the relationships between starch and ruminal acidosis. In ruminants, it is of great importance to determine which cereal grain contains the starch type that is less and difficult to ferment, which can enable them to continue their normal digestive activities without further reducing the ruminal acidity value, and therefore delays the formation of acidity, and to prepare the appropriate diet ingredients. However, since starch is an important content of plants and an important food source for animals, it is understood that there is a need for further investigation in order to fully understand its physiological, biochemical and microbial functionality, efficiency and effects in the organism, as well as its relationship with performances and diseases. To date, studies on starch degradation have mostly been conducted in ruminants consuming a low-starch diet. In the literature, data on starch degradation in ruminants consuming a high-starch diet is limited.

In this study, it was aimed to determine the effects of different starch sources commonly used in ruminant diets on ruminal fermentation and *in situ* digestibility characteristics and some blood parameters related to acidosis in cows.

Material and Methods

The study was carried out as two consecutive trails using 3 non-lactating Holstein with rumen cannulate, aged 6 years and an average live weight of 650 kg, with the decision of the Ministry of Agriculture and Forestry, International Center for Livestock Research and Training directorate, Animal Experiments Local Ethics Committee, numbered 156/18.

In the study, nutrient contents of all feedstuffs used in the experiment were determined in the laboratory of animal nutrition. Based on the determined nutrient contents of these feedstuffs, three different feed mixtures were formulated with similar energy, protein, and starch contents. The first of these mixtures was prepared in such a way that the main starch source was from cracked barley (barley-based ration, group's named "B"), the second main starch source was from cracked wheat (wheat-based ration, group's named "W"), and the third was from cracked corn (corn-based ration, group's named "C"). These three different total mixed diets (TMR) consisted of approximately 70% concentrate and the remaining 30% roughages (consisting of equal proportions of alfalfa grass, wheat straw and corn silage). These TMRs were fed to experimental animals as two meals at 09:00 AM and 08:00 PM.

In the feeding trial, which is the first trail, three different TMRs mentioned above were given to the experimental animals in three periods within a 3×3 Latin square trial design. Animals were randomly assigned to one of three experimental group. Before the experiment, the animals were adapted to TMRs for 15 days, and in this process, the amount of concentrated feed mixes consumed by the animals were gradually increased to 70% of the total diet. At the beginning of each period, the body weights of the experimental animals were weighed. Each trial period was planned to be 18 days in total. In the first 10 days, the determination of feed consumptions and the adaptation of the experimental animals to the formulated diets, and also in the next five days the amounts of feed consumption were determined. On the 16th day, blood samples were taken from the jugular vein to determine blood gases and some blood biochemical parameters. Also, on the 16^{th} day of the experiment, approximately 50 ml of rumen fluids were taken from the rumen at 0-, 2-, 4-, 6-, 8- and 10- hours post-feeding, through rumen cannulas, and the pH of the rumen fluid was quickly determined using a portable digital pH-meter. In addition, for volatile fatty acids (VFA_s) analysis, samples were taken from these ruminal fluids in 10 ml to plastic Falcon® tubes (consisting of 9 ml of rumen fluid and 1 ml of HCl acid diluted with 50/50 distilled water) and these tubes were preserved in the cold chain and then, stored in a deep freezer at -18°C. The probability of animals experiencing ruminal acidosis during these last five days of each period was also monitored.

As a part of the second trail, on the 17^{th} and 18^{th} days of each period, incubation of feedstuffs in rumen was performed by using Dacron sacs (R510) with pore size of 40-50 µ to determine the "*in situ*" degradability rates of nutrient contents. In this Dacron bag trial, barley, wheat, and corn samples were ground to pass through a sieve with 2 mm pores. From these ground feed samples, approximately 3 g were placed in Dacron bags with 5×10 cm dimensions, and then incubated in the rumen of cannulated cows for 0-, 2-, 4-, 8-, 12-, 24- and 48- hours. After the incubation, the bags were washed with tap water until the washing water became clear (approximately 15 minutes), and then the bags were dried in the drying oven. Also, after drying, Dacron bags were weighed to determine the dry matter and starch degradability (Hassan and Karsli, 2023).

The experimental diets were formulated to be isocaloric, isonitrogenous and contained 32% starch from different starch sources, and dry matter (DM), organic matter (OM), crude protein (CP), ash contents of diets were determined according to Weende analysis methods (AOAC, 2006); neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents according to the method reported by Van Soest et al. (1991) and starch contents were analyzed according to Ewers polarimetric method (ISO, 1997). Botanical compositions of TMRs are presented in Table 1; Nutrient contents of feedstuffs used in TMRs and *in situ* digestion experiment are presented in Table 2.

The biochemical parameters in the blood samples were determined with a spectrophotometric autoanalyzer (Mindray BS-800M, Shenzhen, China), and blood gases and ions were determined using the RAPID lab® 1265 (Siemens Medical Diagnostics, Bayer, Tarrytown, NY, USA) device. Volatile fatty acids (acetic, butyric, propionic) and lactic acid contents of rumen fluid samples were determined using the Shimadzu Prominence LC 20AD HPLC (Shimadzu Corp., Kyoto, Japan) device, by modifying the method reported by Tjardes et al. (2000). Ruminal fluid samples were thawed at room temperature (22°C) before analysis, then mixed by vortexing and the supernatant was clarified by centrifugation. 1 ml of this liquid was filtered by syringe membrane filter (ISOLAB Laborgeräte GmbH, Eschau, Germany) with a pore width of 0.45 µm, specially produced for HPLC analyses. No internal standard was used. A correlation graph was created by preparing 10-20-40-60-100 -200 ppm calibration solutions of acetic, propionic, butyric, and lactic acids (Sigma Aldrich, St. Louis MO, USA) as external standards. Organic acid levels were determined using Inertsil ODS 3 HPLC analysis column (5 µm, 4.6 × 150 mm; GL Sciences Inc., Tokyo, Japan), the filtrate injection volume was 10µl, the mobile phase was 20 mM $(NH_4)H_2PO_4$ buffer solution, the flow rate was 1.5 mL/min, and the column temperature was 30°C. The amount of these four organic acids were determined in mmol/L units.

Preliminary tests were conducted to understand whether parametric test assumptions (normality and homogeneity) were met. Levene's test was performed to determine whether the group variances were homogeneous and it was found that the group variances were homogeneous. For normality, the Shapiro-Wilk test was performed. Box and Whisker

charts were examined, then data were analyzed as 3×3 Latin square method. Statistical analyzes of the data obtained in the experiment were carried out using the SPSS© 15.0 package program. The Duncan test method was used for analysis of variance (one-way ANOVA) to determine the data differences and significance values between the experimental groups and for pair wise comparisons of the means. P<0.05 was accepted as statistically significant. Average of each parameter was expressed as $\bar{x} \pm$ SEM.

Results

In the experiment, nutrient intakes of cattle consuming diets prepared with different cereal grains are shown in Table 3. The consumptions of nutrients, except NDF were similar in cows consuming diets containing different cereal grains as a source of different starch (P>0.05). NDF consumption of cows consuming a diet containing barley was found to be lower than the others (P<0.05). In general, while *in*

Table 1. Nutritional composition of TMR

	Barley Mix			Wheat Mix			Corn Mix		
Feed raw materials	**TMR* (kg)**	**%**	**% DM**	**TMR* (kg)**	**%**	**% DM**	**TMR*, (kg)**	**%**	**% DM**
Corn silage	12.5	43.3	19.15	12.5	43.40	18.95	12.5	43.33	18.93
Alfalfa	1.2	4.19	6.02	1	3.47	4.96	1.4	4.85	6.94
Wheat straw	1.2	4.19	6.17	1.4	4.86	7.13	1	3.47	5.09
Wheat bran	-	-	-	1	3.47	5.09	0.7	2.43	3.56
Barley	8	27.93	39.86	-	-	-	-	-	-
Wheat	-	-	-	7	24.30	34.59	-	-	-
Corn	-	-	-	-	-	-	6.8	23.57	33.51
Rice	1	3.49	4.87	0.85	2.95	4.10	0.8	2.77	3.85
Sunflower seed meal	2	6.98	10.02	2.3	7.99	11.40	2.8	9.71	13.87
Soybean meal	2.5	8.73	12.56	2.5	8.68	12.43	2.6	9.07	12.92
Limestone	0.25	0.87	1.35	0.25	0.87	1.34	0.25	0.87	1.34

** TMR, total mixed ration; DM, dry matter (kg).*

Table 2. Nutrient contents of feedstuffs used in TMRs and *in situ* digestion experiment (analyzed and calculated)

Diet Components	**DM**	**NE_L* (Mcal)**	**CP**	**NDF**	**ADF**	**EE**	**Ash**	**Starch**	**Ca* (g/kg)**	**P* (g/kg)**
In Both Diets and *In Situ* Experiment (%, DM)**										
Corn grain	92.14	2.14	8.53	9.35	1.92	3.85	1.33	74.08	0.3	3.2
Barley grain	92.13	2.00	12.22	20.42	6.25	2.61	3.31	56.81	0.6	3.9
Wheat grain	92.29	2.07	11.21	14.19	4.14	2.00	1.85	66.42	0.5	4.4
In Diets (%, DM)										
Corn silage	30.34	1.40	7.80	45.66	25.79	3.45	6.16	23.90	3	2.7
Alfalfa hay	92.71	0.99	17.47	44.78	32.54	2.00	10.19	3.51	11.6	2.3
Wheat straw	95.16	0.82	2.80	81.59	48.29	1.16	4.10	-	1.7	0.5
Wheat bran	95.09	1.77	14.67	55.71	17.35	4.89	5.43	13.15	1.4	11.7
Rice grain (dehulled)	91.03	2.19	9.39	20.67	6.33	1.23	0.54	87.46	0.5	2.1
Sunflower seed meal	92.60	1.17	27.84	53.11	35.06	1.15	6.39	2.64	4.5	11.2
Soybean meal	92.90	1.98	47.93	15.69	8.05	1.81	7.10	5.56	1.6	7.6
Calculated Nutrient Contents (%, DM)**										
Ration Mixes		**NE_L**	**CP**	**NDF**	**ADF**	**Starch**	**Starch Origin**	**Percentage of forage in TMR**	**Ca**	**P**
Wheat Mix Diet		1.65	16.56	33.45	17.63	32.83	69.02	31.95	0.65	0.51
Barley Mix Diet		1.64	16.72	33.08	17.31	32.57	68.57	32.25	0.66	0.44
Corn Mix Diet		1.65	16.49	31.40	17.12	34.39	71.18	31.87	0.69	0.48

** Values were calculated with the following equation: ME (Mcal/kg) = Digestible energy × 0.82; NE_L (Mcal/kg) = 0.00245 × DE - 0.12; Ca and P values were calculated according to NRC (2001) feed data tables.*

*** DM, dry matter (kg);NE_L, net energy of lactation (Mcal/day); CP, crude protein (kg); NDF, neutral detergent fiber (kg); ADF, acid detergent fiber (kg); Starch (kg).*

situ dry matter degradation was significantly higher for barley and wheat compared with corn, starch degradability values of different cereal grains were significantly different among all three grains (P<0.05; Table 4). Ruminal volatile fatty acids composition ratios and pH values were similar (P>0.05), except 4-hour post-feeding acetic acid values (Table 5). 4-hour post-feeding acetic acid value was significantly higher in cows fed barley-based diet compared with the other two groups (P<0.05). In general, serum and blood gas biochemical parameter values were similar (P>0.05) only serum urea level significantly increased during trail period (P<0.05; Table 6).

Table 3. Nutrient intakes of cattle consuming diets prepared with different cereal grain

	Treatment Groups* (x̄ ± SEM)			
Parameters**	**B**	**W**	**C**	**P-value**
DM (kg)	9.15 ±0.66	9.83 ±0.09	8.76 ±1.10	0.600
NE_L (Mcal/day)	15.00 ±1.08	16.22 ±0.48	14.45 ±1.81	0.590
CP (kg)	1.53 ±0.11	1.65 ±0.10	1.44 ±0.18	0.590
NDF (kg)	1.58 ±0.11^{b}	3.28 ± 0.08^{a}	2.78 ±0.34^{a}	0.030
ADF (kg)	1.58 ±0.11	1.73 ±0.08	1.50 ±0.19	0.260
Starch (kg)	2.97 ±0.21	3.23 ±0.06	3.01 ±0.38	0.760

* *Determined on dry matter basis; B, group were fed with Barley starch; W, group were fed with wheat starch; C, group were fed with corn starch; SEM, standard error of mean.*
** *DM, dry matter;NE_L, net energy of lactation; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.*
^{a,b} Significant differences were found between values with different letters on the same line (P<0.05).

Table 4. *In situ* dry matter and starch digestibility values of different cereal grains used in the experiment

Parameters	Incubation Time (h)	Treatment Groups* (%, x̄ ± SEM)			
		B	**W**	**C**	**P-value**
Dry Matter	0	23.76 ±2.34^{a}	20.23 ±1.87^{a}	14.73 ±2.18^{b}	0.063
	2	38.85 ±1.41^{a}	40.22 ±6.93^{a}	24.22 ±1.59^{b}	0.064
	4	51.73 ±2.69^{a}	49.83 ±4.10^{a}	28.77 ±1.81^{b}	<0.001
	8	64.02 ±2.98^{a}	59.18 ±2.86^{a}	36.78 ±2.53^{b}	<0.001
	12	78.47 ±1.66^{a}	77.50 ±3.16^{a}	47.93 ±1.54^{b}	<0.001
	24	82.55 ±1.14^{a}	85.85 ±1.79^{a}	66.27 ±1.25^{b}	<0.001
	48	85.78±0.28^{b}	89.53 ±0.40^{a}	89.59 ±0.74^{a}	<0.001
Starch	0	32.54 ±2.07^{a}	25.27 ±1.75^{b}	22.43 ±1.98^{b}	0.025
	2	53.79 ±1.07^{a}	49.13 ±5.90^{a}	28.84 ±1.49^{b}	0.006
	4	64.90 ±1.95^{a}	60.18 ±3.25^{a}	33.89 ±1.68^{b}	<0.001
	8	79.52 ±1.70^{a}	68.32 ±2.15^{b}	41.79 ±2.33^{c}	<0.001
	12	95.98 ±0.31^{a}	83.07 ±2.38^{b}	54.54 ±1.34^{c}	<0.001
	24	98.33 ±0.11^{a}	93.40 ±0.84^{b}	74.38 ±0.95^{c}	<0.001
	48	99.59 ±0.01^{a}	98.63 ±0.05^{b}	97.59 ±0.17^{c}	<0.001

* *B, group were fed with barley starch; W, group were fed with wheat starch; C, group were fed with corn starch; SEM, standard error of mean. Determined on dry matter basis.*
^{a,b,c} *Significant differences were found between values with different letters on the same line (P<0.05*).

Table 5. Volatile fatty acids composition and pH values of rumen fluids obtained from cattle consuming diets prepared with different cereal grain

Time (Hour)	Parameters	Treatment Groups* (Mmol/L, x̄ ± SEM)			
		B	W	C	P-value
0th (n =9)	Acetic acid	79.21 ±8.90	65.66 ±6.77	62.85 ±4.42	0.279
	Butyric acid	5.12 ±0.78	4.24 ±0.58	3.77 ±0.09	0.303
	Propionic acid	46.64 ±10.07	34.27 ±5.97	32.06 ±5.53	0.391
	Lactic acid	12.85 ±2.02	9.51 ±1.84	9.42 ±1.81	0.400
	pH	7.00 ±0.10	7.09 ±0.09	7.04 ±0.02	0.750
2nd (n =9)	Acetic acid	65.68 ±1.62	54.92 ±3.92	60.74 ±13.05	0.651
	Butyric acid	8.32 ±1.10	6.46 ±0.53	6.73 ±1.28	0.430
	Propionic acid	38.98 ±1.88	29.15 ±5.84	35.04 ±2.70	0.272
	Lactic acid	12.14 ±0.61	10.67 ±1.32	11.93 ±1.43	0.653
	pH	6.52 ±0.06	6.32 ±0.38	6.66 ±0.05	0.404
4th (n =9)	Acetic acid	96.60 ±3.33[a]	69.16 ±0.42[b]	69.48 ±11.49[b]	0.049
	Butyric acid	9.09 ±0.91	6.68 ±0.62	6.21 ±1.45	0.196
	Propionic acid	60.45 ±5.31	38.16 ±2.92	43.50 ±11.60	0.174
	Lactic acid	15.34 ±0.42	10.87 ±0.32	11.69 ±2.68	0.184
	pH	6.10 ±0.14	6.30 ±0.16	6.48 ±0.11	0.207
6th (n =9)	Acetic acid	77.28 ±5.24	59.64 ±11.52	76.23 ±13.34	0.466
	Butyric acid	6.37 ±0.31	5.11 ±1.16	5.42 ±0.70	0.547
	Propionic acid	44.20 ±1.10	30.46 ±8.22	44.29 ±13.97	0.524
	Lactic acid	12.02 ±1.04	8.79 ±2.09	12.42 ±3.35	0.529
	pH	6.34 ±0.07	6.39 ±0.06	6.54 ±0.10	0.291
8th (n =9)	Acetic acid	74.21 ±9.30	63.00 ±9.78	82.64 ±13.44	0.491
	Butyric acid	6.10 ±0.12	5.01 ±0.94	5.80 ±0.79	0.569
	Propionic acid	39.24 ±7.04	30.32 ±4.95	46.59 ±14.50	0.533
	Lactic acid	12.02 ±1.70	9.92 ±1.56	12.91 ±3.05	0.582
	pH	6.76 ±0.07	6.71 ±0.05	6.72 ±0.20	0.969
10th (n =9)	Acetic acid	83.38 ±15.00	58.12 ±4.69	81.08 ±15.52	0.364
	Butyric acid	5.77 ±0.39	4.23 ±0.81	5.54 ±1.08	0.407
	Propionic acid	49.76 ±10.74	29.49 ±5.35	44.09 ±13.97	0.432
	Lactic acid	14.42 ±3.54	8.86 ±1.83	12.09 ±3.62	0.489
	pH	6.84 ±0.13	6.86 ±0.07	6.85 ±0.14	0.993

** B, group were fed with barley starch; W, group were fed with wheat starch; C, group were fed with corn starch; SEM, standard error of mean; n, number of samples.*

[a,b] Significant differences were found between values with different letters on the same line (P<0.05).

Table 6. Blood and blood gases biochemical parameters values

Parameters	Sampling Time (Hour)	Pre-trial Values	Treatment Groups* (x̄ ± SEM)			P-value
			B (n =6)	W (n =6)	C (n =6)	
Blood Biochemical Parameters						
Glucose (mg/dL)	0th	70.30 ±1.33	72.67 ±1.75	72.83 ±2.01	71.33 ±1.50	0.616
	6th	-	67.83 ±1.87	63.17 ±2.74	65.33 ±1.63	0.328
Total Protein (g/dL)	0th	7.40 ±0.19	7.57 ±0.17	7.37 ±0.20	7.36 ±0.11	0.862
	6th	-	6.88 ±0.46	6.45 ±0.68	6.88 ±0.29	0.796
Triglyceride (mg/dL)	0th	26.80 ±3.40	20.00 ±3.06	21.33 ±1.67	21.33 ±1.76	0.359
	6th	-	17.67 ±0.88	17.33 ±3.28	17.33 ±3.53	0.995
Urea (mg/dL)	0th	16.60 ±0.40[b]	29.67 ±3.18[a]	32.00 ±3.06[a]	29.67 ±4.63[a]	0.004
	6th	-	26.00 ±4.16	27.00 ±1.53	26.00 ±5.51	0.980
Albumin (g/dL)	0th	2.84 ±0.08	2.93 ±0.03	3.00 ±0.10	2.97 ±0.03	0.455
	6th	-	2.73 ±0.09	2.67 ±0.23	2.80 ±0.15	0.859
Blood Gases Biochemical Parameters						
pH	0th	7.47 ±0.01	7.45 ±0.01	7.46 ±0.01	7.41 ±0.03	0.110
	6th	-	7.44 ±0.02	7.45 ±0.01	7.44 ±0.01	0.765
pCO_2 (mmHg)	0th	37.66 ±1.08	37.83 ±1.69	38.43 ±0.62	40.73 ±4.64	0.767
	6th	-	39.00 ±2.31	40.07 ±0.97	38.60 ±0.50	0.776
pO_2 (mmHg)	0th	28.64 ±2.32	34.83 ±1.06	36.97 ±2.09	34.70 ±4.25	0.157
	6th	-	33.43 ±2.94	30.53 ±1.62	31.73 ±2.91	0.735
HCO_3^- (mmol/L)	0th	26.70 ±0.74	25.77 ±1.59	26.67 ±0.15	25.23 ±1.39	0.712
	6th	-	25.93 ±0.84	27.30 ±0.26	25.80 ±0.46	0.205
Na^+ (mmol/L)	0th	135.90 ±3.36	137.63 ±0.37	140.13 ±2.81	137.60 ±1.31	0.759
	6th	-	138.40 ±0.55	140.20 ±0.72	137.47 ±1.04	0.123
K^+ (mmol/L)	0th	3.87 ±0.08	4.06 ±0.15	3.16 ±0.42	3.75 ±0.48	0.268
	6th	-	3.51 ±0.30	3.58 ±0.22	3.53 ±0.26	0.982
Ca^{++} (mmol/L)	0th	1.10 ±0.02	1.13 ±0.04	1.04 ±0.07	1.10 ±0.06	0.572
	6th	-	1.07 ±0.07	0.97 ±0.08	1.06 ±0.06	0.589
Cl^- (mmol/L)	0th	99.80 ±2.65	100.67 ±4.33	104.33 ±0.88	99.67 ±4.37	0.748
	6th	-	106.00 ±2.52	103.33 ±1.45	105.00 ±0.58	0.569
Anion Gap (mmol/L)	0th	13.30 ±1.37	15.27 ±3.56	12.30 ±2.11	16.47 ±2.92	0.637
	6th	-	9.97 ±1.88	13.13 ±0.82	10.23 ±0.82	0.230
Lactate (mmol/L)	0th	1.31 ±0.14	0.66 ±0.10	0.81 ±0.28	1.10 ±0.45	0.276
	6th	-	0.65 ±0.14	0.93 ±0.17	0.66 ±0.15	0.421

** B, group were fed with barley starch; W, group were fed with wheat starch; C, group were fed with corn starch; SEM, standard error of mean; n, number of samples.*

[a,b] Significant differences were found between values with different letters on the same line (P<0.05).

Discussion and Conclusion

Nutrient intakes

Nutrient intakes of cows consuming different starch-based diets was similar, except NDF. Since the chemical composition of the diets prepared in the study were very similar to each other, it was not surprising that the nutrient consumption of the cattle consuming these diets was similar. It is thought that the low NDF consumption in cattle consuming a diet containing barley may be due to feed sorting in this group. Mosavi et al. (2012) reported that the nutrient intakes of dairy cows fed with diets containing barley, corn, wheat and potato-based starch did not change, which are in agreement with the results of the current study. It has been reported that responses of lactating cows to different cereal grains in terms of nutrient intakes depend on the level of dietary inclusion, the basal ration, physical processing of the cereal grains, the composition of a given batch of cereal grain, and the level of dietary intake (Khorasani et al., 2001). Since the characteristics of the diets were very similar in the present study, it was an expected result that the nutrient consumptions were similar.

Ruminal digestibility

In the presented study, when the *in situ* dry matter degradability values are examined, *in situ* DM degradation was similar in barley and wheat, but the DM degradation in both was significantly higher than that of corn from the beginning to the end of incubation times. This clearly shows that barley and wheat begin to undergo rapid digestion as soon as they enter the digestive system and reach an average of 84% within the first 24 hours, while corn follows a slower and stable digestion process within 48 hours (barley = wheat > corn).

When the *in situ* starch degradability values are examined, it was seen that the starch degradability rates of barley and wheat at the beginning are similar until the 4th hour, but after the 4th hour, it was observed that the starch degradation of all three grasses differed significantly in the periods until the end of incubation. Starch degradation was listed from fastest to slowest in barley, wheat and corn. At 12th hour of incubation, the degradation of barley starch was almost complete, while the degradation of wheat starch reached 83%, but only half of the degradation of corn starch was completed. Thus, when the time-dependent starch digestion rates were examined, it was seen that barley starch is digested faster than wheat and wheat is digested faster than corn starch, and that barley and wheat starch are almost complete within the first 24 hours. However, it was determined that the corn starch structure followed a slower and more stable digestion process within 48 hours (barley > wheat > corn).

In studies, it was reported that the ruminal fermentation or digestibility of corn starch is slower than other vegetable starches and it passes into the intestines without ruminal digestion at an average rate of 30% (18-42%). However, it is also known that the increase in the amount of starch intake with the diet increases the amount of starch that escapes from ruminal fermentation (Theurer, 1986; Mills et al., 1999). Similar results were also obtained in the presented study. Moreover, Overton et al. (1995) and Chibisa et al. (2015) also reported similar findings. In an experiment conducted by Hassan and Karsli (2023) in sheep consuming a forage-based diet, it was observed that the rumen starch degradation rates of barley, wheat and corn and the starch degradation values after 48 hours of incubation were higher than the data obtained in the current study. This shows that increasing the concentrated feed content of the diet reduces the rate and level of starch degradation in the rumen.

Ruminal fermentation

When the data obtained in the presented study were evaluated, it was determined that there was no statistically significant difference between the pH's and the acetic, butyric, propionic, lactic acids and total VFA values of the rumen fluid samples taken post-feeding. In the study, it is seen that ruminal pH values remained at neutral (7.00-7.09) levels in all groups at the beginning of the trial, only went down to the lowest value (6.10-6.48) at the 6-hour post-feeding, and then increased again and almost approached the initial values (6.84-6.86) at the 10-hour post-feeding. Ruminal pH values of cows fed corn-based diets were numerically higher compare with other two groups at 2- and 4-hours post-feeding, indicating a slower ruminal starch fermentation in this group. Consequently, it is seen that the pH values of the rumen fluids obtained from the experimental animals during the trial periods never fall below 6.00 to create sub clinical acidosis as it was expected (Oetzel, 2004; Khafipour et al., 2009; Morgante et al., 2009; Danscher et al., 2015).

In studies, it has been reported that high starch diets result in high ruminal VFA (acetic, propionic, butyric, isobutyric and valeric acids) and lactic acid levels, especially in animals with ruminal acidosis (Krause and Otzel, 2006; Plaizier et al., 2008; Zhao et al., 2018). However, based on the results obtained in the presented study, it shows that the preparation of diets with different starch structure does not cause any effect on ruminal fermentation and organic acid formation. The only difference was a significant increase in the level of acetic acid in the barley group at the 4th hour of the digestion process, which can be considered as an indication of the higher fermentability of barley starch. Although the starch levels of the diets in the current study were prepared to be at the high-

est levels of dairy cow diets, the expected risk of acidosis was eliminated because the feed consumption levels of the non-lactating dairy cattle used in the study were very low. For this reason, neither the expected decrease in rumen pH nor the increase in volatile fatty acids of the animals was observed. Only some numerical changes could be seen between groups.

Blood biochemistry

According to the data obtained on blood biochemistry, there was a significant change and increase in blood urea levels in the later stages of feeding with trial diets (independent of the content changes of all three ration mixes) compared to the beginning of the trial. It is thought that this change in urea is probably due to the transition from pre-experimental roughage-based diets to the experimental concentrated-based diets and the increase in nitrogenous microbial fermentation products.

In other blood parameters (glucose, total protein, albumin, and triglyceride), it was determined that there were no statistical differences depending on the dietary content changes. However, it is noticed that all these biochemical parameters examined show a decreasing trend in the advancing process from the first hour to the 6th hour of feeding, but it is understood that this situation is the result of the normal course of food digestion and metabolic process. Silveira et al. (2007) reported that no significant differences were observed in blood glucose levels between groups in feeding with barley and corn starch-based diets, and Cabrita et al. (2009) also reported that similar results in cows fed wheat and corn starch -based diets. Mosavi et al. (2012) found that no significant differences were observed between the groups in serum glucose, triglyceride, total cholesterol, LDL and HDL levels in cows fed barley, wheat, and corn -based diets. Moreover, Zhao et al. (2018) reported that no serious changes were observed in blood glucose levels even in cases ruminal acidosis.

Regarding the blood gas biochemical parameters (pH, pCO_2, pO_2, HCO_3^-, Na^+, K^+, Ca^{+2}, Cl^-, Anion Gap, and lactate) of the experimental animals, it was determined that there were no statistically significant differences among diets or sampling times (0- and 6-hours post-feeding). However, if the data between 0- and 6-hours post-feeding are evaluated, it is seen that normal changes occur at certain rates depending on possible metabolic processes. Morgante et al. (2009) determined that among the blood gas parameters pCO_2, pO_2, HCO_3 and blood pH values were 44.33, 39.76, 29.81, 7.42, respectively, in cattle control group with normal ruminal physiological values. However, they also reported that these values can change if there is a disorder such as ruminal acidosis these values were determined as 50.11, 36.60, 32.39, 7.41, respectively, in cattle with acidosis groups. Moreover, when the literatures are examined, it is seen that blood gas studies in cattle are quite limited. In the present study, it is thought that there is no statistically significant change were observed in rumen fermentation parameters due to low feed intake, as it does not cause any significant change or negatively effects in blood gas parameters of animals fed with diets formulated with different cereal grain-based with different starch types.

In conclusion, it was determined that feeding corn, wheat, and barley -based diets can significantly change *in situ* ruminal dry matter and starch digestibility values in animals according to starch types in these cereal grains, but it did not cause significant changes on ruminal pH and fermentation products (VFAs) and blood biochemical parameters with low feed intake. According to the results obtained, it was determined that feeding cows with corn-based diet may create a more stable ruminal digestibility.

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References

Abdela N. Sub-acute ruminal acidosis (sara) and its consequence in dairy cattle: a review of past and recent research at global prospective. Achievements Life Sci 2016; 10(2): 187-96.

Allen MS, Piantoni P. Carbohydrate nutrition: Managing energy intake and partitioning through lactation. Van Saun RJ. eds. In: Veterinary Clinics of North America: Food Animal Practice. New York: Elsevier Inc., 2014; pp. 577-97.

AOAC. Official methods of analysis of the association of official analytical chemists. Eighteenth Edition. Gaithersburg MD: Association of Official Analytical Chemists, 2006.

Boerman JP, Potts SB, VandeHaar MJ, Allen MS, Lock AL. Milk production responses to a change in dietary starch concentration vary by production level in dairy cattle. J Dairy Sci 2015; 98(7): 4698-706.

Cabrita ARJ, Vale JMP, Bessa RJB, Dewhurst RJ, Fonseca AJM. Effects of dietary starch source and buffers on milk responses and rumen fatty acid biohydrogenation in dairy cows fed maize silage-based diets. Anim Feed Sci Tech 2009; 152 (3-4): 267-77.

Chibisa GE, Gorka P, Penner GB, Berthiaume R, Mutsvangwa T. Effects of partial replacement of

dietary starch from barley or corn with lactose on ruminal function, short-chain fatty acid absorption, nitrogen utilization, and production performance of dairy cows. J Dairy Sci 2015; 98(4): 2627-40.

Danscher AM, Li S, Andersen PH, Khafipour E, Kristensen NB, Plaizier JC. Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. Acta Vet Scand 2015; 57(1): 1-14.

Ferraretto LF. Impact of starch content and digestibility in dairy cattle diets. Twenty-Eighth Annual Florida Ruminant Nutrition Symposium. February, 6-8, 2017; Florida-USA.

Fuentes C, Kang I, Lee J, Song D, Sjöö M, Choi J, Lee S, Nilsson L. Fractionation and characterization of starch granules using field-flow fractionation (FFF) and differential scanning calorimetry (DSC). Anal Bioanal Chem 2019; 411(16): 3665-74.

Giuberti G, Gallo A, Masoero F, Ferraretto LF, Hoffman PC, Shaver RD. Factors affecting starch utilization in large animal food production system: A review. Starch-Stärke 2014; 66(1-2): 72-90.

Gomez LM, Posada SL, Olivera M. Starch in ruminant diets: A review. Rev Colomb Cienc Pec 2016; 29(2): 77-90.

Hassan MAS, Karsli MA. The effect of *Saccharomyces cerevisiae* as a probiotic on the nutrient degradability of some commonly feedstuffs used in Turkey. Turk J Vet Anim Sci 2023; 47(3): 255-69.

Huntington GB, Harmon DL, Richards CJ. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J Anim Sci 2006; 84: 14-24.

ISO. International Organization for Standardization. ISO 10520:1997 Native starch. Determination of starch content. Ewers polarimetric method. https://www.iso.org/standard/18589.html; Accessed Date: 24.09.2023.

Khafipour E, Krause DO, Plaizier JC. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J Dairy Sci 2009; 92(3): 1060-70.

Khorasani GR, Okine EK, Kennelly JJ. Effects of substituting barley grain with corn on ruminal fermentation characteristics, milk yield and milk composition of Holstein cows. J Dairy Sci 2001; 84(12): 2760-9.

Krause MK, Otzel GR. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim Feed Sci Tech 2006; 126(3-4): 215-36.

Mills JAN, France J, Dijkstra J. A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model: 1. Dietary starch characterization and ruminal starch digestion. J Anim Feed Sci 1999; 8(3): 291-340.

Monteils V, Jurjanz S, Blanchart G, Laurent F. Kinetics of ruminal degradation of wheat and potato starches in total mixed rations. J Anim Sci 2002; 80 (1): 235-41.

Morgante M, Gianesella M, Casella S, Ravarotto L, Stelletta C, Giudice E. Blood gas analyses, ruminal and blood pH, urine and faecal pH in dairy cows during subacute ruminal acidosis. Comp Clin Pathol 2009; 18(3): 229-32.

Mosavi GHR, Fatahnia F, Mehrabi AA, Mirzaei Alamouti HR, Darmani Kohi H. Effect of dietary starch source on milk production and composition of lactating Holstein cows. S Afr J Anim Sci 2012; 42(3): 201-9.

NRC (National Research Council). Nutrient Requirements of Dairy Cattle. Seventh Revised Edition. Washington DC: National Academy Press, 2001.

Oetzel GR. Monitoring and testing dairy herds for metabolic disease. Vet Clin North Am Food Anim Pract 2004; 20(3): 651-74.

Overton TR, Cameron MR, Elliott JP, Clark JH, Nelson DR. Ruminal fermentation and passage of nutrients to the duodenum of lactating cows fed mixtures of corn and barley. J Dairy Sci 1995, 78(9): 1981-98.

Plaizier JC, Krause DO, Gozho GN, McBride BW. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. Vet J 2008; 176(1): 21-31.

Qi X, Tester RF. Effect of native starch granule size on susceptibility to amylase hydrolysis. Starch-Stärke 2016; 68(9-10): 807-10.

Silveira C, Oba M, Beauchemin KA, Helm J. Effect of grains differing in expected ruminal fermentability on the productivity of lactating dairy cows. J Dairy Sci 2007; 90(6): 2852-9.

Singh J, Colussi R, McCarthy OJ, Kaur L. Potato starch and its modification. Singh J, Kaur L eds. In: Advances in Potato Chemistry and Technology. London: Academic Press, 2016; pp. 195-247.

Theurer CB. Grain processing effects on starch utilization by ruminants. J Anim Sci 1986; 63(5): 1649-62.

Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci 1991; 74(10): 3583-97.

Wang M, Jiang J, Tan ZL, Tang SX, Sun ZH, Han XF. *In situ* ruminal crude protein and starch degradation of three classes of feedstuffs in goats. J Appl Anim Res 2009; 36(1): 23-8.

Tjardes K, Buskırk D, Allen M, Ames N, Bourquın L, Rust S. Brown midrib-3 corn silage improves digestion but not performance of growing beef steers. J Anim Sci 2000; 78(11): 2957-65.

Zhao C, Liu G, Li X, Guan Y, Wang Y, Yuan X, Sun G, Wang Z, Li X. Inflammatory mechanism of Rumenitis in dairy cows with subacute ruminal acidosis. BMC Vet Res 2018; 14(1): 1-8.

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Prevalence of Cystic Echinococcosis in Cattle Slaughtered in Kastamonu Slaughterhouse and Its Importance in Turkish Economy*

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Abstract: This study was carried out to determine the prevalence of cystic echinococcosis (CE) in cattle slaughtered in the slaughterhouse of Kastamonu Municipality, where cattle breeding is common and to determine the economic loss in the country's economy due to this disease. A total of 5511 bovine livers were examined postmortem for CE between January and December 2021. Of the 5511 cattle, 3872 are male and 1639 are female. In the postmortem examination, the livers of the animals were examined and the number of animals with CE was calculated to be 524 and the prevalence of the disease was 9.51%. In animals, the highest monthly prevalence was observed in July with 14.85% and the lowest prevalence in December with 3.94%. CE was the lowest with 4.94% in winter and the highest with 13.68% in summer. CE was found to be the lowest in male animals with 4.44% in winter and the highest prevalence in summer with 12.70%. The prevalence of the disease in female animals was 6.79% in winter and 21.32% in summer. Livers with CE were destroyed regardless of infection intensity. As a result of this study, with the destruction of 524 livers, 136240 TL (13859 USD) was lost to the Turkish economy. It has been stated that CE is common in Kastamonu as in Turkey and causes great economic losses.
Keywords: Cattle, echinococcosis, economic loss, Kastamonu, zoonosis

Kastamonu Mezbahanesinde Kesilen Sığırlarda Cystic Echinococcosis'in Prevalansı ve Türkiye Ekonomisindeki Önemi

Öz: Bu çalışma, büyükbaş hayvancılığın yaygın olduğu Kastamonu ilinde Belediyeye ait mezbahanede kesilen sığırlarda Kistik Echinococcosis'in prevalansının araştırılması ve bu hastalık nedeniyle ülke ekonomisindeki ekonomik kaybın belirlenmesi amacıyla yapılmıştır. Ocak-Aralık 2021 tarihlerinde toplam 5511 adet sığır karaciğeri kistik echinococcosis yönünden postmortem olarak incelenmiştir. 5511 sığırın 3872 tanesi erkek, 1639 tanesi ise dişidir. Postmortem muayenede hayvanların karaciğerleri incelenmiş kistik echinococcosisli hayvan sayısının 524 olduğu ve hastalığın prevalansının %9.51 olduğu hesaplanmıştır. Hayvanlarda aylık olarak en yüksek prevelans %14.85 ile Temmuz ayı, en düşük prevalans ise %3.94 ile Aralık ayı olarak görülmüştür. Kış mevsiminde %4.94 olarak en düşük, yaz mevsiminde % 13.68 oran ile en yüksek Kistik Echinococcosis belirlenmiştir. Erkek hayvanlarda kış mevsiminde Kistik Echinococcosis sayısında %4.44 ile en düşük, %12.70 ile ise yaz mevsiminde en yüksek prevalans tespit edilmiştir. Hastalığın prevalansı dişi hayvanlarda kış mevsiminde %6.79 olarak görülmüş, yaz mevsiminde ise %21.32 olarak belirlenmiştir. Kistik echinococcosisli karaciğerler enfeksiyon yoğunluğuna bakılmaksızın imha edilmiştir. Bu çalışmanın sonucu olarak, 524 adet karaciğerin imhasıyla beraber 136240 TL (13859 USD) Türkiye ekonomisinde kayıp şekillenmiştir. Kistik echinococcosisin Türkiye genelindeki gibi Kastamonu ilinde de yaygın olduğu ve büyük ekonomik kayıplara sebep olduğu açıklanmıştır.
Anahtar kelimeler: Echinococcosis, ekonomik kayıp, Kastamonu, sığır, zoonoz

Introduction

The amount of animal protein consumed per capita is one of the most important criteria for the development level of countries. In underdeveloped and developing countries, the increase in animal product production is less than the increase in population. Regardless of the level of development, ensuring the food security of the society is one of the basic responsibilities of every country. Animal husbandry is a sector that makes significant contributions to the country's economy and it is necessary to provide the highest level of importance for food safety in our country. Ensuring the continuity of food security is one of the cornerstones for the development of the country. It is known that parasitic infections cause low yield in cattle bree-

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ding (Uslu et al., 2021; Kücükyağlıoğlu and Uslu, 2022).

Echinococcus granulosus, which is the causative agent of the disease, is a helminthozoonosis caused by larval form of hydatid cyst, which lives in the small intestines of dogs, wolves, jackals and other canidae and develops in mammals such as sheep, goats, cattle and humans as intermediate hosts (Umur and Aslantas, 1993; Altintas, 1998; Senlik, 2012). In addition to the environment, host and intermediate host factors, human habits also have a great impact on the spread and interregional differences of CE (Yıldız and Tuncer, 2005).

Symptoms of CE can cause serious losses in labor and economy. It can also result in death in humans and animals. The prevalence of CE in humans in Turkey has been reported as 1/2.000 (Acıöz et al., 2021). In addition to the economic loss in the destruction of livers infected with CE, the cost of treatments applied in human medicine is also important as it causes morbidity and mortality (Balkaya and Simsek, 2010).

Reasons such as illegal slaughtering of animals, stray dogs, inadequate slaughterhouse conditions and inability to destroy diseased organs play a role in the spread of the disease. The spread of the disease occurs through irregular parasitic applications of domestic dogs and close contact with stray dogs (Arslan and Umur, 1997).

Diagnosis is difficult as the disease does not show significant symptoms in intermediate hosts such as cattle, sheep and goats (Senlik, 2000). It has been reported that using ultrasonography in diagnosis is not cost effective and practical (Eckert and Deplazes, 2004). The definitive diagnosis of the disease is made through postmortem examination (Senlik, 2012).

Studies showing the prevalence of CE by months and seasons are limited in our country and in the world (Azami et al., 2013). It has not been determined that any research has been carried out in Kastamonu regarding CE. It is thought that this study conducted in Kastamonu will contribute to the literature.

In the study we conducted in Kastamonu, where livestock breeding is common, it was aimed to determine the damage to the country's economy by grouping the prevalence of CE in cattle slaughtered in abattoirs according to month, season and gender.

Material and Method

Study area

According to the data of the Turkish Statistical Institute, Kastamonu province has an important place in the country's livestock with 276859 cattle in 2021 (TSI, 2021).

Study period and animals

The research was carried out in cattle slaughtered in the slaughterhouse of Kastamonu Municipality between January and December 2021.

In this study, postmortem examination of 5511 bovine livers for CE was performed. After postmortem examination, domestic and imported beef and dairy cattle slaughtered were evaluated by classifying them according to gender, month and season.

Postmortem examinations

In the study, CE in other organs was not included in the evaluation since CE is mostly found in the liver and the economic value of the liver is high.

During the examinations of the internal organs, the liver was first examined macroscopically, then by superficially stroking and applying pressure, the presence of hardness and swelling in the inner parts of the liver, as well as the presence of a section with a knife, the internal parts of the organ were examined for CE. All infected livers were destroyed regardless of the number of cysts and the degree of infection.

Calculation of economic losses

The damage caused to the national economy due to the destruction of the liver infected with CE was determined as 65 TL/kg on average and the average liver weight of cattle was 4 kg over the offal prices in 2021. Since the entire liver is destroyed regardless of the degree of infection, the economic loss caused by CE in an animal was calculated as 65x4kg=260 TL on average.

Total economic loss was calculated following, formula; TEL = NLCE X CPL

TEL: Total economic loss (TL)

NLCE: Number of livers with CE

CPL: Current price of the liver

Data management and statistical analysis

Excel program was used in the documentation process and the data were; The comparison of CE according to months, seasons and genders was evaluated by performing a Fisher’s exact chi-square and Bonferroni corrected Post Hoc tests in the

''SPSS29.0 statistical program (IBM Corporation, Armonk, New York, USA.)''. For the significance level of the tests, P<0.001 was accepted. Categorized data are shown with frequency and percentage values.

Results

In the study, 3872 of the 5511 cattle slaughtered in Kastamonu Municipality slaughterhouse are male and 1639 of them are females. Livers of slaughtered animals were examined for CE in postmortem examination.

Table 1 shows data on the number of cattle slaughtered and the number of infected animals by month. When the number of infected animals was analyzed by months, a statistically significant difference was found (P<0.001). Letters that differ from each other are categories with statistically significant differences. Significant for animals infected between January (4.17), February (7.02), March (10.93), April (9.94), May (13.36), June (12.24), July (14.85), August (13.62) and September (10.13). difference was found. However, there is no significant difference between January and October (6.12), November (4.69) and December (3.94). In the postmortem examination, it was determined that the number of animals with CE was 524 and the prevalence of the disease was 9.51%. The month with the highest prevalence was found to be July with 14.85% and the lowest prevalence was determined as December with 3.94%.

Table 2 shows the data on the number of cattle slaughtered and the number of infected animals according to the seasons. When the number of infected animals was analyzed by months, a statistically significant difference was found (P<0.001). Different letters next to the infection rates are the categories that have a statistical difference. While there was no statistically significant difference in the number of infected animals between January (10.9) and February (13.7), a difference was found between January and March (7.1) and April (4.9) (P<0.001). The rates of seasonally infected cattle with CE are indicated in the table as winter in December-January-February, spring in March-April-May, summer in June-July-August and autumn in September-October- November. In the study, it was observed that animals with CE were slaughtered at the lowest rate as 4.94% in the winter season and at the highest rate with 13.68% in the summer season

Table 1. Cystic echinococcosis numbers by months

Months	Number of Slaughtered Cattle	Number of Infected Animals	Infected Rate (%)
January	192	8	4.17^{a}
February	413	29	7.02^{b}
March	302	33	10.93^{c}
April	674	67	$9.94^{b,c}$
May	262	35	13.36^{d}
June	531	65	$12.24^{c,d}$
July	680	101	14.85^{d}
August	514	70	13.62^{d}
September	464	47	$10.13^{c,d}$
October	343	21	$6.12^{a,b}$
November	426	20	4.69^{a}
December	710	28	3.94^{a}
Total	5511	551	9.51

The difference between groups with different letters in the same column is statistically significant (P<0.001).
$^{a-d}$: There is a statistical difference between different letters and letter groups.

Table 2. Cystic echinococcosis numbers by seasons

Seasons	Number of Slaughtered Cattle	Number of Infected Animals	Infected Rate (%)
Spring	1238	135	10.9^{a}
Summer	1725	236	13.7^{a}
Autumn	1233	88	7.1^{b}
Winter	1315	65	4.9^{b}

The difference between groups with different letters in the same column is statistically significant (P<0.001).
^{a,b}: There is a statistical difference between different letters and letter groups.

Table 3 shows data on the number of male cattle slaughtered by season and the number of infected male animals. When the number of infected animals was analyzed by months, a statistically significant difference was found (P<0.001). Different letters next to the infection rates are the categories that have a statistical difference. There was a significant difference in the number of infected male animals between spring (9.8) and summer (12.7) and winter (4.4) in terms of infected male animals but there was no significant difference between spring and autumn (6.7) and winter (4.4) and autumn (6.7). The number of male cattle with CE is the lowest with 4.4% in winter and the highest prevalence with 12.7% in summer.

Table 3. Cystic echinococcosis numbers in male animals by seasons

Seasons	Number of Slaughtered Cattle	Number of Infected Animals	Infected Rate (%)
Spring	1080	106	9.8[a]
Summer	1528	194	12.7[b]
Autumn	1148	77	6.7[a,c]
Winter	1035	46	4.4[c]

The difference between groups with different letters in the same column is statistically significant (P<0.001).
[a-c]: There is a statistical difference between different letters and letter groups.

Table 4 shows data on the number of female cattle slaughtered by season and the number of infected females. When the number of infected animals was analyzed by months, a statistically significant difference was found (P<0.001). Different letters next to the infection rates are the categories that have a statistical difference. There was a significant difference in the number of infected female animals between spring (18.4) and winter (6.8) in terms of infected female animals but there was no significant difference between spring and summer (21.3) and autumn (12.9). While the prevalence was low in female animals at 6.79% in winter, the prevalence was calculated as 21.32% in summer.

Table 4. Cystic echinococcosis numbers in female animals according to seasons

Months	Number of Slaughtered Cattle	Number of Infected Animals	Infected Rate (%)
Spring	158	29	18.4[a]
Summer	197	42	21.3[a]
Autumn	85	11	12.9[a,b]
Winter	280	19	6.8[c]

The difference between groups with different letters in the same column is statistically significant (P<0.005).
[a-c]: There is a statistical difference between different letters and letter groups.

Regardless of the density or type of cysts (small, large, calcified) in the livers with infection in the study, all of them were destroyed. A total of 524 livers were destroyed and since the price of a cattle liver in 2021 was 260 TL, the total loss was calculated as 260x524=136 240 TL. When the USD exchange rate is calculated as 9.83 TL on average during the dates of the study, it is seen that the economic damage to the country is approximately 13859 USD.

Discussion and Conclusion

The CE, which has a high spread in Turkey, causes serious economic losses as well as the damage it causes to animal and human health (Düzlü et al., 2010). CE which has a high spread in Turkey, causes serious economic losses as well as the damage it causes to animal and human health (Düzlü et al., 2010). The prevalence of CE in cattle was observed at low rates of 0.002%-8.28% in countries such as Nigeria, Brazil, China, Saudi Arabia and Iran (Onah et al., 1989; Artures et al. 1996; He and Wang, 2001; Ibrahim 2010; Azami et al., 2013), and at rates of 11.3%-19.4% in countries such as Libya, India, Iran, Kenya and Ethiopia (Sarma et al., 2000; Dalimi et al., 2002; Njoroge et al., 2002; Tashani et al., 2002; Kumsa 2019). While the prevalence is as low as 0.21%-9.4% in Turkey's Muğla, Kayseri, Sivas, Kars, Elâzığ, Konya and Ankara provinces (Öge et al., 1998; Acıöz et al., 2008; Düzlü et al., 2010; Demir and Mor, 2011; Baspınar et al., 2014; Acıöz et al., 2021; Kücükyağlıoğlu and Uslu, 2022). It was determined at higher rates such as 11.6%-56.5% in the Thrace region, Burdur, Kırıkkale, Samsun, Afyonkarahisar, Kars, Sivas, Van and Erzurum provinces (Toparlak and Gül, 1989; Celep et al., 1990; Umur and Aslantas, 1993; Yıldız and Tuncer, 2005; Ulutaş Esatgil and Tüzer, 2007; Köse and Kırcalı Sevimli, 2008; Balkaya and Simsek, 2010; Erol et al., 2021). The result obtained in this study (9.51%) was found to be close to the rates in Saudi Arabia (8.28%) and

Ankara in Turkey (9.4%).

Although CE is widely distributed in the world, CE cases are more common especially in rural areas of underdeveloped countries (Dar and Alkarmi, 1997). The prevalence of CE, which is seen in almost every region of the world, varies from country to country. The climate of the region, ecological structure, animal breeding methods, age and species of animals, techniques used in researching their prevalence and data collection etc. many biotic and abiotic factors such as CE are thought to be effective in determining the prevalence (Senlik, 2000; Eckert et al., 2004; Düzlü et al., 2010; Almalki et al., 2017). The reasons for this difference are as follows. In studies carried out to date, it has been determined that butchers do not have enough information about how CE disease occurs (Aydın et al., 2015). In the study conducted in Iran, they detected CE at the most 7.89% in the spring and at least 4.6% in the winter season; (Azami et al., 2013) in our study, the highest prevalence was 13.68% in summer and 4.94% in winter season. In another study in Elâzığ, they reported highest prevalence in winter with 9.87% and the lowest prevalence as 4.17% in spring (Baspınar et al. 2014). Studies indicating the prevalence of CE by months and seasons are limited. While it was reported that the prevalence of CE was higher in October in Algeria, the highest rate of infection was observed in July in our study (Ayad et al., 2019). Although the rates detected in the winter months in our study are similar to the rates in the winter months in Iran, we think that it is not possible to draw a consistent conclusion according to seasons and months because many factors are effective in the spread of the disease.

In a study conducted in Aydın, the prevalence of CE was reported to be 2.09% in males and 14.31% in female cattle. In this study, it was determined that it was 8.83% in males and 14.02% in female cattle. Although the prevalence in female cattle was similar to the study conducted in Aydın, it was observed that the rate in males was 4 times on average (Bağdatlıoğlu, 2019). It is thought that the reason for this difference is the number of slaughtered animals and the excess of males in livestock.

It has been reported that CE causes economic damage of 4 billion USD worldwide (Uslu et al., 2021). In a country-wide study, it was reported that the loss of CE, which causes a decrease in meat, milk, fleece and fertility in ruminants, to the country's economy in 2008 was 89.2 million USD (Sarıözkan and Yalcın, 2009). In a study conducted in Erzurum, in the CE examination of 1066 sheep and 530 cattle after slaughter, the infection rate in cattle was reported as 46.41% and the economic loss was calculated as 2300 USD (Arslan and Umur, 1997). Although it is seen that there are infected animals about 5 times as many as our study, it is seen that the economic loss is below our study. In a study conducted in Konya, the prevalence of hydatid cysts in cattle was reported as 9.40% (Gıcık et al., 2004). Although the rate of 9.40% in Konya is similar to the rate of 9.51% in our study, it is different from our study when the CE infection values are examined by month. The prevalence was highest in October (75.3%) and lowest in June (15.1%). It has been calculated that the destruction of the infected liver and lungs causes an annual economic loss of 52 264 USD (Dik et al., 1992). This value shows that there is a high economic loss according to our study. In the study conducted in the same region in Konya in cattle, it was reported that the prevalence of hydatid cyst was around 5.60% (Civi et al., 1995). In a study conducted by Küçükyağlıoğlu and Uslu (2022) on 49 545 cattle between 2018 and 2019 in Konya, it was reported that the livers of 1947 (3.93%) cattle were destroyed and an economic loss of 56 434 USD (384 400 TL) occurred. Although the rate seems low according to our research, the total value of the economic loss due to the excess amount of animals examined was found to be higher than our study (Kücükyağlıoğlu and Uslu, 2022). In the study conducted to investigate the economic losses caused by CE in ruminant animals in Burdur province, an annual loss of 583 USD was observed with the destruction of the infected liver and lungs of 183 cattle out of 1355 cattle (Umur and Aslantas 1993). Considering the ratio, 13.5% is higher than our study but it remains low in terms of USD.

Hydatic cyst is a zoonotic disease that continues to be an important problem in terms of public health in many parts of the World and our region. Control and prevention measures should be carried out together to prevent the spread of the disease. For the control of the disease, public awareness, control and treatment of dogs, prevention of offal and raw meat consumption by dogs, regular inspection and effective control of slaughterhouses, cooperation with public health authorities for the eradication of the disease show the importance of working. For this purpose, uncontrolled animal slaughter should not be done. In addition, infected organs should be disposed of properly (burned in ovens, buried in deep pits) and never fed to dogs. Considering the life cycle of the parasite, the routine examination and treatment of stray dogs by municipalities or private veterinary clinics is also important for public health. Legal animal slaughter should be adopted as a control measure and stray dogs roaming around the slaughterhouse should be removed if possible.

It has been observed that the dog population has increased abnormally in our country in recent years and it has been determined that the streets, walking areas and parks are wandered by stray dogs. We think that hydatidosis transmitted from these animals will threaten public health more in the coming years and cause much higher economic losses.

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References

Acıöz M, Çeliksöz A, Özçelik S, Değerli S. Prevalence of hydatid cyst in cattle slaughtered between April and May 2005 in Sivas. Turkiye Parazitol Derg 2008; 32(3): 205-7.

Acıöz M, Bozkaya F, Zorbozan H, Yılmaz AI. Economic importance of hydatid cyst in slaughtered cattle of Muğla province. Turkiye Parazitol Derg 2021; 45(2): 117-20.

Almalki E, Al-Quarishy S, Abdel-Baki AS. Assessment of prevalence of hydatidosis in slaughtered Sawakny sheep in Riyadh city, Saudi Arabia. Saudi Journal of Biological Sciences 2017; 24: 1534-7.

Altintas N. Cystic and alveolar echinococcosis in Turkey. Ann Trop Med Parasitol 1998; 92(6): 637-42.

Arslan MO, Umur S. The distribution and economic importance of hydatidosis in sheep and cattle slaughtered in Erzurum slaughterhouses. Kafkas Univ Vet Fak Derg 1997; 3(2): 167-1.

Artures CF, Souza RM, Ribeiro RPM. Prevalence and geographical distrubution of bovine hydatidosis in the state of Minas Gerais, Brasil. Arg Bras Med Vet Zootec 1996; 48(5): 623-7.

Ayad A, Benhanifia M, Balla EH, Moussouni L, Ait-Yahia F, Benakhla AA. Retrospective survey of fasciolosis and hydatidosis in domestic ruminants based on Abattoirs' Data in Bejaia Province, Algeria. Veterinaria 2019; 68(1): 47-51.

Aydın MF, Gökmen S, Koc S, Adıgüzel E, Kocaman H, Cöplü M, Sahin A. Evaluation the knowledge levels regarding hydatid cyst among butchers in Karaman province of Turkey. Van Vet J 2015; 26 (3): 147-50.

Azami M, Anvarinejad M, Ezatpour B, Alirezaei M. Prevalence of hydatidosis in slaughtered animals in Iran. Turkiye Parazitol Derg 2013; 37(2): 102-6.

Bağdatlıoğlu AI. Aydın yöresinde mezbahada kesilen sığırlarda bazı cestod larvalarının (*Hidatid kist, Cysticercus bovis, Cysticercus tenuicollis*) yayılışı, Yüksek lisans tezi, Aydın Adnan Menderes Üniv Sağ Bil Ens, Aydın 2019; p.8.

Balkaya I, Simsek S. Prevalence and economic importance of hydatidosis and fasciolosis in slaughtered cattle in Erzurum province of Turkey. Kafkas Univ Vet Fak Derg 2010; 16(5): 793-7.

Baspınar S, Kaplan M, Kelestemur N. The Prevalance of cystic echinococcosis in live stock slaughtered between 2008-2012. Fırat Univ Health Sci Vet J 2014; 28(2): 89-92.

Celep A, Açmam M, Çetindağ M, Coşkun SZ, Gürsoy S. Helminthological studies on cattle from the Samsun region. Etlik Vet Microbiol J 1990; 6(6): 117-30,

Civi S, Güler S, Kesci S. According to the records of Konya Meat And Fish Institution and Konet facilities, economic losses due to hydatid cyst. Turkiye Parazitol Derg 1995; 19(2): 237-42.

Dalimi A, Motamedi GH, Hosseini M, Mohammadian B, Malaki H, Ghamari Z, Far FG. Echinococcosis/ hydatidosis in Western Iran. Vet Parasitol 2002; 105(2):161-71.

Dar FK, Alkarmi T. Public health aspects of cystic echinococcosis in the Arab countries. Acta Trop 1997; 67(1-2): 125-32.

Demir P, Mor N. Kars Seasonal Distribution and economic importance of cystic echinococcosis in cattle slaughtered at Kars Municipal Abattoir, Turkey. Turkiye Parazitol Derg 2011; 35: 185-8.

Dik B, Cantoray R, Handemir E. the distribution and economic importance of hydatidosis in small and bovine animals slaughtered in Konya Meat and Fish Institution Combine. Turkiye Parazitol Derg 1992; 16: 91-9.

Düzlü O, Yıldırım A, Sarıözkan S, Inci A. The Economic importance of cystic echinococcosis in sheep and cattle from three different slaughterhouses in Kayseri region. Erciyes Univ Vet Fak Derg 2010; 7(1): 7-11.

Eckert J, Deplazes P. Biological, epidemiological and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev 2004; 17: 107-35.

Erol U, Urhan OF, Şahin ÖF, Altay K. Sivas ilinde sığırlarda kist hidatik'in yaygınlığının araştırılması . EDUVET Uluslarası Veteriner Bilimleri Kongresi, 25 -27 Haziran, 2021; Online-Türkiye.

Gıcık Y, Arslan MÖ, Kara M, Köse M. The prevalence of cystic echinococcosis in cattle and sheep slaughtered in the Kars province. Turkiye Parazitol Derg 2004; 28(3): 136-9.

He DL, Wang HA. Report on the epidemiological evaluation of hydatid disease in zeku county, qinghai province. Endem Dis Bull 2001; 16(4): 36-

8.

Ibrahim MM. Study of cystic Echinococcosis in slaughtered animals in Al Baha Region, Saudi Arabia: Interaction between some biotic and abiotic factors. Acta Trop 2010; 113(1): 26-33.

Köse M, Kırcalı Sevimli F. Prevalence of cystic echinococcosis in slaughtered cattle in Afyonkarahisar. Turkiye Parazitol Derg 2008; 32(1): 27-30.

Kumsa B. Cystic Echinococcosis in slaughtered cattle at addis ababa abattoir enterprise, Ethiopia. Vet Anim Sci 2019; 7: 100050.

Kücükyağlıoğlu A, Uslu U. Prevalence and economic significance of hidatidosis in cattle slaughtered at an abattoir in Konya, Turkey. Turkiye Parazitol Derg 2022; 46(3): 207-12.

Njoroge EM, Mbithi PMF, Gathuma JM, Wachira TM, Gathura PB, Magambo JK, Zeyhle EA. Study of cystic echinococcosis in slaughter animals in three selected areas of northern Turkana, Kenya. Vet Parasitol 2002; 104(1): 85-91.

Onah DN, Chiejina SN, Emehelu CO. Epidemiology of echinococcosis/hydatidosis in Anambra State, Nigeria. Ann Trop Med Parasitol 1989; 83(4): 387-93.

Öge H, Kalınbacak F, Gıcık Y, Yıldız K. Ankara yöresinde kesilen koyun, keçi ve sığırlarda bazı metasestodların (*Hidatid Kist, Cysticercus tenuicollis, Cysticercus bovis*) yayılışı. Ankara Univ Vet Fak Derg 1998; 45: 123-30.

Sarıözkan S, Yalcın C. Estimating the production losses due to cystic echinococcosis in ruminants in Turkey. Vet Parasitol 2009; 163(4): 330-4.

Sarma MD, Deka DK, Borkakoty MR. Occurrence of hydatidosis and porcine cysticercosis in Guwahati city. J Vet Parasitol 2000; 14(2): 173-4.

Senlik B. Prevalence of hydatidosis and its relationship with age, race and gender in sheep from Bursa region. Turkiye Parazitol Derg 2000; 24: 304-8.

Senlik B. Echinococcosis. Turk Clinics J Vet Sci 2012; 3(2): 88-96.

Tashani OA, Zhang LH, Boufana BA, Jegi A, McManus DP. Epidemiology and strain characteristics of *Echinococcus granulosus* in the Benghazi area of Eastern Libya. Ann Tropic Med Parasitol 2002; 96(4): 369-81.

Toparlak M, Gül Y. The prevalence of hydatidosis in animals slaughtered in the municipal slaughterhouse in Van province. Ankara Univ Vet Fak Derg 1989; 36(1): 129-37.

Turkish Statistical Institute (TSI). Databases, Livestock Statistics. https://biruni.tuik.gov.tr/medas/?kn=101&locale=tr; Accessed Date: 29.05.2022.

Ulutaş Esatgil M, Tüzer E. Prevalence of hydatidosis in slaughtered animals in Thrace, Turkey. Turkiye Parazitol Derg 2007; 31(1): 41-5.

Umur S, Aslantas O. The Spread and economic importance of hydatidosis in ruminants slaughtered in kars municipality slaughterhouse. Turkiye Parazitol Derg 1993; 17(2): 27-34.

Uslu U, Kücükyağlıoğlu A, Senlik B. Prevalence of liver hydatidosis and its economic significance in sheep slaughtered in a private abattoir in Konya. Turkiye Parazitol Derg 2021; 45(1): 5-10.

Yıldız K, Tuncer C. Prevalence of hydatid cysts in cattle in the province of Kırıkkale. Turkiye Parazitol Derg 2005; 29(4): 247-50.

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Relationship Between Growth Performance, Passive Immunity and Health In Preweaned Lambs*

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Abstract: This study was designed to investigate associations between transfer of passive immunity, selected illnesses (diarrhoea, pneumonia, and fatigue anorexia syndrome-FAS) and growth performance [body weight (BW) and average daily gain (ADG)] in preweaned lambs. A total of 347 lambs were blood sampled at 24±1 h of age after birth and the serum IgG concentration for each lamb was measured after colostrum intake using a commercial ELISA kit. Lambs were weighed on a scale immediately after birth (before colostrum intake) and reweighed on day 28 (end of neonatal period) and day 84 (age of weaning). Lambs ADG was calculated from 0 to 28 d of life, from 29 to 84d of life, and from 0 to 84 d of life. Growth performance (BW and ADG) of lambs with diarrhoea and FAS in the neonatal period and with pneumonia in the postneonatal period was significantly lower than that of healthy lambs of the same period (P<0.05). BW of lambs with serum IgG concentration at 24th hour after the birth (SIgGC-24)<600 mg/dL and <1000 mg/ml was significantly lower on days 28 and 84 than that of lambs with SIgGC-24 >600 mg/dL and >1000 mg/ml (P<0.001). Similarly, the ADG of lambs with SIgGC-24 <600 mg/dL and <1000 mg/ml was significantly lower on days from 0-28, 29-84 and 0-84 of age than those of lambs with SIgGC-24 >600 mg/dL and >1000 mg/ml (P<0.001). In conclusion, our findings show that low serum IgG concentration in lambs, as well as the presence of pneumonia, diarrhea and FAS, reduces growth performance in pre-weaning lambs.
Keywords: Growth, health, IgG cut off, lambs, performance

Sütten Kesilmiş Kuzularda Büyüme Performansı, Pasif Bağışıklık ve Sağlık Arasındaki Ilişki

Öz: Bu çalışmanın amacı, sütten kesim dönemi kuzularda pasif bağışıklık transferi ile bazı hastalıklar (ishal, pnömoni, fatigue anoreksi sendromu-FAS) ve büyüme performansı [vücut ağırlığı (BW) ve ortalama günlük ağılık artışı (ADG)] arasındaki ilişkiyi araştırmaktır. Toplam olarak 347 kuzudan kolostrum alınımından ya da doğumdan sonra 24±1 saat kan örnekleri toplanmış ve her bir kuzu için serum IgG konsantrasyonu ticari bir ELISA kiti kullanılarak ölçülmüştür. Kuzular doğumdan hemen sonra (kolostrum alımından önce) tartılmış ve 28. günde (neonatal dönemin sonunda) ve 84. günde (sütten kesim yaşı) tekrar tartılmıştır. Kuzuların ADG'si yaşamın 0 ila 28. günleri arasında, yaşamın 29 ila 84. günleri arasında ve yaşamın 0 ila 84. günleri arasında hesaplanmıştır. Neonatal dönemde ishalli ve FAS tanılı ve postneonatal dönemde pnömonili kuzuların büyüme performansı (BW ve ADG) aynı dönemdeki sağlıklı kuzulara göre önemli ölçüde düşük bulundu (P<0.05). Doğumdan sonraki 24. saatte serum IgG konsantrasyonu (SIgGC-24) <600 mg/dL ve <1000 mg/ml olan kuzuların canlı ağırlıkları 28. ve 84. günlerde SIgGC-24 >600 mg/dL ve >1000 mg/ml olan kuzulardan önemli ölçüde daha düşüktü (P <0.001). Benzer şekilde, SIgGC-24 <600 mg/dL ve <1000 mg/ml olan kuzuların günlük canlı ağırlık artışının 0-28, 29-84 ve 0-84 yaş günlerinde SIgGC-24 >600 mg/dL ve >1000 mg/ml olan kuzulardan önemli ölçüde daha düşüktü (P<0.001). Sonuç olarak, bulgularımız düşük serum IgG konsantrasyonunu ile birlikte pnömoni, ishal ve FAS bulunması kuzularda sütten kesim öncesi büyüme performansını azaltmaktadır.
Anahtar kelimeler: Büyüme performansı, IgG eşik değerleri, kuzu, sağlık

Introduction

Neonatal diseases are the most common problem in animal husbandry. Diseases such as diarrhea, pneumonia, and FAS in preweaning lambs cause significant economic losses every year due to high mortality rate, decreased growth performance and treatment expenses (Gökçe et al., 2014; Kenyon et al., 2019). Preweaning colostrum management is an important predictor of lamb health and growth performance and should be assessed regularly. The first step in in-

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creasing farm yield and profitability is to ensure adequate passive transfer (Elsohaby et al., 2019). Growth in the preweaning period is influenced by many different factors, including passive colostral immunity, disease, nutrition, management practices, and environmental conditions.

The syndesmochorial structure of the ovine placenta does not allow transfer of maternal immunoglobulins to the fetus during pregnancy. Therefore, the lamb is a gammaglobulinemic at birth and dependent on passive transfer of IgG from maternal colostrum within the first hours of life. The transfer of maternal antibodies is critical for protection of new born lambs as their immune systems is not yet fully competent. The passive transfer of colostral immunity occurs through the absorbtion of colostral Ig by pinocytosis in the small intestinal epithelium (Kozyr et al., 2019). Newborn lambs absorb colostral IgG during the first 24 hours after birth; after which the absorption capacity decreases over time as the digestive system matures. Therefore, the 24th hour after birth is the best time to determine the passive immune status in the lamb sera (Alves et al., 2015). Studies suggest that adequate IgG absorption is associated with reduced morbidity due to infection and therefore reduced use of antibiotics, and improved growth performance (Aydogdu et al., 2018; Agenbag et al., 2021; Johnson et al., 2022). Thus, determination of serum IgG concentrations in new born lamb is essential in order to take appropriate measures to reduce morbidity, mortality and improve growth performance (Vatankhah, 2013; Santiago et al., 2020). However, to date, there has been little research into developing strategies for optimizing passive immunity transfer in lambs, especially in Turkey.

Low serum IgG concentration absorbed from colostrum is defined as failure of passive transfer (FPT) (Tsiligianni et al., 2012; Hernández-Castellano et al., 2014; Gökçe et al., 2021a). FPT has been associated with many neonatal ruminant diseases, including the respiratory system, diarrhea, septicemia, and omphalophlebitis (Herndon et al., 2011). FPT also increases the duration of illness, contagiousness, or pathogen shedding, and reduces growth in preweaning lambs (first few months of life) (Massimini et al., 2006; Andres et al., 2007; Turquino et al., 2011, Atkinson et al., 2017, Bond, 2020). Recommended best practices to prevent FPT include feeding a minimum of 50 ml/kg of colostrum within 6 h of lambing in a total of 150-290 ml/kg in 18-24 h, to have sufficient passive immunity (Aydoğdu et al., 2018).

There is no internationally accepted threshold value for FPT in lambs. For this reason, studies to date have generally used two different thresholds of IgG <600 and <1000 mg/dl at 24 hours after birth, indicating inadequate passive transfer (Gilbert et al., 1988; Britti et al., 2005; Gökçe et al., 2019). Poor health and growth in young lambs can have lasting effects on their development and future production. Improving growth performance also motivates producers. The effect of diseases such as pneumonia and diarrhoea on the growth performance of lambs is not well understood. This may be due to the length of time required for disease detection, health examination and observation.

This study was designed to determine the relationship between the transfer of passive immunity (24^{th} h postpartum IgG concentration) and growth performance (weight gain-WG and average daily gain-ADG) in preweaned dairy lambs in relation to selected diseases, as there seems to be a lack of knowledge about it.

Materials and Methods

The study protocol was authorized by the Institutional Ethics Committee for the Care and Use of Animals, Kafkas University (KAU-HADYEK, 2008-23).

Animals

The details of the study design are given elsewhere (Gokce et al., 2013). Briefly, a longitudinal observational study was designed in which 347 Akkaraman crossbred lambs from two neighbouring farms with similar management practices and feeding regimes in Kars, Turkey, agreed to participate. All ewes were housed as set out by management procedures of the farms. Ewes were not given any drugs or other substances during gestation or parturition. Flock management was typical of the region, with lambs being born in winter (December to February) or spring (March to May) and was supervised by stockmen during the entire lambing period and lambs were allowed to suckle colostrum on their own within 24 hours of birth. Lambs and their dams were housed in individual pens for up to seven days, after which the lambs were, then moved to group pens, allowed to suckle twice a day (morning and evening), fed hay only for three weeks after the first week of life, and straw and commercial growth feed (Bayramoglu AS, Türkiye) in addition to hay for three months. The lambs were intensively reared.

Daily weight gain

The study included only lambs that were regarded as healthy after birth. Plastic ear tags were placed on each ear of the lambs shortly after birth. The lambs were weighed at birth before colostrum intake (n=347) using a scale [CASIA DB2-150 kg (±30 g)] and then let to naturally suckle their dams. The lambs were not given any vaccinations, drugs or other substances during the study period. The lambs were weighed again on day 28 (at the end of the neonatal period, n=291) and day 84 (weaning time, n=290) using the same scale. Lambs ADG was calculated

from 0 to 28 d of life, from 29 to 84d of life, and from 0 to 84 d of life as previously reported (Elsohaby et al., 2019).

Blood sampling

All lambs were blood sampled at 24±1 h after birth, provided they had received colostrum, by jugular vein puncture into an 8.5 mL clot-activated tube (BD Vacutainer, BD, Franklin Lakes, NJ). Serum was obtained following centrifugation at 4000 rpm for 30 minutes and stored at -20^{o}C until analyses.

IgG assays

A commercially available ELISA test (Bio-X Competitive ELISA Kit for Ovine blood serum IgG Assay-BIO K 350, Bio-X Diagnostics, Belgium) was used to measure serum IgG concentrations in lambs. The test was performed and interpreted according to the manufacturer's instructions.

Clinical examination

Routine clinical examinations of lambs were undertook to determine clinical problems (diarrhea, pneumonia, suspected septicemia, fatigue-anorexia syndrome, other or unknown) in neonatal lambs based on case definition as previously described (Gökçe and Erdoğan, 2009). Lamb health was monitored on daily visits during the neonatal period (0-28 days) and every two days after neonatal period until weaning.

Statistical analysis

Data was entered on to a database (Microsoft access). The distribution of the data was tested for conformity to a normal distribution by the Shapiro-Wilk test. Mean ± SE (standard error) values for serum IgG concentrations and growth performance (ADG and WG) was calculated. The results of clinical examination were categorized in term of health as healthy and sick and life period as the neonatal (first four weeks of life) and postneonatal (the period from 5 to 12 weeks after birth). An independent two-sample t-test was used to compare SIgGC-24 and in different categories of health status in both periods. In addition, the same test was used to compare growth performance (ADG and WG) and different categories of health status such as diarrhea and pneumonia in neonatal and postneonatal periods. An independent two-sample t- test was used to compare the growth performance (ADG and WG) of the lambs in neonatal and postneonatal period according to the post-colostral (24th hour after birth) IgG cut-off point (<600 versus >600 and <1000 versus >1000 mg/dl) (Gilbert et al., 1988; Britti et al., 2005; Gökçe et al., 2019). Correlations between growth performance and SIgGC-24 for selected diseases were determined using Pearson correlation test. Lambs that died, sold or not measured between the two periods were excluded from the growth performance analyses. P value <0.05 was considered as significant.

Results

Health status

The morbidity and mortality rates in the neonatal period were 17.3% (60/347) and 3.7% (13/347) respectively. The proportions of diarrhoea, pneumonia, suspected septicaemia and fatigue anorexia syndrome (FAS) in neonatal lambs were 9.2% (32/347), 1.7% (6/347), 3.2% (11/347) and 3.2% (11/347) respectively. Of the deaths in this period, 10 lambs died of suspected of septicemia and 9 in the first week of life.

The proportions of sick and dead lambs in the post neonatal period were 32.4% (108/333) and 4.5% (15/333) respectively. Most common postneonatal health problems were diarrhoea (18.6%, 62/333), pneumonia (7.5%, 25/333), suspected septicemia (1.2%, 4/333) and others/unknown causes (5.1%, 17/333).

Growth performance and IgG concentrations

The serum IgG concentration at 24 hours after birth (SIgGC-24) ranged from 8 to 5302 mg/mL (2199±1160). The mean live body weight measured at birth, 28 and 84 days after birth was 4.06±0.646 g (2.260 to 5.900 g), 9.281±1.164 g (5.800 to 14.020 g) and 20.789±4.057 g (11.850 to 29.800 g), respectively. Mean ADG for 0-28, 29-84 and 0-84 days were 0.184±0.047 g/d (0.103 to 0.449 g/d), 0.210±0.044 g/d (0.105 to 0.449g/d), and 0.247±0.048 g/d (0.141 to 0.355 g/d), respectively.

A comparison of growth performance in relation to the health status of pre-weaned lambs is shown in Table 1. Growth performance (BW and ADG) of lambs with diarrhoea and FAS in the neonatal period and with pneumonia in the postneonatal period was significantly lower than that of healthy lambs of the same period (P<0.05).

Changes in growth performance at different serum IgG thresholds are shown in Figure 1. The BW of lambs with SIgGC-24 <600 mg/dL was significantly lower (P<0.001) at 28 and 84 days of age (5.91 kg and 14.26 kg, respectively) than that of lambs with SIgGC-24 >600 mg/dL (8.91 kg and 20.53 kg, respectively) (Figure 1A). Similarly, lambs with SIgGC-24 <600 mg/dL had significantly lower ADG from days 0-28, 29-84 and 0-84 of age (0.07 g, 0.16 g and 0.17 g, respectively) than lambs with SIgGC-24 >600 mg/dL on the same days (0.18 g, 0.21 g and 0.24 g, respectively) (P<0.001) (Figure 1B).

Lambs with SIgGC-24 <1000 mg/dL had significantly lower BW on days 28 and 84 (7.14 kg and 16.19 kg, respectively) than those with SIgGC-24 >1000 mg/dLon the same days (8.98 kg and 20.72 kg, respectively), (P<0.001) (Figure 1C). Similarly, lambs with

Table 1. Growth performance of pre-weaned lambs in relation to health status

	Period*			
	Neonatal (first 4 weeks after birth)		Postneonatal (5-12 weeks after birth)	
Clinical Diagnosis	**ADG (g/day) (Mean ± SE)**	**BW (kg) (Mean ± SE)**	**ADG (g/day) (Mean ± SE)**	**BW (kg) (Mean ± SE)**
Diarhoea	0.137±0.01 (n=30) **P<0.001**	7.7±0.39 (n=30) **P=0.002**	0.173±0.007 (n=60) **P<0.001**	9.73±0.41 (n=60) **P<0.001**
Pneumonia	0.113±0.03 (n=6) **P=0.126**	6.77±0.89 (n=6) **P=0.126**	0.182±0.009 (n=20) **P=0.018**	10.22±0.55 (n=20) **P=0.018**
FAS*	0.133±0.02 (n=9) **P=0.048**	7.03±0.76 (n=9) **P=0.049**	None	None
Other	None	None	0.193±0.006 (n=12) **P=0.326**	10.84±0.35 (n=12) **P=0.359**
Healthy (n)	0.178±0.003 (n=272)	9.09±0.11 (n=272)	0.28±0.003 (n=213)	12.15±0.19 (n=213)

Mean ± SE (standart error), n= number of lambs, None: no cases, ADG=Average Daily Gain, BW=Body Weight, FAS=Fatigue-Anorexia Syndrome (Mismothering, hypotermia and starvation), significantly different from healthy lambs. * Exclusion of lambs unavailable (death, sale, not measured) between neonatal and post-neonatal period accounts for the difference in numbers.*

SIgGC-24 <1000 mg/dL was significantly lower the ADG on days from 0-28, 29-84 and 0-84 age (0.13 g, 0.16 g and 0.19 g, respectively) than those with SIgGC-24 >1000 mg/dL (0.18 g, 0.21 g and 0.25 g, respectively), (P<0.001) (Figure 1D).

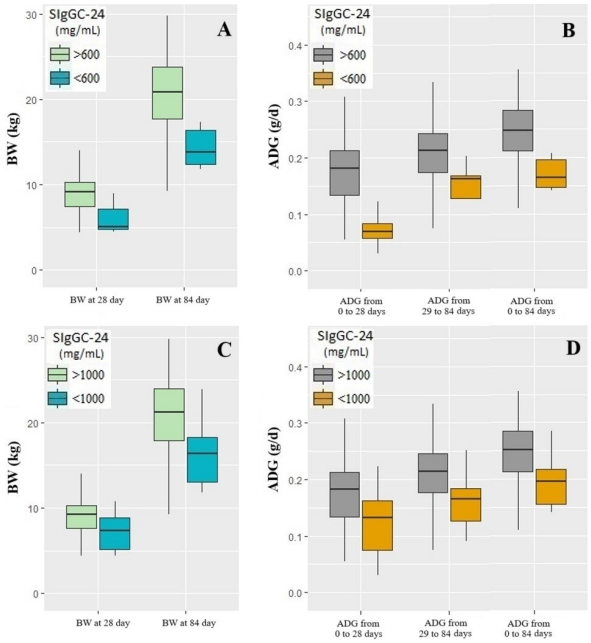

Figure 1: Box plots illustrating variability in (A, C) body weight (BW) at 28 days and weaning age; average daily gain (ADG) from 0 to 28, 29 to 84, and 0 to 84 days of life (B, D) in Akkaraman lambs with adequate transfer of passive immunity (IgG>600 and IgG>1000 mg/dL) and failure of transfer of passive immunity (IgG<600 and IgG<1000 mg/dL).

Discussion and Conclusion

Growth performance and morbidity rates have been shown to be suitable indicators for assessing lamb health and welfare at flock level. Growth performance reflects appropriate nutrition and feeding strategies for lambs, but it can be affected if lambs are sick or stressed. Colostrum management is also an important predictor of health and growth performance and should be assessed early in life. Passive transfer of colostral immunity (PTCI) may better reflect health and flock management, whereas FPT requires monitoring of individuals that may require additional treatment and preventive measures (Gökçe et al., 2019). In addition to the implications for infections, lambs with sufficient colostral immunoglobulins may develop a more efficient metabolic system and achieve normal growth, in contrast to lambs with FPT who have reduced feed intake (Massimini et al., 2007).

The present study evaluated passive immunity and diseases on growth performance of pre-weaned lambs. Studies to date have reported that there is no single threshold value of IgG indicating FPT in lambs (Gilbert et al., 1988; Britti et al., 2005; Gökçe et al., 2019). Therefore, two different previously suggested values threshold (serum IgG <600 mg/mL and <1000 mg/mL) were used in our study.

Despite the ambiguity of the results, studies, especially in calves (Caldow et al., 1988), disclosed a positive association between sufficient colostral immunity and growth performance (Atkinson et al., 2017; Al and Sayed-Ahmed, 2020), while FPT together with diseases reduced growth performance (Gokce et al., 2013; Windeyer et al., 2014). This was the case in our study where FPT and concurrent disease were associated with poor growth performance in pre-weaned lambs. Poor growth performance is to be

expected as FPT causes an inability to overcome infections (diarrhoea, pneumonia), resulting in disease onset, which consequently leads to reduced intestinal nutrient utilization and feed intake, thus depriving the animal of nutrients required for growth and immunity. This may explain the reduced growth performance of lambs with FPT compared to those with adequate PTCI in our study. Similar associations were reported by reseracher in calves and lambs (Elsohaby et al., 2019; Pesca et al., 2020). Studies also reported local protective action of colostrum in the intestine (Nocek et al., 1984) and enhanced growth through morphological changes and functional maturation of the gastrointestinal tracts of neonates (Gascoigne and Davies, 2019; Elsohaby et al., 2019; Bond, 2020). Since colostrum is known to contain many substances (lymphocytes, cytokines, lactoferrin, acute phase proteins, growth factors, fat, lactose, vitamins, minerals, carnitine, antioxidant, enzymes, etc.) in addition to immunoglobulins (Mastellone et al., 2011; Hernández-Castellano et al, 2014, Hedegaard and Heegaard, 2016; Ahmadi et al., 2016; Gökçe et al., 2021a; Gökçe et al., 2021b; Agenbag et al., 2021; Gökçe et al., 2022), it is possible that colostrum could have influenced the growth response or the immune and metabolic systems of the Akkaraman lambs in this study

In conclusion, our results indicate that low serum IgG concentration in lambs reduces growth performance in preweaning lambs, as well as pneumonia, diarrhoea and FAS. It may also suggest that appropriate colostrum management may help to maintain health and growth performance, thus improving productivity and profitability of preweaning lambs on sheep farms. However, further studies are needed to better understand the relationship between growth performance, disease and FPT.

References

Agenbag B, Swinbourne AM, Petrovski K, van Wettere WH. Lambs need colostrum: A review. Livest Sci 2021; 251: 104624.

Ahmadi M, Boldura O, Milovanov C, Dronca D, Mircu C, Hutu I, Tulcan C. 2016. Colostrum from different animal species-A product for health status enhancement. Bulletin UASVM Anim Sci Biotech 2016; 73(1): 2016.

Ali MAE, Sayed-Ahmed ME. Relationship between passive immunity levels and morbidity, mortality and growth rates of friesian calves in Egypt. J Anim Poult Prod 2020; 11(12): 629-36.

Alves AC, Alves NG, Ascari IJ, Junqueira FB, Coutinho AS, Lima RR, Abreu LR. Colostrum composition of Santa Inês sheep and passive transfer of immunity to lambs. J Dairy Sci 2015; 98(6): 3706 -16.

Andrés S, Jiménez A, Sánchez J, Alonso JM, Gómez L, Lopez F, Rey J. Evaluation of some etiological factors predisposing to diarrhoea in lambs in "La Serena" (Southwest Spain). Small Rum Res 2007; 70(2-3): 272-5.

Atkinson DJ, Von Keyserlingk MAG, Weary DM. Benchmarking passive transfer of immunity and growth in dairy calves. J Dairy Sci 2017; 100(5): 3773-82.

Aydogdu U, Coskun A, Yuksel M, Basbug O, Agaoglu ZT. The effect of dystocia on passive immune status, oxidative stress, venous blood gas and acid-base balance in lambs. Small Rum Res 2018; 166: 115-20.

Bond C. Evaluation of lamb colostrum supplements. Vet Rec 2020; 187(11): e100.

Britti D, Massimini G, Peli A, Luciani A, Boari A. Evaluation of serum enzyme activities as predictors of passive transfer status in lambs. J Am Vet Med Assoc 2005; 226(6): 951-5.

Caldow GL, White DG, Kelsey M, Peters AR, Solly KJ. Relationship of calf antibody status to disease and performance. Vet Rec 1988; 122(3): 63-5.

Elsohaby I, Cameron M, Elmoslemany A, McClure J, Keefe G. Effect of passive transfer of immunity on growth performance of preweaned dairy calves. Can J Vet Res 2019; 83(2): 90-6.

Gascoigne E, Davies P. An approach to neonatal lamb post-mortem examinations. Livestock 2019; 24(4): 193-8.

Gilbert RP, Gaskins CT, Hillers JK, Parker CF, McGuire TC. Genetic and environmental factors affecting immunoglobulin G1 concentrations in ewe colostrum and lamb serum. J Anim Sci 1988; 66(4): 855-63.

Gokce E, Atakisi O, Kirmizigul AH, Unver A, Erdogan HM. Passive immunity in lambs: Serum lactoferrin concentrations as a predictor of IgG concentration and its relation to health status from birth to 12 weeks of life. Small Rum Res 2014; 116(2-3): 219-28.

Gokce E, Erdogan HM. An epidemiological study on neonatal lamb health. Kafkas Univ Vet Fak Derg 2009; 15(2): 225-36.

Gokce E, Kirmizigul AH, Atakisi O, Kuru M, Erdogan HM. Passive immunity in lambs: Colostral and serum γ-glutamyltransferase as a predictor of IgG concentration and related to the diseases from birth to 12 weeks of life. Vet Med 2021a; 66(2): 45-57.

Gökçe E, Atakişi O, Kırmızıgül AH, Erdoğan HM. Risk factor sassociated with passive immunity, health, birth weight and growth performance in lambs: II. Effects of passive immunity and some risk factors on growth performance during the first 12 weeks of life. Kafkas Univ Vet Fak Derg 2013; 19(4): 619-27.

Gökçe E, Atakişi O, Kırmızıgül AH, Erdogan HM. Interrelationships of serum and colostral IgG (passive immunity) with total protein concentrationsand health in lambs. Kafkas Univ Vet Fak Derg 2019; 25(3): 387-96.

Gökçe E, Cihan P, Atakişi E, Kırmızıgül AH, Erdoğan HM. Oxidative stress in neonatal lambs and its relation to health status and passive colostral immunity. Vet Immunol Immunopathol 2022; 251: 110470.

Gökçe E, Sözmen M, Gülmez C, Bozukluhan B, Gökce G, Atakişi E, Erdoğan HM. Carnitine concentrations in healthy and septicaemia suspected neonatal calves and its relation to passive immunty. Turk J Vet Anim Sci 2021b; 45(2): 229-37.

Hedegaard CJ, Heegaard PM. Passive immunisation, an old idea revisited: basic principles and application to modern animal production systems. Vet Immunol Immunopathol 2016; 174: 50-63.

Hernández-Castellano LE, Almeida AM, Ventosa M, Coelho AV, Castro N, Argüello A. The effect of colostrum intake on blood plasma proteome profile in newborn lambs: Low abundance proteins. BMC Vet Res 2014; 10(1):1-9.

Herndon CN, Shanthalingam S, Knowles DP, Call DR, Srikumaran S. Comparison of passively transferred antibodies in bighorn and domestic lambs reveals one factor in differential susceptibility of these species to Mannheimia haemolytica-induced pneumonia. Clin Vaccine Immun 2011; 18(7): 1133-8.

Johnson T, Jacobson BT, Jones, K, Mosdal C, Jones S, Vitkovic M, Bimczok D. Transfer and persistence of bovine immunoglobulins in lambs fed a colostrum replacer. Vet Rec 2022; 191(10): 1974.

Kenyon PR, Roca Fraga FJ, Blumer S, Thompson AN. Triplet lambs and their dams–a review of current knowledge and management systems. New Zealand J Agric Res 2019; 62(4): 399-437.

Kozyr VS, Antonenko PP, Mylostyvyi RV, Suslova NI, Skliarov PM, Reshetnychenko OP, Pushkar TD, Sapronova VO, Pokhyl OM. Effect of herbal feed additives on the quality of colostrum, immunological indicators of newborn calves blood and growth energy of young animals. Theor Appl Vet Med 2019; 7 (3): 137-42.

Massimini G, Mastellone V, Britti D, Lombardi P, Avallone L. Effect of passive transfer status on preweaning growth performance in dairy goat kids. J Am Vet Med Assoc 2007; 231(12): 1873-7.

Massimini G, Britti D, Peli A, Cinotti S. Effect of passive transfer status on preweaning growth performance in dairy lambs. J Am Vet Med Assoc 2006; 229(1): 111-5.

Mastellone V, Massimini G, Pero ME, Cortese L, Piantedosi D, Lombardi P, Avallone L. Effects of passive transfer status on growth performance in buffalo calves. Asian-Australas J Anim Sci 2011; 24(7): 952-6.

Nocek JE, Braund DG, Warner RG. Influence of neonatal colostrum administration, immunoglobulin, and continued feeding of colostrum on calf gain, health, and serum protein. J Dairy Sci 1984; 67(2): 319-33.

Pesca C, Forti K, Felici A, Scoccia E, Forte C, Antenucci P, Crotti S. Enzootic pneumonia in sheep: ewe and lamb immune response after Mannheimia haemolytica vaccine administration under field condition in Italy. Large Anim Rev 2020; 26(2): 73-6.

Santiago MR, Fagundes GB, do Nascimento DM, Faustino LR, da Silva CMG, Dias FEF, Cavalcante TV. Use of digital Brix refractometer to estimate total protein levels in Santa Inês ewes' colostrum and lambs' blood serum. Small Rum Res 2020; 182: 78-80.

Tsiligianni T, Dovolou E, Amiridis GS. Efficacy of feeding cow colostrum to newborn lambs. Livestock Sci 2012; 149(3): 305-9.

Turquino CF, Flaiban KKM, Lisbôa JAN. Transferência de imunidade passiva em cordeiros de corte manejados extensivamente em clima tropical. Pesqui Vet Bras 2011; 31(3): 199-205.

Vatankhah, M. Relationship between immunoglobulin concentrations in the ewe's serum and colostrum, and lamb's serum in Lori-Bakhtiari sheep, Iran. J Appl Anim Sci 2013; 3(3): 539-44.

Windeyer MC, Leslie KE, Godden SM, Hodgins DC, Lissemore KD, LeBlanc SJ. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. Prev Vet Med 2014; 113(2): 2

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Bibliometric Analysis and Science Mapping on RNA-seq and Gene Expression in Sheep

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Abstract: This study aimed to determine the development of research articles on 'RNA-sequencing and gene expression' in sheep between 2011-2023 in the Web of Science (WoS) database by scientific mapping method. In this regard, 205 articles were examined in the first search using the relevant keywords, and 124 articles suitable for analysis. They were analysed with the Shiny web application of the Bibliometrix R package, and VOSviewer. The results determined that the country with the most publications in the relevant field was China, the related institution with the most studies was "The University of Edinburgh", and the journal with the most publications was "PLoS One". According to the keyword analysis, the trend topics started from studies on granulosa cells and showed into research areas such as immune response, growth, pathway and meat quality. In the abstract analysis, the word 'transcriptome' and words such as mammary gland, muscle, fertility, Peripheral Blood Mononuclear Cells (PBMCs), lactation, fat storage were found together, while the words forming the parasitic agents, drug resistance, miRNA studies were clustered in different groups. The scarcity of the articles obtained in the analysed period reveals the openness of the study area. It can be suggested that the researchers who will plan to work on this subject can plan studies on the identification of variants belonging to different sheep breeds, resistance to antiparasitic drugs used in sheep, meat yield, disease resistance, reproductive tissues and organs, as well as designing all these studies as study subjects based on climate change and global warming factor.
Keywords: Bibliometric analysis, gene expression, RNA-sequencing, sheep

Koyun Türünde RNA-Dizileme ve Gen İfadesi Üzerine Bibliyometrik Analiz ve Bilimsel Haritalama

Öz: Bu çalışmada, Web of Science (WoS) veri tabanında 2011-2023 yılları arasında koyunlarda 'RNA dizileme ve gen ifadesi' konulu araştırma makalelerinin gelişiminin bilimsel haritalama yöntemi ile belirlenmesi amaçlanmıştır. Bu kapsamda, ilgili anahtar kelimeler kullanılarak yapılan ilk taramada 205 makale incelenmiş ve 124 makale analize uygun bulunmuştur. Bu makaleler Bibliometrix R paketinin Shiny web uygulaması ve VOSviewer ile analiz edilmiştir. Sonuçlar, ilgili alanda en çok yayın yapılan ülkenin Çin, en çok çalışma yapılan ilgili kurumun "The University of Edinburgh", en çok yayın yapılan derginin ise "PLoS One" olduğunu ortaya koymuştur. Anahtar kelime analizine göre trend konuları granüloza hücreleri üzerine yapılan çalışmalardan başlayarak bağışıklık tepkisi, büyüme, yolak ve et kalitesi gibi araştırma alanlarına doğru ilerlemiştir. Özet analizinde 'transkriptom' kelimesi ile meme bezi, kas, fertilite, PBMC, laktasyon, yağ depolama gibi kelimeler bir arada bulunurken, parazitik ajanlar, ilaç direnci, miRNA çalışmalarını oluşturan kelimeler farklı gruplarda kümelenmiştir. İncelenen dönemde elde edilen makalelerin azlığı çalışma alanının açıklığını ortaya koymaktadır. Bu konuda çalışma planlayacak araştırmacıların farklı koyun ırklarına ait varyantların belirlenmesi, koyunlarda kullanılan antiparaziter ilaçlara direnç, et verimi, hastalıklara direnç, üreme doku ve organları gibi konularda çalışmalar planlayabilecekleri gibi tüm bu çalışmaları iklim değişikliği ve küresel ısınma faktörüne dayalı çalışma konuları olarak tasarlamaları önerilebilir.
Anahtar kelimeler: Bibliyometrik analiz, gen ifadesi, koyun, RNA-dizileme

Introduction

Bibliometric analyses are important for providing a comprehensive evaluation of the publications reported in terms of institutions, authors, sources, citations, countries (quality, quantity and impact) used. Although there are other types of research, such as meta-analyses and systematic reviews, bibliometric analyses require better management and more resources in the field of research and statistical measurement. (Salinas-Ríos, 2022). With the advancement of bioinformatics algorithms, technologies in the field of molecular genetics rapidly advancing and the decreasing costs of methods and curiosity in planning research in different species is increasing day by day. One of these methods is RNA-seq, transcriptome analysis (Kukurba and Montgomery, 2015). There are no bibliometric study compiling studies in the field of gene-expression and RNA-seq in the sheep species.

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In the study planned for this purpose in the manual search conducted with the keywords "RNA-seq and gene expression" in sheep, the first identified study was a composite transcriptome study using RNA-seq analysis in sheep with a delayed bone healing model, which serves as a model organism (Jäger et al., 2011). Because sheep is a model organism for humans, several studies including a study on polycystic ovary syndrome in sheep by Sinha et al. (2020), a study by Dou et al. (2020) analyzing transcriptional regulation in visceral and subcutaneous fat tissues in female sheep exposed to prenatal bisphenol-A. Another study by Quttainah et al. (2022) where RNA-seq analysis was conducted to obtain transcriptomic information following an experimental design created in sheep related to heart failure observed in humans have been conducted on sheep.In addition to these studies on model organisms, studies related to meat and fat metabolism development have also been identified. Armstrong et al. (2018) conducted a study in which gene expression profiles were analysed in 9 different muscle species to explain the high degree of phenotypic differences observed in histochemical and metabolic parameters related to meat quality among different muscles. Bakhtiarizadeh et al. (2019) conducted a study on fat-tail metabolism in fat-tail sheep. Farhadi et al. (2023) reported the examination of gene expression profiles of fat tissue in thin-tailed and fat-tailed male sheep breeds using RNA-seq analysis method. RNA-seq and gene expression studies related to milk yield in sheep are as follows. Wang et al. (2020) stated a mammary gland transcriptome study in lactating and non-lactating Small-tailed Han ewes; Farhadian et al. (2022) stated a transcriptome analysis by RNA-seq method from milk collected at two different stages of lactation in Ghezel ewes. Suárez-Vega et al. (2023) indicated study in which the relationship between high and low feed efficiency and milk yield was determined by milk somatic cell transcriptome. Some studies related to fleece formation and molecular development in sheep; Lv et al. (2022) stated a study in which miRNA and mRNA profiles of hair follicles in skin tissue of Hu sheep were examined by RNA-seq method; Shi et al. (2022) indicated a study in which signaling pathways and key genes associated with wool density in Hetian sheep were determined.

A study by Li et al. (2020) investigated the lung tissue response to experimental Mycoplasma pneumonia infection in Argali hybrid sheep at the transcriptome level. A transcriptomic meta-analysis on unannotated long non-coding RNAs associated with immune response in sheep was performed by Bilbao-Arribas and Jugo (2022). A study by Kyselová et al. (2023); examined the Caseous Lymphadenitis immune response in sheep at the whole blood transcriptome level. Another study also investigated the Caseous Lymphadenitis immune response in sheep at the whole blood transcriptome level (Lins et al., 2023) and reported the RNA-seq analysis of abomasal tissues against Haemonchus contortus resistance in Santa Ines and Ile de France breed dairy lambs.

When previous studies are examined, it has been reported that many studies have been carried out in the field investigated with the reproductive system in sheep. Many studies on the reproductive system of sheep have been carried out. The effect of poor maternal nutrition during the gestational period on prenatal muscle development and growth was determined by RNA-seq analysis in a study performed by Gauvin et al. (2020). Chang et al. (2022) reported a study investigating the FecB genotype associated with fertility in small-tailed Han sheep and transcriptome analysis in thyroid tissue related to different genotypes; Chen et al. (2022) reported a study in which miRNA-mRNA analysis of adrenal glands playing a role in the reproductive system in sheep was determined by RNA-seq method. Sadeghi et al. (2022) investigated the lncRNA-miRNA, mRNA and ceRNA network in Romanov and Baluchi sheep in good and poor genetic value animals to understand the molecular mechanisms responsible for fertility in sheep. Li et al. (2022) investigated the effect of high altitude on alternative splicing and gene expression of ovarian follicle development in Tibetan sheep. In a study of Liu et al. (2023) reproductive system-related genes were identified in 10 different tissues in Xinggao sheep The effect of melatonin on the morula stage of sheep vitrified embryos was examined at the transcriptome level in a study conducted by Ji et al. (2023). In general, the reviewed literatures were determined as studies in the fields of model organism, meat, milk, wool production, reproductive system, and immune system.

Through bibliometric analyses, important trends in the research area have been reported in literature, journals, authors, keyword analyses, and institutions. Especially with the use of integrated software with Scopus and Web of Science (WoS) databases, these analyses can be conducted. The bibliometric development of gene expression and RNA-seq studies in sheep species is not known. Therefore, in this study, it was aimed to identify RNA-seq and gene expression studies in sheep species in the WoS database between 2011-2024 and to analyse these studies by bibliometric analyses on the basis of citation, author, institution, country and keyword.

Material and Methods

Database creation and bibliometric analyses

The Web of Science database search included literature from 01.01.2011 to 01.01.2024. The search question used to analyze the scientific publications was designed as follows: (All Fields (AF) = ("ovine"

OR "sheep" OR "lamb") AND AF= ("gene expression" AND "RNA-seq"). All records and reference information of the identified literatures were converted into a Plain Text File (.txt). Bibliometric analyses were performed using the R-based Bibliometrix package Biblioshiny version 4.1.4 (Aria and Cuccurullo, 2017) and VOSviewer software, version 1.6.20 (van Eck and Waltman, 2010). The WoS search query identified 205 literatures on 'RNA-seq and gene expression' in sheep from 2011-2023. Relevant literatures were analysed for title and abstract content. After eliminating irrelevant literatures (RNA-seq studies on bacteria causing disease in sheep, RNA-seq studies on viruses, goat-related RNA-seq studies not directly related to sheep), a total of 124 studies were analysed. In the Bibliometrix package, articles were filtered by year and language and analyses were initiated on 124 final articles (articles, papers: early access). Among the analyses performed, annual scientific production of countries and institutions, bibliographic merging with sources, Most Cited Articles and Most Influential Authors, trending topics and common word analyses constituted the main data information.

Results

Quantitative analysis of publication

The main data information analyzed in the Bibliometrix program is presented in Table 1.

Table 1. Main data information

Main information about data	
Timespan	2011:2023
Sources (Journals, Books, etc)	58
Documents	124
Annual Growth Rate %	27.23
Document Average Age	4.6
Average citations per doc	12.79
References	6795
Document contents	
Keywords Plus (ID)	648
Author's Keywords (DE)	344
Authors	
Authors	767
Authors of single-authored docs	0
Authors collaboration	
Single-authored docs	0
Co-Authors per Doc	7.94
International co-authorships %	32.26

The graph of the annual increase of the studies from January 2011 to January 2024 is shown in Figure 1. It has been observed that the number of studies has started to increase since 2014. In particular, it was determined that the number of studies produced in 2020 reached the highest level in the examined research area.

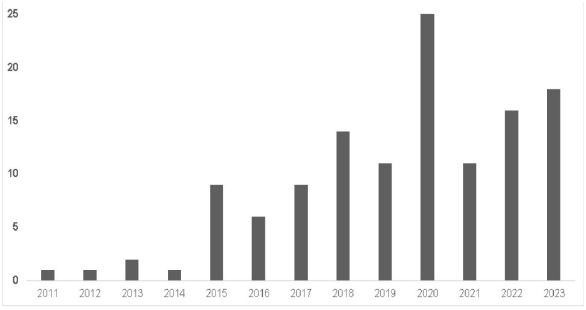

Figure 1: Annually number of publications on gene expression and RNA-seq studies in sheep.

Analysis of countries and institutions

According to the results of the analysis of the publication production of RNA-seq and gene expression research in sheep, there was an increase in countries such as Australia, the United Kingdom, Spain, the USA, and China since 2014, with China being at the highest level in this increase. Considering the most appropriate links identified by the authors of the relevant articles, the University of Edinburgh had the highest number of articles (n=22) which was closely followed by ICAR-Indian Veterinary Research (15 articles) and ICAR-Indian Council of Agricultural Research (13 articles). Figure 2 shows a three-domain plot using the Sankey plot describing the interaction between institutions, countries and journals. The findings indicated that the United Kingdom, India, China, and France were the dominant countries in terms of countries, institutions and resources.

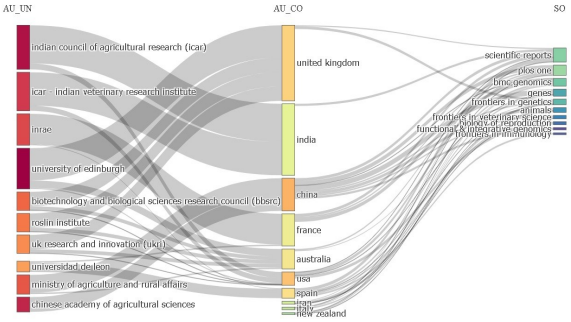

Figure 2: Three-field plot showing the network between institutions (left), countries (middle), and journals (right).

Bibliographic coupling with sources

A total of 62 sources were generated from research articles, and the full counting method was applied with a minimum threshold of 5. Only 6 sources met these criteria. For each of the 6 sources, the total strength of bibliographic coupling links with other sources was calculated (Table 2). The analysis revealed 10 links and total link strength of 530, forming 1 cluster with 5 items. The cluster comprised 5 elements. The source with the highest total link strength was "Scientific Reports", followed by "PLoS One".

Table 2. The top most 10 strong bibliographic coupling with sources

Rank	Sources	Clusters	Links	Total link strength	Documents	Citations
1	PLoS One	1	4	215	11	180
2	Scientific Reports	1	4	257	9	259
3	Frontiers in Genetics	1	4	219	7	53
4	BMC Genomics	1	4	215	7	209
5	Genes	1	4	154	7	51

Among the top 10 journals, "PLoS One" ranked first as the journal with the highest number of published articles. As indicated in Table 4, the number of publications in Frontiers in Veterinary Science, which has the highest Journal Impact Factor (JIF), is still low (Table 3).

Table 3: Top 10 journals with the most papers

Rank	Journals	Documents	JIF Percentile	JIF Quartile
1	PLoS One	11	88.39	Q2
2	Scientific Reports	9	70.5	Q2
3	Animals	7	81.5	Q1
4	BMC Genomics	7	70.6	Q2
5	Frontiers in Genetics	7	65.2	Q2
6	Genes	7	61.7	Q2
7	Frontiers in Veterinary Science	4	92.0	Q1
8	Biology of Reproduction	3	66.1	Q2
9	Frontiers in Immunology	3	78.6	Q1
10	Functional & Integrative Genomics	3	47.1	Q3

JIF: Journal Impact Factor

The Bradford area reported PLoS One, Scientific Reports, BMC Genomics, Frontiers in Genetics, was identified as the area where the distribution of articles across journals was analyzed and showed the inaugural academic articles relevant to the area under investigation.

Highly cited articles and most influential authors

Table 4 presents the top 5 most cited research articles. "A high-resolution atlas of gene expression in the domestic sheep (Ovis aries)" performed by Clark et al. (2017). Published in 2017 was the most cited article with 90 citations in 5 years in the field of RNA-seq and gene expression in sheep.

Table 4. Highly cited articles

Rank	Title	Paper	Total Citations	TC per Year	Normal-ized TC
1	A high resolution atlas of gene expression in the domestic sheep (Ovis aries).	Clark et al. (2017)	90	11.25	4
2	Effects of early feeding on the host rumen transcriptome and bacterial diversity in lambs.	Wang et al. (2016)	75	8.33	2.76
3	Genome-wide transcriptome analysis of mRNAs and microRNAs in Dorset and Small Tail Han sheep to explore the regulation of fecundity.	Miao et al. (2016)	60	6	1.72
4	Composite transcriptome assembly of RNA-seq data in a sheep model for delayed bone healing.	Jäger et al. (2011)	55	3.92	1
5	Genome-wide mRNA-seq profiling reveals predominant down-regulation of lipid metabolic processes in adipose tissues of Small Tail Han than Dorset sheep.	Miao et al. (2015)	54	5.4	1.55

TC: Total citations

In Figure 3, the most prolific authors in the field of gene-expression and RNA-seq studies are shown based on the number of publications attributed to each author. The size of the circles in the visualization indicates the number of publications authored by a person in a given year, while the density of the circles represents the number of citations received by the author in the same year. As the number of publications and citations increased, the size and density of the circles increased proportionally. In particular, authors such as Arranz JJ, Guteierrez-GIL B, and Suarez-Vega A (Suárez-Vega et al., 2018; Chitneedi et al., 2020; Suárez-Vega et al., 2023) were found to have continuity in their publications from 2015 until 2023. Authors such as Bush SJ, Clark EL, Hume DA and Mcculloch MEB (Clark et al., 2017; Bush et al., 2017; 2018; 2019; 2020; Salavati et al., 2019) were found to be active in terms of publications and citations in 2017 and 2020.

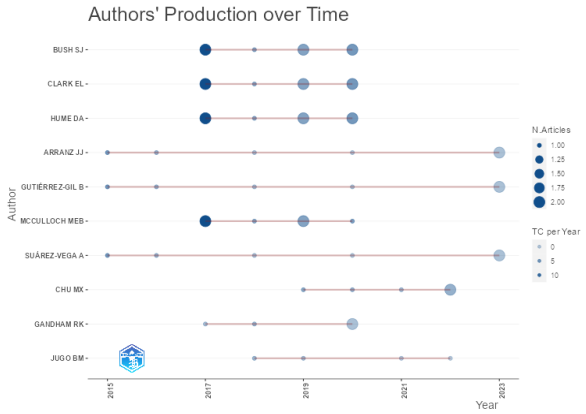

Figure 3. Production of the most productive authors over time.

Keyword analysis

Keyword analysis is crucial for gaining insight into key issues, focus areas, and trends in a research field. Such analyses enable researchers to quickly grasp the most discussed topics and key concepts. The word cloud highlighted the words "rna-seq", "growth", "and expression" but also different terms as identified in Figure 4.

Figure 4. Keyword analysis results for the research area.

Figure 5. Trend topics across years for RNA-seq and gene-expression studies in sheep.

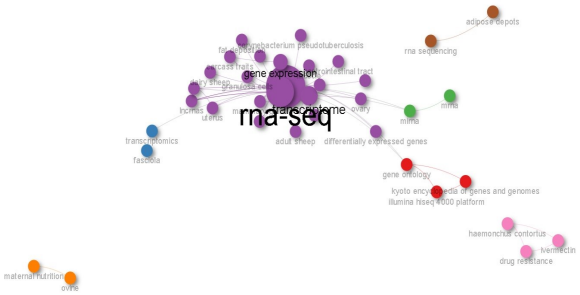

Figure 6. The result of a network of keywords that coword in the field of gene-expression and RNA-seq studies in sheep.

Discussion and Conclusion

Bibliometric analyses are studies in which scientific articles are evaluated both mathematically and statistically. The first study was reportedly conducted by Alan Pitchard in 1969 (Salinas-Ríos, 2022). These analyzes are carried out in many fields of science, but with the development of algorithms, it can be used more effectively in recent years. When the increasing graph of the studies conducted in the investigated field according to years was examined, there was an increase in the studies since 2011 and this increase reached the highest level in 2020. In 2021, it was determined that the decrease in 2023 was temporary and an increase was observed again in 2023. The top 10 journals with the highest number of publications in the field of RNA-seq and gene expression in sheep species were determined as WoS categories; Multidisciplinary Sciences, Agriculture, Dairy & Animals Science, Genetics & Heredity, Veterinary Science, Reproductive Biology, Immunology. The reported research areas can be defined as the intersection of RNA-seq and gene expression studies in sheep.

According to Bradford's law, the top three journals with the most publications were "PLoS One", Frontiers in Genetics", "Animals", while the three most cited journals were "Bioinformatics", "Nucleic Acid Research" and "PLoS One". Clark et al. (2017) was the most cited author in the related research field with 90 citations during the analysis period. When the subject of the study was examined, it was confirmed that a high-resolution gene expression atlas of domestic sheep was created and the researchers benefited from this article as a reference source. It was determined that the authors who produced continuous publications over time were Arranz JJ, Guterrez-Gil B and Suarez-Vega A (Suárez-Vega et al., 2018; Chitneedi et al., 2020; Suárez-Vega et al., 2023; Alonso-Garcia et al., 2023). In addition, Bush SJ, Clarck EL, Hume DA and McCulluch MEB (Clark et al., 2017; Bush et al., 2017; 2018; 2019; 2020; Salavati et al., 2019; 2020; Tsang et al., 2020) were determined as the authors working together with the highest number of local citations. When the fields of study of the most cited researchers were examined, it was determined that they were on early fat storage in sheep, perirenal fat transcriptome (Suárez-Vega et al., 2018; Alonso-Garcia et al., 2023), resistance to gastrointestinal nematodes (Chitneedi et al., 2020), milk transcriptome (Suárez-Vega et al., 2023). Other highly cited researchers worked on lncRNAs (Bush et al., 2018), gastrointestinal tract transcriptome at different developmental stages (Bush et al., 2019), species-specific mammalian macrophage response to Lipopolysaccharide (Bush et al., 2020). It was determined that the identified authors have been studying and publishing in the relevant research field for many years. According to the keyword analyses, the words "rna-seq", "transcriptome", "sheep", "mammary gland", " lncRNA ", "prolificacy" and "gene expression" were highlighted . The United Kingdom, China and India were the top three countries where the most articles were published whereas the top three institutions were University of Edinburgh, Indian Veterinary Research Institute and Indian Council of Agricultural Research (ICAR).

According to the trend subject analyses, it was determined that in the first years, granulosa cell studies were emphasized in the abstract, while in the following years, immune response, pathway, receptor, growth, and meat quality studies came to the fore. It can be recommended that researchers should design their researches in this respect.

Seven different clusters were determined according to the results of keyword analysis in sheep species. These clusters were identified as miRNA, fasciola, drug resistance, maternal nutrition, adipose storage, prolificacy, Corynebacterium pseudotuberculosis, lncRNA, ovary, mammary gland, lactation. In addition to the areas where keywords are specified, studies should be planned by identifying keywords that are

not included in the clusters. Studies planned in the reported areas can provide researchers with the opportunity for discussion. Keywords can also shed light on the future in studies to be planned in unreported areas. The results of analyses indicated that the studies conducted in the field of RNA-seq and gene expression in sheep species since 2011 are still in the development stage. It is necessary to carry out gene expression studies specific to different breeds, in different tissues, in different disease or application situations, such as the studies reported by Li et al. (2020) or Li et al. (2022) in the manual review. Especially in cattle species, studies measuring the responses to heat stress have started to be carried out. Research on this subject can also be planned in sheep species (Khan et al., 2020; Czech et al., 2022). Results were also obtained regarding the journals in which the planned studies could be published on Q1 and Q2 WOS cathegories.

This is the first bibliometric analysis planned in the field of RNA-seq and gene expression in sheep with the obtained results, predictions are provided for researchers to plan their studies in this field and they can carry out their planned studies with which institutions and organizations. However, the scarcity of the articles obtained in the analysed period reveals the openness of the study area. It can be suggested that the researchers who will plan to work on this subject can plan studies on the identification of variants belonging to different sheep breeds, resistance to antiparasitic drugs used in sheep, meat yield, disease resistance, reproductive tissues and organs, as well as designing all these studies as study subjects based on climate change and global warming factor. In the light of the present study, it can be recommended that researchers who plan to work in this field should direct their studies and plan their experimental designs in the field of RNA-seq and gene expression in sheep by examining the reported analyses.

References

Alonso-García M, Suárez-Vega A, Fonseca PAS, Marina H, Pelayo R, Mateo J, Arranz JJ, Gutiérrez-Gil B. Transcriptome analysis of perirenal fat from Spanish Assaf suckling lamb carcasses showing different levels of kidney knob and channel fat. Front Vet Sci 2023; 10: 1150996.

Aria M, Cuccurullo C. Bibliometrix: An R-tool for comprehensive science mapping analysis. J Informetr 2017; 11: 959-75.

Armstrong E, Iriarte A, Nicolini P, De Los Santos J, Ithurralde J, Bielli A, Bianchi G, Peñagaricano F. Comparison of transcriptomic landscapes of different lamb muscles using RNA-Seq. PLoS One 2018; 13(7): e0200732.

Bakhtiarizadeh MR, Salehi A, Alamouti AA, Abdollahi-Arpanahi R, Salami SA. Deep transcriptome analysis using RNA-Seq suggests novel insights into molecular aspects of fat-tail metabolism in sheep. Sci Rep 2019; 9(1): 9203.

Bilbao-Arribas M, Jugo BM. Transcriptomic meta-analysis reveals unannotated long non-coding RNAs related to the immune response in sheep. Front Genet 2022; 13: 1067350.

Bush SJ, McCulloch MEB, Summers KM, Hume DA, Clark EL. Integration of quantitated expression estimates from polyA-selected and rRNA-depleted RNA-seq libraries. BMC Bioinformatics 2017; 18: 301.

Bush SJ, Muriuki C, McCulloch MEB, Farquhar IL, Clark EL, Hume DA. Cross-species inference of long non-coding RNAs greatly expands the ruminant transcriptome. Genet Sel 2018; 50(1): 20.

Bush SJ, McCulloch MEB, Muriuki C, Salavati M, Davis GM, Farquhar IL, Lisowski ZM, Archibald AL, Hume DA, Clark EL. Comprehensive transcriptional profiling of the gastrointestinal tract of ruminants from birth to adulthood reveals strong developmental stage specific gene expression. G3 (Bethesda) 2019; 9(2): 359-73.

Bush SJ, McCulloch MEB, Lisowski ZM, Muriuki C, Clark EL, Young R, Pridans C, Prendergast JGD, Summers KM, Hume DA. Species-specificity of transcriptional regulation and the response to lipopolysaccharide in mammalian macrophages. Front Cell Dev Biol 2020; 8: 661.

Chang C, He X, Di R, Wang X, Han M, Liang C, Chu M. Thyroid transcriptomic profiling reveals the follicular phase differential regulation of lncRNA and mRNA related to prolificacy in Small Tail Han sheep with two FecB genotypes. Genes 2022; 13 (5): 849.

Chen Y, Liu Y, Chu M. miRNA-mRNA analysis of sheep adrenal glands reveals the network regulating reproduction. BMC Genom Data 2022; 23(1): 44.

Czech B, Wang Y, Wang K, Luo H, Hu L, Szyda J. Host transcriptome and microbiome interactions in Holstein cattle under heat stress condition. Front Microbiol 2022; 13: 998093.

Chitneedi PK, Arranz JJ, Suárez-Vega A, Martínez-Valladares M, Gutiérrez-Gil B. Identification of potential functional variants underlying ovine resistance to gastrointestinal nematode infection by using RNA-Seq. Anim Genet 2020; 51(2): 266-77.

Clark EL, Bush SJ, McCulloch MEB, Farquhar IL,

Young R, Lefevre L, Pridans C, Tsang HG, Wu C, Afrasiabi C, Watson M, Whitelaw CB, Freeman TC, Summers KM, Archibald AL, Hume DA. A high resolution atlas of gene expression in the domestic sheep (Ovis aries). PLoS Genet 2017; 13(9): e1006997.

Dou J F, Puttabyatappa M, Padmanabhan V, Bakulski KM. Developmental programming: Transcriptional regulation of visceral and subcutaneous adipose by prenatal bisphenol-A in female sheep. Chemosphere 2020; 255: 127000.

Farhadi S, Hasanpur K, Ghias JS, Palangi V, Maggiolino A, Landi V. Comprehensive gene expression profiling analysis of adipose tissue in male ındividuals from fat- and thin-tailed sheep breeds. Animals 2023; 13(22): 3475.

Farhadian M, Rafat SA, Panahi B, Ebrahimie E. Transcriptome signature of two lactation stages in Ghezel sheep identifies using RNA-Sequencing. Anim Biotecnol 2022; 33(2): 223-33.

Gauvin MC, Pillai SM, Reed SA, Stevens JR, Hoffman ML, Jones AK, Zinn SA, Govoni KE. Poor maternal nutrition during gestation in sheep alters prenatal muscle growth and development in offspring. J Anim Sci 2020; 98(1): skz388.

Jäger M, Ott CE, Grünhagen J, Hecht J, Schell H, Mundlos S, Duda GN, Robinson PN, Lienau J. Composite transcriptome assembly of RNA-seq data in a sheep model for delayed bone healing. BMC Genom 2011; 12: 158.

Ji P, Liu Y, Yan L, Jia Y, Zhao M, Lv D, Yao Y, Ma W, Yin D, Liu F, Gao S, Wusiman A, Yang K, Zhang L, Liu G. Melatonin improves the vitrification of sheep morulae by modulating transcriptome. Front Vet Sci 2023; 10: 1212047.

Khan A, Dou J, Wang Y, Jiang X, Khan MZ, Luo H, Usman T, Zhu H. Evaluation of heat stress effects on cellular and transcriptional adaptation of bovine granulosa cells. JABS 2020; 11: 25.

Kyselová J, Tichý L, Sztankóová Z, Marková J, Kavanová K, Beinhauerová M, Mušková M. Comparative characterization of imune response in sheep with caseous lymphadenitis through analysis of the whole blood transcriptome. Animals (Basel) 2023; 13(13): 2144.

Kukurba KR, Montgomery SB. RNA squencing and analysis. Cold Spring Harb Protoc 2015; 11: 951-69.

Li W, Zeng W, Jin X, Xu H, Fang X, Ma Z, Cao G, Li R, Ma L. High-Altitude stress orchestrates mrna expression and alternative splicing of ovarian follicle development genes in Tibetan sheep. Animals (Basel) 2022; 12(20): 2812.

Li Z, Du Z, Sun Y, Wang J, Liu H, Yang Y, Zhao N. Comprehensive RNA-Seq profiling of the lung transcriptome of Argali hybrid sheep in response to experimental Mycoplasma ovipneumoniae infection. Res Vet Sci 2020; 132: 57-68.

Lins JGG, Albuquerque ACA, Almeida FA, Britton C, Malossi C, Araújo-Júnior JP, Louvandini H, Amarante AFT. Abomasal RNA-seq reveals a strong local cellular response in suckling lambs with resistance against Haemonchus contortus. Int J Parasitol 2023; 53(13): 739-49.

Liu Z, Fu S, He X, Dai L, Liu X, Narisu Shi C, Gu M, Wang Y, Manda Guo L, Bao Y, Baiyinbatu Chang C, Liu Y, Zhang W. Integrated multi-tissue transcriptome profiling characterizes the genetic basis and biomarkers affecting reproduction in sheep (Ovis Aries). Genes 2023; 14(10): 1881.

Lv X, Chen W, Wang S, Cao X, Yuan Z, Getachew T, Mwacharo JM, Haile A, Sun W. ıntegrated hair follicle profiles of micrornas and mrnas to reveal the pattern formation of Hu sheep lambskin. Genes 2022; 13(2): 342.

Miao X, Luo Q, Qin X, Guo Y, Zhao H. Genome-wide mRNA-seq profiling reveals predominant down-regulation of lipid metabolic processes in adipose tissues of Small Tail Han than Dorset sheep. Biochem Biophysic Res Commun 2015; 467(2): 413-20.

Miao X, Luo Q, Qin X. Guo Y. Genome-wide analysis of microRNAs identifies the lipid metabolism pathway to be a defining factor in adipose tissue from different sheep. Sci Rep 2016; 5: 18470

Quttainah M, Raveendran VV, Saleh S, Parhar R, Aljoufan M, Moorjani N, Al-Halees ZY, AlShahid M, Collison KS, Westaby S, Al-Mohanna F. Transcriptomal insights of heart failure from normality to recovery. Biomolecules 2022; 12(5): 731.

Sadeghi M, Bahrami A, Hasankhani A, Kioumarsi H, Nouralizadeh R, Abdulkareem SA, Ghafouri F, Barkema HW. lncRNA-miRNA-mRNA ceRNA network ınvolved in sheep prolificacy: An integrated approach. Genes 2022; 13(8): 1295.

Salavati M, Bush SJ, Palma-Vera S, McCulloch MEB, Hume DA, Clark EL. Elimination of reference mapping bias reveals robust immune related allele-specific expression in crossbred sheep. Front Genet 2019; 10: 863.

Salinas-Ríos K, López A. Bibliometrics, a useful tool within the field of research. Journal of Basic and

Applied Psychology 2022; 3: 9-16.

Shi R, Li S, Liu P, Zhang S, Wu Z, Wu T, Gong S, Wan Y. Identification of key genes and signaling pathways related to Hetian sheep wool density by RNA-seq technology. PLoS One 2022; 17(5): e0265989.

Sinha N, Roy S, Huang B, Wang J, Padmanabhan V, Sen A. Developmental programming: prenatal testosterone-induced epigenetic modulation and its effect on gene expression in sheep ovary. Biol Reprod 2020; 102(5): 1045-54.

Suárez-Vega A, Arranz JJ, Pérez V, de la Fuente LF, Mateo J, Gutiérrez-Gil B. Early adipose deposits in sheep: comparative analysis of the perirenal fat transcriptome of Assaf and Churra suckling lambs. Anim Genet 2018; 49(6): 605-17.

Suárez-Vega A, Frutos P, Gutiérrez-Gil B, Esteban-Blanco C, Toral PG, Arranz JJ, Hervás G. Feed efficiency in dairy sheep: An insight from the milk transcriptome. Front Vet Sci 2023; 10: 1122953.

Tsang HG, Clark EL, Markby GR, Bush SJ, Hume DA, Corcoran BM, MacRae VE, Summers KM. Expression of calcification and extracellular matrix genes in the cardiovascular system of the healthy domestic sheep (Ovis Aries). Front Genet 2020; 11: 919.

van Eck NJ, Waltman L. Software Survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics 2010; 84: 523-38.

Wang W, Li C, Li F, Wang X, Zhang X, Liu T, Nian F, Yue X, Li F, Pan X, La Y, Mo F, Wang F, Li B. Effects of early feeding on the host rumen transcriptome and bacterial diversity in lambs. Sci Rep 2016; 6: 32479.

Wang J, Zhou H, Hickford J GH, Hao Z, Shen J, Luo Y, Hu J, Liu X, Li S. Comparison of the transcriptome of the ovine mammary gland in lactating and non-lactating Small-Tailed Han sheep. Front Genet 2020; 11: 472.

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Effects of Microplastics on Animal Health and Nutrition*

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Abstract: Macro plastics are defined as plastics that are larger than 20 cm. Plastics that measure between 5-20 cm are referred to as mesoplastics, while those between 1-5 mm are known as large microplastics. Plastics that measure between 1-1000 µm are called small micro plastics, and those that are smaller than 1000 µm are referred to as nano-plastics. Microplastics are particles that result from the degradation of plastic products or are specifically produced in the form of small pieces. They are considered to be less than 5 µm in size. Micro plastics have come to the fore in recent years and are pollutants of major concern to the environment. Plastic materials are commonly used on farms, but they can have negative effects on farm animals. Ruminants such as cattle, sheep and goats require cellulolytic micro-organisms for fiber digestion in their diet. The micro biota of the digestive system varies according to dietary habits. The digestive system and other organs can be adversely affected by sudden changes and damage to the micro biota. The ingestion of large plastic materials causes rumen complications such as rumen atony, indigestion and tympani in livestock. Ingested plastic fragments degrade in the digestive tract, increasing the number of small particles likely to be ingested. In a recent study, the presence of low-density micro plastics in sheep feces suggests that animals can ingest micro and macro plastics from their environment and feed. The increase in demand for the consumption of plastics worldwide is increasing the production of plastics. This situation causes the presence of micro plastics to increase rapidly day by day. Even if the production of plastics decreases, the continuous degradation of plastic waste in the earth will continue the formation of micro plastics and cause environmental pollution. The effects of microplastics in our country should be investigated by conducting detailed studies from the perspective of veterinary medicine.
Keywords: Animal nutrition, micro plastic, sustainability

Mikroplastiklerin Hayvan Sağlığı ve Beslenme Üzerine Etkileri

Öz: Makroplastikler >20 cm altında kalan plastiklerdir. 5-20 cm arasında kalan plastikler mesoplastikler, 1-5 mm arasında olanlar büyük mikroplastiklerdir. 1-1000 µm yer alan plastikler küçük mikroplastikler; <1000 µm altında kalan mikroplastikler nanoplastikler olarak adlandırılırlar. Mikroplastikler, plastik ürünlerin parçalanmasıyla oluşan veya özellikle küçük parçalar şeklinde üretilen, boyutu 5 µm'den daha küçük kabul edilen parçacıklardır. Son yıllarda gündeme gelmiş olup; çevre için büyük öneme sahip kirleticilerdir. Plastik malzemeler çiftliklerde de sıklıkla kullanılan ürünlerdir. Bu malzemelerin çiftlik hayvanları üzerinde olumsuz etkileri olabilir. Sığır, koyun, keçi gibi ruminantlar tükettikleri yemlerdeki lif sindirimi için selülolitik mikroorganizmalara ihtiyaç duyarlar. Sindirim sistemi mikrobiyotası beslenme alışkanlığına göre değişiklik gösterir. Mikrobiyotada meydana gelen ani değişimler ve hasarlar sindirim sistemi ve diğer organları olumsuz etkileyebilir. Büyük ebatlardaki plastik materyallerin yutulması besi hayvanlarında rumen atonisi, hazımsızlık ve timpani gibi rumen komplikasyonlarına neden olur. Yutulan plastik parçaları sindirim sisteminde parçalanarak emilme olasılığı yüksek olan küçük parçacıkların sayısını arttırır. Yapılan çalışmalarda koyun dışkısında düşük yoğunluklu mikroplastik varlığının tespit edilmesi çiftlik hayvanlarının çevrelerinden ve yemlerinden mikro ve makroplastikleri alabileceklerini göstermektedir. Dünya genelinde plastik tüketimine olan talebin artması plastik üretimini de arttırmaktadır. Bu durum mikroplastik varlığının her geçen gün hızla artmasına neden olmaktadır. Plastik üretimi azalsa dahi yeryüzünde var olan plastik atıkların devamlı parçalanması sonucu mikroplastik oluşumu devam edecek ve çevresel kontaminasyona sebep olacaktır. Mikroplastiklerin ülkemizdeki etkileri veteriner hekimliği açısından detaylı çalışmalar yapılarak araştırılmalıdır.
Anahtar kelimeler: Hayvan besleme, mikroplastik, sürdürülebilirlik

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Introduction

The production of plastics has become an environmental hazard due to the discovery of synthetic polymers (Hidalgo-Ruz et al., 2012). The most commonly

used synthetic plastics include polyethylene (low and high density), polystyrene, polyvinyl chloride, polypropylene, and polyethylene terephthalate. Polymers are used in the textile, fiber and synthetic leather industries; packaging and wrapping materials, toys; building and construction materials, drainage pipes; electronics, automotive, aircraft, and railway industries; dental and prosthetic materials, lenses; medical and disposable materials (Bansal and Singh, 2022). Micro plastics are present in various cosmetic products, including face wash gels, creams, and makeup (Auta et al., 2017). Plastics are synthetic polymers composed of long chains of carbon, hydrogen, oxygen, and chlorine atoms. They are in high demand and production due to their durability, lightness, low cost, and versatility (Bansal and Singh, 2022). Micro plastics are particles that are produced by the degradation of plastic products or in the form of particularly small pieces, considered to be smaller than 5 µm in size. They have been on the agenda in recent years. They are pollutants of great importance to the environment (Rainieri and Barranco, 2018). The environmental impact of micro plastics is influenced by several factors. Micro plastics of different sizes and polymers have different sorption and desorption times. Additionally, the concentration of micro plastics in soil is an important factor (Wang et al., 2019). Microplastics in soil can impede the absorption of water and nutrients by plants. These micro plastics negatively affect the biochemical structure of plant tissues, the root structure of the plant and the microorganism activity of the soil in which the plant grows (De Souza Machado et al., 2019). Micro plastics inhibit the growth and reproduction of microorganisms in soil and pose a threat to the soil biome by disrupting microbial diversity (Wang et al., 2019). The most frequently isolated polymers from water samples are polyethylene, polypropylene, polystyrene, and polycarbonates. Polystyrene polymers can cause oxidative stress by producing free oxygen radicals. Additionally, they have genotoxic effects on aquatic organisms by inhibiting DNA repair (Koelmans et al., 2019).

Table 1. Synthetic polymers commonly used

Polyester	High-density polyethylene (HDPE)
Polyethylene (PE)	Polycarbonate (PC)
Polyethyleneterephthalate (PET)	Cellulose acetate
Polypropylene (PP)	Cellulose nitrate
Polystyrene (PS)	Polylactic acid (PLA)
Polyvinyl chloride (PVC)	Melamine
Alkyd, polyurethane (PUR)	Polybutylene succinate (PBS)
Nylon (polyamide) (PA)	Polyhydroxyalkanoates
Polymethyl methacrylate (PMMA)	Polyethylene sülfonlar (PES)
Polyacrylonitrile (PAN)	Styrene-butadiene rubber (SBR)
Polyvinyl alcohol (PVA)	Polyvinyl acetate (PVA)
Poly acrylonitrile butadiene styrene (PABS)	

Classification of micro plastics

Micro plastics are classified based on their size, morphology, density and material composition (Amelia et al., 2021). Macro plastics are plastics below >20 cm. Plastics between 5-20 cm are meso plastics, and plastics between 1-5 mm are large micro plastics. Plastics between 1-1000 µm are called small micro plastics; micro plastics <1000 µm are called nanoplastics (Hanvey et al., 2017). According to another classification method, micro plastics are classified as primary and secondary micro plastics. Industrially produced as microbeads of different sizes, primary micro plastics are used in personal care products or as raw materials for the manufacture of various products. Plastics can degrade and break down in the environment due to exposure to oxygen, heat and radiation. These micro plastics formed as a result of physical and chemical decomposition are secondary micro plastics (Barnes et al., 2009; Rillig, 2012; Andrady, 2017). The main synthetic polymers commonly used today are given in the table (Bansal and Singh, 2022).

According to their morphology, microplastics are classified as fibers, fragments, beads, and films (Amelia et al., 2021).

Transmission pathways

Plastics can enter the soil in different ways. These ways include agricultural interventions, the usage of sewage and sludge in agricultural lands, and indiscriminate disposal of plastics into the environment (Rodriguez-Seijo et al., 2019). Studies show that micro plastics have been found in sewage treatment plants. Micro plastics of low density and small size in soil and water can be transported by wind and deposited back into soil and water through precipitation events (Lee et al., 2022). Plastics used in agriculture can also accumulate agrochemicals, making them a source of primary pollutants. Micro plastics can also accumulate pesticides. This creates a larger environmental problem. In a study of earthworms, it was

reported that they ingest micro plastics through the digestive tract or the skin and carry them deep into the soil through their movements (Rodriguez-Seijo et al., 2019). Exposure to plastics is more likely in urban center and areas with factories producing synthetic polymers. Plastic pellets can leak into the environment during production, transportation, recycling, or use (Andrady, 2017). According to Prata et al. (2022), mammals are primarily exposed through respiration and diet. Micro plastics are often found in the fiber structure of the atmosphere. Textile products are dispersed into the environment under the influence of the atmosphere and human activities. Micro plastics in the atmosphere have the biggest role in the pollution of the water environment. Accordingly, the routes of exposure to micro plastics have expanded from contaminated food and beverages in the food chain to inhalation (D'Angelo and Meccariello, 2021). Microplastics are taken into the gastrointestinal tract by drinking contaminated water or using such water to wash food, and by consuming fish living in the sea and oceans. Aquatic animals also ingest micro or nanoplastics through their gills (Bansal and Singh, 2022). Plastic materials are commonly used in farms for feed transport pipes, taps, drinkers, and plastic bottles for disinfectants or medicines. However, overtime, these plastics break down due to chemical, physical, and biological reactions, forming micro plastics. As a result, microplastics can be transported in these ways and be a source of microbial contamination for livestock and poultry. Additionally, microplastics from the environment can enter manure during the composting process. Composting animal manure is a common method, but it can also pose a threat to the ecological system due to the presence of micro plastics. A study reported that microplastic contamination, including PP, PE, and PR fibers and fragments, occurred in farm and poultry enterprises. This provides evidence that the direct application of manure can potentially contaminate soil with micro plastics (Wu et al., 2021). Micro plastics and nanoplastics not only act as environmental pollutants but also pose a hazard by interacting with toxic metals such as cadmium and mercury (Yong et al., 2020).

Importance of micro plastics in animal nutrition

Micro plastics are present in both land and water ecosystems (Akçay et al., 2020). Exposure to micro plastics is significant for poultry and other livestock in land ecosystems. It is not yet clear whether plastic species are included in the food chain after they are broken down into micro plastics. However, it has been observed that microplastics enter the food chain when animals consume feed and food contaminated with micro plastics. Microplastics that enter the aquatic ecosystem accumulate in the intestines of animals living in this ecosystem. It has been reported that these microplastics will not have a direct impact on human health since the intestines of aquatic animals offered for human consumption are removed before consumption. However, as the removed intestines are added to animal feed, animal health and indirectly human health are affected. (Atakan et al., 2021). A study was conducted on chickens and the area where they were raised to determine the transfer of low-density polyethylene (LDPE) plastic residues. The study examined the feces, gizzards, soil, and earthworms in which the chickens live, as well as the feed they eat. While 0.87±1.9 particles/g micro plastics were found in soil samples, 1.8±28.8 particles/g micro plastics were found in earthworms, 82.3±129.8 particles/g in chicken feces, and 10.2±13.8 particles/g in chicken gizzards. No micro plastics were found in the feed (Huerta et al., 2017). Ruminants, such as cattle, sheep, and goats, require cellulolytic microorganisms to digest the fiber in their feed. The micro biota of the digestive system varies depending on dietary habits. Sudden changes or damage to the micro biota can have adverse effects on the digestive system and other organs. Ingestion of large plastic materials can cause rumen complications, such as rumen atony, indigestion, and tympani, in livestock (Ramachandraiah et al., 2022). Ingested plastic fragments break down in the digestive tract, increasing the number of small particles that are likely to be absorbed. According to a study by Beriot et al. (2021), the presence of low-density micro plastics in sheep feces suggests that livestock may ingest micro and macro plastics from their environment and feed. Micro plastics are anthropogenic pollutants found in soil, oceans, air and biota, especially in urban environments (Prata et al., 2021). In a study conducted on dogs and cats living in Porto, micro plastics were detected in postmortem kidney, lung, ileum, liver, and blood samples using Nile Red Staining and Micro-Raman Spectroscopy methods (Prata et al., 2022). Micro plastics can carry pathogenic microorganisms and alter the microbial diversity of the environment. Micro plastics carry antimicrobial resistance genes, which can persist due to their effects on the carbon cycle and metabolism of micro biota (Wu et al., 2021; Eckert et al., 2017). Additionally, micro plastics damage gastrointestinal villi, leading to reduced nutrient absorption and feed intake in animals (Wu et al., 2021). According to Wang et al. (2019), micro plastics ingested by animals cannot be digested and can cause obstructions in the gastrointestinal tract. In a study by Lei et al. (2018), polystyrene nano- and micro plastics were found to damage cholinergic and GABAergic neurons. In studies on fish, it was observed histopathologically that micro plastics accumulate in the intestines, gills and livers of larvae and adult fish (Lu et al., 2016). The main pathological symptoms of micro plastic and nanoplastic toxicity in the intestine are disruption of epithelial integrity, inflammation, oxidative stress, changes in intestinal biomarkers and disruption of intestinal biota (Chen et al., 2018). When fish ingest micro plastics, changes in liver metabolites and liver enzymes can occur. In

some cases, micro plastics have also been found in the brains of fish, where significantly inhibited acetyl cholinesterase activity has been observed (Ding et al., 2018). Barboza et al. (2019) observed that wild fish consumed by humans, which had microplastics in their intestines and other tissues, had significantly higher levels of lipid peroxidation and acetylcholinesterase in their brains, gills, and dorsal muscles compared to fish without micro plastics. In a study on mice, micro plastics and nanoplastics were detected in the intestine, liver, and kidney. The distribution of microplastics in tissues is influenced by particle size. A study found that micro plastics with a diameter of 20 µm were evenly distributed among all tissues, whereas those with a diameter of 5 µm accumulated more in the intestine. The data indicate that micro plastics accumulate not only in the digestive system but also in other tissues through the circulatory system. In mice exposed to micro plastics, researchers observed a decrease in ATP concentration and an increase in LDH activity in the liver, as well as disrupted lipid metabolism (Deng et al., 2017). High concentrations of micro plastics and nanoplastics are cytotoxic, and cell death can occur through necrotic plasma membrane rupture or programmed cell death. Plastic-associated surfactants can disrupt the lipid layer of the plasma membrane at high concentrations. They can also inhibit cellular signaling processes that rely on cellular surface structures, such as proteoglycans, extracellular matrix components, and ligand-receptor interactions, even at moderate concentrations. As a result, cellular physiology may be affected to varying degrees. Nanoplastics are taken up by endocytosis, which depends on the cell type, and nanoplastics released into the cytosol can affect key organelles such as mitochondria or the nucleus, as well as cellular events such as mitotic spindle formation during cell division and chromosome migration. Micro plastics and nanoplastics can disrupt transport events along the exocytosis pathway within cells, which may hinder the expression of vital signaling receptors or membrane transporters. Additionally, the accumulation of nanoplastics in endosomes or lysosomes can lead to the degradation of these organelles, ultimately inhibiting macrophage and autophagic cell death (Yong et al., 2020). A study conducted on female mice exposed to micro plastics found that these animals experienced tissue damage, impaired immune response, decreased live births in offspring, changes in sex ratio, decreased body weight, and changes in lymphocyte composition in the spleen (Park et al., 2020). Additionally, micro plastics have been observed to cause inflammation in male reproductive cells and abnormal spermatozoon formation (D'Angelo and Meccariello, 2021). A study conducted by Hou et al. (2020) found that adding different doses of micro plastics to the drinking water of male mice reduced the number of live spermatozoa in the epididymis and caused morphologically abnormal spermatozoa.

The negative effects of microplastics were observed in many systems of the organism, particularly the digestive system. In order to prevent this situation, it is necessary to be familiar with the methods of analysis that can detect the presence of microplastics in any substance that is contaminated with microplastics.

Microplastic analysis methods

Collection of samples

Micro plastic samples are collected using selective, bulk, and reduced volume sampling methods. Selective sampling is used when plastic debris is visible to the naked eye. This method is easy and straightforward. However, this method has the disadvantage of only detecting larger micro plastics and being unable to detect them when mixed with other substances. Bulk sampling is a method of sampling without reducing the volume of the material to be sampled. However, this method negatively affects the representativeness of the entire sample as it only allows for a small sample to be collected. This method ensures better representativeness of the entire sample. On the other hand, reduced volume sampling involves rapid filtration to reduce the volume of the sample, with a small portion retained for analysis. Esmeray and Armutcu (2020) found that rapid filtration leads to the discarding of a large portion of the sample and a significant loss of micro plastics.

Preparation of samples

Density separation

The density of plastic varies depending on the type of polymer and manufacturing process. Density values can range from 0.8 to 1.4 cubic centimeters. In order to determine the density of the plastic in the sample, saturated solutions are used; such as sodium chloride (NaCl), sodium iodide, zinc chloride and sodium polytungstate solutions. The sample is mixed with a saturated solution and shaken to separate light particles from heavy particles. The sediment settles to the bottom while the low-density plastic fragments remain on the surface. The supernatant, which contains the low-density plastic fragments, is extracted. Saturated sodium chloride solution is commonly used to raise the density of the sample for density separation purposes. NaCl solution can be used to extract micro plastics of low density such as polyethylene, polypropylene, and polystyrene. However, it is not effective for separating micro plastics with higher density such as polyvinylchloride and polyethylene terephthalate (Hidalgo-Ruz, 2012; Esmeray and Armutcu, 2020). To separate high density micro plastics, it is recommended to use higher density salt solutions such as sodium iodide (NaI), zinc chloride (ZnCl), or sodium

polytungstate (SPT) (Esmeray and Armutcu, 2020).

Elimination

Micro plastics can be separated from samples by using sieves of different sizes. The remaining samples are collected after sieving. This process categorizes micro plastics according to their size (Hidalgo-Ruz, 2012), reducing the sample volume for extraction (Esmeray and Armutcu, 2020).

Digestion

Micro plastics are persistent and widespread pollutants, which raises concerns about their negative effects. To conduct laboratory toxicity experiments and biomonitoring, it is necessary to remove micro plastics from biological samples using easy and efficient digestion procedures. These procedures typically involve the use of alkaline and acid agents, as well as enzymes (Prata et al., 2021). Samples collected from the environment may contain a variety of organic matter and should be treated accordingly. This process presents challenges in identifying and categorizing micro plastics. The digestion process aims to remove the mixed organic matter in the collected samples (Wang and Wang, 2018). In the case of water and sediment samples, a mixture of 30% hydrogen peroxide (H_2O_2) and sulfuric acid (H_2SO_4) is used (Imhof et al., 2012). Organic material is digested using nitric acid (HNO_3, 22.5 M), hydrogen peroxide (H_2O_2, 32.6 M) and sodium hydroxide (NaOH, 52.5 M) (Claessens et al., 2013). Cleaning the sample with distilled water and ultrasonic cleaning can prevent surface adhesions of the plastic material.

Filtering

In the filtration method, liquid samples containing plastic fragments are passed through filters using a vacuum. The filters separate micro plastics from liquids by allowing only liquid substances to pass through. The size of the filter papers used varies between 1-1.6 µm or 0.45-20 µm (Hidalgo-Ruz et al., 2012; Wang and Wang, 2018). The liquid samples used can quickly clog the filter media because they are full of microscopic particles or debris. Various auxiliary measures can mitigate this issue. These include reducing the solution volume, settling the liquids for a longer time to facilitate the separation of heavier solid particles from the supernatant, performing a pre-filtration step using a filter with a larger pore size, or adding chemicals such as ferrous sulfate to the liquid to flocculate the solid fraction (Wang and Wang, 2018). To remove micro plastics from the aqueous supernatant, tweezers can be used after density separation with fresh water before filtration. Alternatively, for larger particles, water samples can be sieved through a 500 µm pore size sieve (Hidalgo-Ruz et al., 2012). The commonly used filters include glass fibers, nitro-cellulose, polycarbonate membranes, zooplankton, and isoporous filters (Wang and Wang, 2018).

Diagnosis and identification

In order to identify micro plastics, a visual inspection of the concentrated sample residue is required. This can be done with the naked eye or through a microscope. To avoid misidentification of micro plastics, plastic particle selection should be standardized (Hidalgo-Ruz et al., 2012). Once the samples collected from the field are prepared in the laboratory, various approaches can be used to identify microplastics. For this purpose, the analysis of mesoplastics, microplastics, and nanoplastics is conducted using optical, spectroscopic, or thermo-analytical techniques. Spectroscopic and imaging techniques are used to visualize mesoplastics, microspectroscopy and fluorescence techniques are used to visualize microplastics, and electron microscopy is used to visualize nanoplastics (Esmeray and Armutcu, 2020; Wang and Wang, 2018).

Optical techniques

Optical identification is a technique performed with the naked eye or with an optical microscope. This is the most commonly used technique. Shapes and colors are used to determine whether the material examined is micro plastic or not. Microplastic particles are not organic or cellular, and if they are in the form of fibers, they have consistent thickness and color along the entire length. The particles are clear and uniformly colored. To confirm transparent and white particles, high magnification or fluorescence microscopy is necessary. This method can be expensive but is suitable for high volume samples where analytical instruments are not available. It is important to note that weathered microplastics may undergo changes in morphology. Errors in identification can be introduced by the researcher making the identification, the sample matrix, the particle shape and size, or the microscope used, so it is important to be objective and accurate in the identification process. In some suspicious cases, spectroscopy and analytical techniques should be employed (Wang and Wang, 2018).

Scanning electron microscopy (SEM)

The SEM method involves exposing the sample surface to a high intensity electron beam. This produces high-resolution images of the sample, which are scanned in a raster scanning model. The method allows for the display of surface details of the examined sample at high magnification ratios, making it possible to determine the organic-inorganic impurities of the material. SEM has been successfully used to study the surface properties of micro plastics. However, this technique requires significant time and effort for sample preparation, making it unsuitable for pro-

cessing large numbers of samples (Wang and Wang, 2018).

Fourier transform infrared spectroscopy (FTIR)

The principle of FTIR analysis is based on three different modes of operation. Fourier Transform Infrared Spectroscopy (FTIR) is a method of analysis that operates in three different modes: transmission, reflection, and attenuated total reflection (ATR). It is important to note that FTIR analysis is based on objective measurements and not subjective evaluations. To hold the samples in place during the scanning process, a water-resistant and mechanically stable filter substrate with pores to allow filtration of aqueous samples is used. The filter material must also give a minimal spectral response. The transmission mode of analysis involves the beam passing through the sample and being collected. However, this mode is not suitable for colored materials due to their high absorption of the beam, resulting in weak or no beam reaching the detector. Reflection mode, on the other hand, is not affected by this issue. In reflection mode, the incident beam is reflected off the IR reflective substrate and passes through the sample. Attenuated total reflection involves using an ATR crystal, a high refractive index material, which is placed in optical contact and beamed onto the surface. ATR-FTIR is a fast method that requires minimal sample preparation. However, the crystal material can degrade over time due to surface scratching or cracking. It is important to ensure that the crystal material used is covered by the particle under investigation, as small-sized fragments in the crystal may not produce the desired spectrum (Xu et al., 2019).

Raman spectroscopy

Raman spectra are recorded by a Raman microscope system with a laser wavelength of 633 nm and 50x magnification (Imhof et al., 2012). This method is frequently used and reliable for the determination of microplastics. A laser beam is applied to the sample, and the molecular and atomic structure of the sample causes the beams to give light frequencies in the form of absorption, scattering or reflection, known as Raman shift. Different spectra are produced for each of the polymers under investigation. Raman spectroscopy is advantageous for analyzing a large number of samples of microplastics, providing non-destructive chemical characterization. It has the advantage of high spatial resolution, wide spectral range, narrow spectral bands, and lower sensitivity to water interference. This method enables the detection of microplastics as small as 1 µm. Chemicals associated with microplastics, such as dyestuffs, can harm the accuracy of the analysis (Wang and Wang, 2018).

Pyrolysis gas chromatography-mass spectrometry (PYR-GC-MS)

Pyrolysis Gas Chromatography-Mass Spectrometry analyses the thermal degradation products of microplastics. It provides a chemical analysis of microplastics. In this method, solid polymers are processed with a minimal amount of sample. Unlike the FTIR method, this method provides detailed information on the chemical and organic composition of polymers at the same time. This method is insensitive to contamination of the sample being analyzed with contaminants. Small amounts of sample are used for measurement. One single particle is analyzed per cycle. Each measurement takes 30-100 minutes. There is limited applicability for analyzing large sample volumes. Since micro plastic particles are manually placed in the pyrolysis tube, particles large enough to be manually manipulated (>100 µm) are suitable for analysis. Thermo-analytical methods are destructive, provide only chemical characterization, and do not provide detailed information on the morphology of microplastics. Therefore, they should be used in addition to spectroscopic methods (Wang and Wang, 2018).

Conclusion

As the demand for plastic consumption increases worldwide, so does the production of plastic. This situation causes the presence of microplastics to increase rapidly day by day. Even if the production of plastics decreases, the formation of microplastics will continue as a result of the continuous degradation of plastic waste in the earth and will cause environmental pollution (Çağlayan and Aytan, 2021). Plastic pollution has become a global issue. Plastics not only pollute the soil and water but also indirectly pollute the products made from these sources. Alternatives to the use of plastic products in agriculture and animal husbandry should be developed, or disposal methods and protocols should be established after the use of these products. A review of the literature reveals a lack of information on microplastics in feed and their effects on livestock. In vivo and in vitro studies on this topic will contribute to the prevention of plastic pollution, which has become a major problem today.

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References

Akçay S, Törnük F, Yetim H. Mikroplastikler : Gıdalarda bulunuşu ve sağlık üzerine etkileri. Ejosat 2020; 20: 530-8.

Amelia TSM, Khalik WMAWM, Ong MC, Shao YT, Pan HJ, Bhubalan K. Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans. Prog Earth Planet Sci 2021; 8: 12.

Andrady AL. The plastic in microplastics: A review. Mar Pollut Bull 2017; 119: 12-22.

Atakan O, Yüceer M, Caner C. Mikroplastikler ve gıda güvenliği. Akademik Gıda 2021; 433-41.

Auta HS, Emenike CU, Fauziah SH. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. Environ Int 2017; 102: 165-76.

Bansal OP, Singh A. A review on microplastic in the soils and their impact on soil microbes , crops and humans. Int J Res 2022; 10: 245-73.

Barboza LGA, Lopes C, Oliveira P, Bessa F, Otero V, Henriques B, Raimundo J, Caetano M, Vale C, Guilhermino L. Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. Sci Total Environ 2019; 717: 134625.

Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc Lond B Biol Sci 2009; 364: 1985-98.

Beriot N, Peek J, Zornoza R, Geissen V, Huerta Lwanga E. Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain. Sci Total Environ 2021; 755: 142653.

Çağlayan HS, Aytan Ü. Mikroplastiklerin deniz çevresinde neden olduğu etkiler. Doğanın Sesi Dergisi 2021; 6: 44-56.

Chen L, Hu C, Lok-Shun Lai N, Zhang W, Hua J, Lam PKS, Lam JCW, Zhou B. Acute exposure to PBDEs at an environmentally realistic concentration causes abrupt changes in the gut microbiota and host health of zebrafish. Environ Pollut 2018; 240: 17-26.

Claessens M, Van Cauwenberghe L, Vandegehuchte MB, Janssen CR. New techniques for the detection of microplastics in sediments and field collected organisms. Mar Pollut Bull 2013; 70: 227-33.

D'Angelo S, Meccariello R. Microplastics: A threat for male fertility. Int J Environ Res Public Health 2021; 18: 1-11.

De Souza Machado AA, Lau CW, Kloas W, Bergmann J, Bachelier JB, Faltin E, Becker R, Görlich AS, Rilling MC. Microplastics can change soil properties and affect plant performance. Environ Sci Technol 2019; 53: 6044-52.

Deng Y, Zhang Y, Lemos B, Ren H. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci Rep 2017; 7: 1-10.

Ding J, Zhang S, Razanajatovo RM, Zou H, Zhu W. Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (*Oreochromis niloticus*). Environ Pollut 2018; 238: 1-9.

Eckert EM, Di Cesare A, Kettner MT, Arias-Andres M, Fontaneto D, Hans-Peter G, Corno G. Microplastics increase impact of treated wastewater on freshwater microbial community. Environ Pollut 2017; 234: 495-502.

Esmeray E, Armutcu C. Mikroplastikler, çevre ve insan sağlığı üzerine etkileri ve analiz yöntemleri. Düzce Üniversitesi Bilim ve Teknoloji Dergisi 2020; 8: 839-68.

Hanvey JS, Lewis PJ, Lavers JL, Crosbie ND, Pozode K, Clarke OB. A review of analytical techniques for quantifying microplastics in sediments. Anal Methods 2017; 9: 1369-83.

Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ Sci Technol 2012; 46: 3060-75.

Hou B, Wang F, Liu T, Wang Z. Reproductive toxicity of polystyrene microplastics: In vivo experimental study on testicular toxicity in mice. J Hazard Mater 2020; 405: 124028.

Huerta Lwanga E, Mendoza Vega J, Ku Quej V, Chi J de los A, Sanchez del Cid L, Segura GE, Gertsen H,Salánki T,van der Ploeg M, Koelmans AA, Geissen V. Field evidence for transfer of plastic debris along a terrestrial food chain. Sci Rep 2017; 7: 1-7.

Imhof HK, Schmid J, Niessner R, Ivleva NP, Laforsch C. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnology and Oceanography: Methods 2012; 10: 524-37.

Koelmans AA, Hazimah N, Nor M, Hermsen E, Kooi M, Mintenig MS, De Franced J. Microplastics in freshwaters and drinking water: critical review and

assessment of data quality. Water Res 2019; 155: 410-22.

Lee M, Kim H, Ryu H. Review on invasion of microplastic in our ecosystem and implications. Sci Prog 2022; 105: 1-23.

Lei L, Liu M, Song Y, Lu S, Hu J, Cao C, Xie B, Shi H, He D. Polystyrene (nano)microplastics cause size-dependent neurotoxicity, oxidative damage and other adverse effects in *Caenorhabditis elegans*. Environ Sci 2018; 5: 2009-20.

Lu Y, Zhang Y, Deng Y, Jiang W, Zhao Y, Geng J, Ding L, Ren H. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environ Sci and Technol 2016; 50: 4054-60.

Park EJ, Han JS, Park EJ, Seong E, Lee GH, Kim DW, Son HY, Han HY, Lee BS. Repeated-oral dose toxicity of polyethylene microplastics and the possible implications on reproduction and development of the next generation. Toxicol Lett 2020; 324: 75-85.

Prata JC, João P, Lopes I, Andrady AL, Duarte AC, Rocha-santos T. A one health perspective of the impacts of microplastics on animal , human and environmental health. Sci Total Environ 2021: 777.

Prata JC, Patrício Silva AL, Dias-pereira P, Costa JP, Dias-Pereira P, Carvalho A, Fernandes AJS, da Costa FM, Duarte AC, Santos TR. Microplastics in internal tissues of companion animals from urban environments. Animals 2022; 12: 1979.

Prata JC, Sequeira IF, Monteiro SS, Silva ALP, da Costa JP, Dias-Pereira P, Fernandes AJS, da Costa FM, Duarte AC, Rocha-Santos T. Preparation of biological samples for microplastic identification by Nile Red. Sci Total Environ 2021; 783: 147065.

Rainieri S, Barranco A. Microplastics, a food safety issue? Trends Food Sci Technol 2018; 84: 55-7.

Ramachandraiah K, Ameer K, Jiang G, Hong GP. Micro- and nanoplastic contamination in livestock production: Entry pathways, potential effects and analytical challenges. Sci Total Environ 2022; 844: 157234.

Rillig MC. Microplastic in terrestrial ecosystems and the soil? Environ SciTechnol 2012; 46: 6453-4.

Rodríguez-Seijo A, Santos B, Ferreira Da Silva E, Cachada A, Pereira R. Low-density polyethylene microplastics as a source and carriers of agrochemicals to soil and earthworms. Environ Chem 2019; 16: 8-17.

Wang J, Liu X, Li Y, Powell T, Wang X, Wang G, Zhang P. Microplastics as contaminants in the soil environment: A mini-review. Sci Total Environ 2019; 691: 848-57.

Wang W, Wang J. Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. TrAC 2018; 108: 195-202.

Wu RT, Cai YF, Chen YX, Yang YW, Xing SC, Liao XD. Occurrence of microplastic in livestock and poultry manure in South China. Enviro Pollut 2021; 277: 116790.

Xu JL, Thomas KV, Luo Z, Gowen AA. FTIR and Raman imaging for microplastics analysis: State of the art, challenges and prospects. TrAC - Trends Anal Chem 2019, 119: 115629.

Yong CQY, Valiyaveettil S, Tang BL. Toxicity of microplastics and nanoplastics in mammalian systems. Int J Environ Res Public Health 2020; 17: 1509.

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Atıf yapmak için: Özçavuşoğlu Ş, Abozaid AMA, Aslan Ö. Bir tavşanda hipokalsemi olgusu. Erciyes Üniv Vet Fak Derg 2024; 21(2):140-142

Öz: Bu olgunun materyalini, Erciyes Üniversitesi Veteriner Eğitim, Araştırma ve Uygulama Hastanesi'ne lateral pozisyonda, titreme şikayeti ile getirilen dişi bir tavşan oluşturdu. Anamnezde, tavşanın iki ay arayla iki doğum yaptığı ve ikinci doğumundan 20 gün sonra sık sık nefes aldığı ve kasılma şikayetinin başladığı öğrenildi. Tavşanın klinik muayenesinde, generalize tetanik kasılmalar, taşipne, taşikardi ve 36.6°C vücut sıcaklığı belirlendi. Biyokimyasal analizde hipokalsemi (4.05 mg/dl) tespit edildi. Emziren bir tavşanda vakaya, anamnez, klinik muayene ve laboratuvar bulgularının sonucunda hipokalsemi teşhisi konularak 0.5 ml %10 kalsiyum glukonat damar içi uygulandı. Tedaviden yaklaşık bir saat sonra tavşanın klinik semptomlarının düzeldiği ve tetanik kasılmaların kaybolduğu gözlendi. Literatür taramalarında tavşanlarda hipokalsemi ile ilgili fazla kaynağa rastlanmamıştır. Tetanik kasılma, taşikardi ve hipotermi görülmesi nedeniyle olgunun sunulmasının önemli olduğu düşünüldü.
Anahtar kelimeler: Eklampsi, hipokalsemi, tavşan

A Case of Hypocalcemia in a Rabbit

Abstract: This case report describes a female rabbit presented to the Erciyes University Veterinary Training, Research, and Application Hospital in lateral recumbency with a complaint of muscle tremors. Upon obtaining the history, it was revealed that the rabbit had given birth twice within a two-month interval, and approximately 20 days after the second birth, she began experiencing frequent breathing and muscle Tremors. Clinical examination revealed generalized tetanic contractions, tachypnea, tachycardia, and a body temperature of 36.6°C. Biochemical analysis revealed hypocalcemia (4.05 mg/dl). Based on the history, clinical examination, and laboratory findings, the case was diagnosed as hypocalcemia in a lactating rabbit, and 0.5 ml of 10% calcium gluconate was administered intravenously. Approximately one hour after treatment, the rabbit's clinical symptoms improved, and the tetanic contractions disappeared. A literature review did not yield many sources regarding hypocalcemia in rabbits. Given the presence of tetanic contractions, tachycardia, and hypothermia, the presentation of this case is considered important.
Keywords: Eclampsia, hypocalcemia, rabbit

Giriş

Hipokalsemi, yani serum kalsiyum seviyesi düşüklüğü, hayvanlarda gebeliğin son aşamalarında, doğum öncesi dönemde veya artan kalsiyum gereksinimlerinin bir sonucu olarak emzirme döneminde oluşabilir. Bu durum, çeşitli klinik belirtilerle ortaya çıkabilir ve hemen ele alınmazsa hastanın yaşamı için bir risk oluşturabilir (Goldfarb ve Negrea, 2012).

Tavşanlarda hipokalsemi (kalsiyum eksikliği) nadiren gözlenmekte olup, yetersiz diyet alımına bağlı hipoalbuminemi, metabolik anormallikler, endokrin dengesizlikler vb. çok sayıda faktörden kaynaklanabilir. Gebelik ve emzirme döneminde artan kalsiyum gereksinimi, dişi tavşanlarda sıklıkla gözlemlenen bir olgudur. Yetersiz kalsiyum alımı veya yararlanımı, hipokalsemiye yol açan, olumsuz bir kalsiyum dengesine neden olabilir. Tavşanlarda hipokalsemi; gebelik toksemisi, laktasyon, diyetle yetersiz kalsiyum ve D vitamini alımı, paratiroidektomi ve dengesiz beslenme gibi Ca:P oranındaki bozulmaya neden olan çeşitli faktörlerden kaynaklanabilir (Donnelly, 2003; Melillo, 2007).

Hipokalseminin ilk belirtileri, semptom yokluğundan anoreksiya, yüzü ovuşturma, sinirlilik, kas seğirmesi (kulak ve yüz) ve sert bir yürüyüş gibi spesifik olmayan belirtilere kadar değişebilir. Şiddetli vakalarda, tetani ve nöbetlere yol açan nöromüsküler disfonksiyonlar meydana gelebilir. Hipokalseminin teşhisi, kandaki kalsiyum, fosfor, magnezyum ve glikoz düzeylerinin ölçülmesini içerir (Barlet, 1980). Hipokalsemi, serum kalsiyum seviyeleri 12 mg/dL'nin altına düşen tavşanlarda teşhis edilir (Melillo, 2007).

Bu vaka raporunun amacı, tavşanlarda hipokalsemi ile ilgili fazla veri olmaması ve klinik olarak tetanik kasılma ve taşikardiye rağmen hipotermi görülen bir vakanın klinik açıdan önemli olmasıdır.

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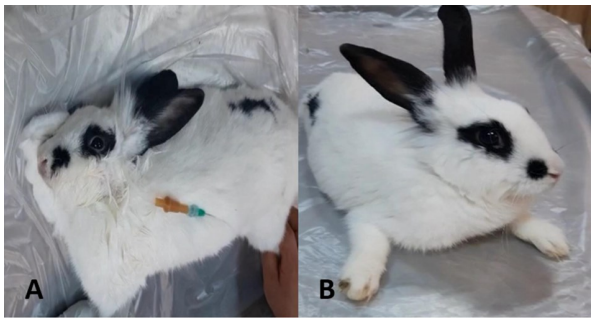
Olgu

Bu olgunun materyalini, Erciyes Üniversitesi Veteriner Fakültesi Eğitim Araştırma ve Uygulama Hastanesi İç Hastalıkları Anabilim Dalı kliniğine titreme, hızlı solunum ve ishal şikayetleri ile getirilen 10 aylık, dişi, 1.33 kg ağırlığındaki tavşan oluşturdu. Alınan anamnezde tavşanın iki ay önce ilk doğumunu, 20 gün önce ikinci doğumunu yaptığı, ilk doğumundan beş, ikinci doğumundan dört yavrusu olduğu, tüm yavruların ise sağlıklı olduğu ve hayvanın daha önce herhangi bir hastalık geçirmediği bilgisi alındı. Hayvanın yulaf, elma, havuç, yonca ve marul ile beslendiği ve su tüketiminin fazla olduğu öğrenildi.

Klinik muayenede tavşanın lateral pozisyonda yattığı, salya artışı, tetanik kasılmaları olduğu ve etrafa ilgisinin olmadığı gözlendi. Vücut sıcaklığı 36.6 °C, solunum sayısı 148/dk tespit edildi, nabız taşikardi sebebiyle sayılamadı (Şekil 1). Natif dışkı muayenesinde her hangi bir paraziter patojene rastlanmadı.

Tavşanın serum kalsiyum değerlerine bakıldı. Serum kalsiyum seviyesi 4.05 mg/dl olarak tespit edildi.



Şekil 1: A-Tedavi öncesi, B-Tedavi sonrası.

Yapılan klinik ve laboratuvar muayeneler sonucunda tavşana laktasyon döneminde gelişen hipokalsemi teşhisi konuldu. Tedavisinde 0.5 ml askorbik asit (Vitce®, Sanovel), 0.5 ml B kompleks vitaminleri (Armavit-B®, Arma), 0.5 ml %10 kalsiyum glukonat (Cal-Mix®, Bavet) ve %0.9 izotonik uygulandı. Uygulamalardan yaklaşık bir saat sonra tavşanda titremelerin durduğu, lateral pozisyondan sternal pozisyona geçtiği, solunumunun düzenlendiği ve etrafa ilgisinin olduğu belirlendi.

Hastaya günde bir damla olacak şekilde 50000 I.U. vitamin D (Devit-3®, Deva) önerildi. Hastanın emzirmesi kesildi ve beslenme planı değiştirildi. Emzirme döneminde dengeli ve yaşa uygun beslenmenin yanı sıra yem katkı maddelerinin kullanılması tavsiye edildi.

Üç gün sonra kontrole gelen tavşanın alınan anamnezinde iştahın ve hareketlerin normale döndüğü bildirildi. Tekrarlanan serum kalsiyum analizinde kalsiyum değerinin 15.99 olduğu belirlendi

Tartışma ve Sonuç

Hipokalsemi, yüksek mortalite sergileyen ve tavşanlarda kandaki total kalsiyum düzeyinin 12.00 mg/dL'nin altına düşmesi ile karakterize akut bir durumdur (Melillo, 2007). Bu durum genellikle gebelik sırasında veya laktasyonun ilk üç haftasında görülür. Bu olgu ise doğumdan üç hafta sonra gerçekleşmiştir. Hipokalsemi, artan kalsiyum gereksinimleri sonucu, gebeliğin son dönemlerinde, özellikle doğum öncesi dönemde veya laktasyon sırasında meydana gelebilir. Hipokalsemi ile gebelik toksemisi arasında potansiyel bir ilişki vardır. Düşük kalsiyum alımı ve yüksek fosfor alımı buna katkıda bulunan faktörlerdir. Tavşanlarda, diğer memelilerden %30-50 daha yüksek serum kalsiyum konsantrasyonu sağlayan kalsiyum metabolizması bulunur (Donnelly, 2003).

Hipokalsemi sığırlarda peripartal dönemde görülmesi nedeniyle süt humması olarak adlandırılırken (Ibrahim ve Kirmani, 2021), köpeklerde eklamsia olarak bilinmektedir (Singh ve ark., 2017). Eklampsi, köpeklerde ve daha az ölçüde kedilerde görülen hipokalsemi kaynaklı kritik ve yaşamı tehdit eden bir durumu temsil eder. Eklampsi gelişimine katkıda bulunan faktörler arasında yetersiz besin alımı, düşük serum albümin seviyeleri, artmış laktasyon ve paratiroid bez disfonksiyonu bulunmaktadır. Dişi köpeklerde puerperal tetaninin patogenezinin önemli bir yönü, özellikle laktasyon döneminde yüksek kalsiyum kaybından kaynaklanan kalsiyumun hücre dışı sıvı girişi ve çıkış hızları arasındaki dengesizliği içermek gibi görünmektedir (Pathan ve ark., 2011). Eklampsi, kan kalsiyum seviyesinin azalması ile karakterize ciddi bir tıbbi durumu temsil eder. Etkilenen köpeklerde, sinir sinyal iletimi ve kas işlevi üzerinde dinamik bir etki yaratır, sonuç olarak huzursuzluk ve sinirlilik hali görülür. Durum ilerledikçe, etkilenen dişi köpekler yürüme yeteneklerini kaybedebilir, uzuvları sertleşebilir, kas titremeleri, göz seğirmeleri gözlenebilir ve solunum hızı da genellikle artar (Pathan ve ark., 2011). İneklerde hipokalsemide ise hafif bir hastalık durumunda, iştah kaybı, yemlerin tekrar yutulması (ruminasyon) ve dışkılama süreçlerinde zorlanma (defekasyon) gibi belirtiler ortaya çıkar. Bu duruma Apetit-Ruminasyon-Defekasyon (ARD) sendromu denir. Sorun daha ciddi hale geldikçe, ayakta durma güçlüğü yaşanır ve vücut uzuvlarının uç kısımları soğumaya başlar. Hipokalsemi (kalsiyum eksikliği) ilerledikçe, ayakta duramayan hayvanlarda bilinç kaybı yaşanır ve mide boşalma yeteneğini kaybetme sonucu karın şişkinliği (timpani) meydana gelir. Agoni, kalsiyum eksikliğinin doğrudan etkisinden ziyade, timpani sonucu oluşan solunum zorluğundan kaynaklanır (Salmanoglu ve Salmanoglu, 1998). Doğum sonrası tavşanlarda hipokalsemi, ekstraselüler kalsiyum girişi ile artan emzirmeden kaynaklanan verim arasındaki dengenin bozulmasıyla oluşur (Donnelly, 2003). Bu vakada gözlenen sinirsel belirtilerin, solunum güçlüğü, salivasyon artışı, nöbetler ve yan yatma gibi klinik bulguların,

kalsiyum kaybı nedeniyle sinir membranları boyunca artan iyon geçirgenliği ve ardışık nöromüsküler tetaninin oluşması ile ilişkili olduğu düşünülmüştür.

Hipokalseminin tetani, nöbet ve deliryum dahil olmak üzere birçok yaygın nörolojik belirtisi vardır. Bu da hipokalseminin merkezi sinir sisteminde uyarılabilirliği artırmada rol oynadığı bildirilmektedir (Han ve ark., 2015). Sunulan vakada görülen hipokalsemik tetani bu literatürle uyumludur.

Hipokalsemi hem ST segment modifikasyonuna hem de QT aralığının uzamasına neden olabilir ve şiddetli olduğunda hayatı tehdit eden ventriküler aritmilere yatkınlık yaratabilir (Cecchi ve ark., 2015). Sunulan vakada literatürle uyumlu olarak taşikardi belirlenmiştir.

Hipokalsemik tetanisiye benzer nörolojik semptomların paraziter enfeksiyonlar (Ensefalitozoonozis, *Baylisascaris procyonis* infestasyonu (serebrospinal nematodiazis), toksoplasmozis, sarkosistis), bakteriyel enfeksiyonlar (Listeriozis, *Francisella tularensis*), toksikasyonlar (Kurşun, toksik bitkiler, rodentisidler ve herbisidler), gebelik toksemisi, hipoksi, neoplazi, elektrolit anormallikleri, epilepsi (özellikle beyaz kürklü ve mavi gözlü tavşanlarda), kardiyovasküler hastalık (örn. arterioskleroz), kuduz ve bazı hastalıkların son aşamalarında da (örn. viral hemorajik hastalık, karaciğer yetmezliği, septisemi, böbrek yetmezliği, bağırsak tıkanıklığı ve ileus) görüldüğü göz önünde bulundurulmalıdır (Keeble ve ark., 2016). Bu olguya anamnez, klinik muayene, laboratuvar bulguları ve kalsiyum tedavisi ile kısa sürede hayvanın vital bulgularının normale dönmesi nedeniyle hipokalsemi teşhisi konuldu.

Köpek ve kedilerde sıkça 40.5°C'yi aşan bir vücut sıcaklığı görülürken, 41.6°C gibi yüksek sıcaklıklar da yaygındır. Bu sıcaklık artışı genellikle kas aktivitesindeki artışla ilişkilidir (Pathan ve ark., 2011). İneklerde hipokalsemi olgularında hipotermi görülmektedir (Salmanoglu ve Salmanoglu, 1998). Sunulan vaka raporunda köpeklerde görülen eklampside olduğu gibi tetanik kasılmalar görülmesine rağmen ineklerde olduğu gibi hipotermi belirlenmiştir. Zhang ve ark. (2009) yaptıkları çalışmada adenozin 5′-monofosfat (AMF) düzeyi ile hipokalsemi ve hipotermi arasında ilişki olduğunu göstermişlerdir (Zhang ve ark., 2009). AMF'nin artışı hipokalsemi ve hipotermi oluşumunu tetiklemektedir. Sunulan çalışmada hipokalsemide artan kas aktivitesi nedeniyle arttığı düşünülen AMF'nin hipotermi oluşum mekanizmasında etkili olabileceği düşünülmüştür.

Sonuç olarak, tavşanda belirlenen hipokalsemide klinik olarak köpeklerde gözlenen eklampsia puerparalis gibi tetanik kasılma ve taşikardiye rağmen ineklerde gözlenen hipokalsemiye benzer şekilde hipoterminin gözlenmesi nedeniyle sunulan vakanın literatüre katkı sağlayacağı düşünülmüştür. Sunulan vaka raporunda tavşanda teşhis edilen hipokalsemide doğru teşhis koymak ve uygun tedaviyi derhal uygulamak çok önemlidir. Bu nedenle tavşanlarda tetanik kasılmalarla seyreden zehirlenmeler, bakteriyel, viral ve paraziter enfeksiyonlar vb vakalarla ayırt edici olarak akla hipokalseminin gelmesi önemlidir.

Kaynaklar

Barlet JP. Plasma calcium, inorganic phosphorus and magnesium levels in pregnant and lactating rabbits. Reprod Nutr Dev 1980; 20: 647-51.

Cecchi E, Grossi F, Rossi M, Giglioli C, De Feo ML. Severe hypocalcemia and life-threatening ventricular arrhytmias: Case report and proposal of a diagnostic and therapeutic algorithm. Clin Cases Miner Bone Metab 2015; 12(3): 265-8.

Donnelly TM. Textbook of rabbit medicine. Lab Anim (NY) 2003; 32: 26-7.

Goldfarb S, Negrea LA. Hypocalcemia and hypercalcemia. Nephrol Secrets 2012: 546-50.

Han P, Trinidad BJ, Shi J. Hypocalcemia-induced seizure: Demystifying the calcium paradox. ASN Neuro 2015; 7(2): 1-9.

Ibrahim N, Kirmani A. Milk fever in dairy cows: A systematic review. Res J Biol 2021; 9(3): 1-11.

Keeble E, Meredith A, Richardson J. Rabbit Medicine and Surgery. Second Edition. New York: CRC Press, 2016.

Melillo A. Rabbit clinical pathology. J Exot Pet Med 2007; 16: 135-45.

Pathan MM, Siddiquee GM, Latif A, Das H, Khan MJZ, Shukla MK. Eclampsia in the dog: An overview. Vet World 2011; 4(1): 45-7.

Salmanoğlu R, Salmanoğlu B. Puerperal hipokalsemili ineklerde kan kalsiyum düzeyleri ve klinik gözlemler. Ankara Univ Vet Fak Derg 1998; 45(1): 151-7.

Singh KP, Singh RV, Singh P, Singh SK. Management of eclampsia in bitches. International Journal of Veterinary Sciences and Animal Husbandry 2017; 2(5): 11-2.

Zhang F, Wang S, Luo Y, Ji X, Nemoto EM, Chen J. When hypothermia meets hypotension and hyperglycemia: The diverse effects of adenosine 5'-monophosphate on cerebral ischemia in rats. J Cereb Blood Flow Metab 2009; 29: 1022-34.

Yazım Kuralları

1. Erciyes Üniversitesi Veteriner Fakültesi Dergisi'nde veteriner bilimlerini ilgilendiren alanlarda orijinal araştırmalar, olgu sunumları, araştırma notları, kısa bildiri, derleme ve editöre mektup yayımlanır.
2. Dergide yayımlanacak yayınlar için resmi dil Türkçe'dir. İngilizce yazılmış eserler de yayımlanabilir. **İngilizce hazırlanmış makalelerin yayımlanmasına öncelik verilir**.
3. Yayınlar A4 tipi formatta, çift aralık, Arial, 10 punto ve iki yana yaslı olarak yazılmalıdır. Her kenardan 2.5 cm boşluk bırakılarak, sayfaların sağ altına numara verilmelidir. Resimler, şekiller ve kaynaklar dâhil orijinal makaleler ve derlemeler 14, olgu sunumları, araştırma notu ve kısa bildiriler 7 sayfayı geçmemelidir.
4. Yazılar, ercvet@gmail.com adresine gönderilmelidir. Yazışmalar için, makale kapak sayfasında, sorumlu yazarın yazar adı, unvanı, ORCID numarası ve E-posta adresi yazılmalıdır.
5. Daha önce kongrelerde tebliğ edilmiş ve özeti yayımlanmış çalışmalar, bu durum kapak sayfasında belirtilmek üzere kabul edilir.
6. Araştırma herhangi bir kuruluş tarafından desteklenmiş ise kapak sayfasında dipnot olarak belirtilir.
7. Kapak sayfasında Türkçe makale başlığı (koyu ve ilk harfleri büyük), İngilizce başlık (ilk harfler büyük), kısa başlık (40 karakteri geçmemeli ve ilk kelimenin ilk harfi büyük, diğerleri küçük olarak yazılmalıdır), yazar adları (unvansız), çalıştıkları kuruma ait bilgiler (soyadı üstüne numara konulup dipnot olarak) verilmelidir.
8. Türkçe ve İngilizce özetlerin bir sonraki sayfaya yazılması gerekir. Bu sayfa, paragrafsız olarak Türkçe ve İngilizce özetleri (en fazla 250 kelime) içermelidir. Anahtar kelimeler özetlerin altına alfabetik olarak (virgülle ayrılmış şekilde) yazılmalıdır. Yalnızca ilk anahtar kelime büyük harfle başlamalıdır. **Türkçe Bilmeyen yazarlar için Türkçe özet ve anahtar kelimeler yazma zorunluluğu bulunmamaktadır.**
9. Araştırma makalesi; Kapak Sayfası - Özet (Türkçe ve İngilizce) - Anahtar kelimeler (Türkçe ve İngilizce), Giriş, Gereç ve Yöntem, Bulgular, Tartışma ve Sonuç, Teşekkür, Kaynaklar, Tablo ve Şekiller, Sorumlu yazar (Correspondence Author) bölümlerini içerecek şekilde düzenlenmelidir. Metin içindeki tüm başlıklar koyu yazılmalıdır. Metin içinde paragraf girintisi yapılmamalı, devamlı satır numarası verilmelidir.
10. Derlemeler, orijinal olması, en son yenilikleri içermesi, yazarların konu ile doğrudan ilişkili **en az 3 adet** çalışmalarının olması ve bunların derleme içinde kullanılması durumunda yayınlanmak üzere kabul edilebilecektir. Derlemeler kapak sayfası, Özet (Türkçe ve İngilizce), Anahtar kelimeler (Türkçe ve İngilizce), Giriş, konunun kendine ait alt başlıkları, Sonuç, Kaynaklar, Tablo ve Şekiller ve Sorumlu yazar (Correspondence) bölümlerini içerecek şekilde düzenlenmelidir.
11. Olgu Sunumları, Özet (Türkçe ve İngilizce), Anahtar kelimeler (Türkçe ve İngilizce), Giriş, Olgu(lar), Tartışma ve Sonuç, Kaynaklar, Tablo ve Şekiller ve Sorumlu yazar bölümlerini içermelidir.
12. Etik kurul onayı gerektiren çalışmalarda Etik Kurul onayı alınan kurumun adı ve onay numarası, çalışmanın Gereç ve Yöntem kısmında belirtilmelidir.
13. Tablo ve şekillerin metinde geçeceği yer, altı ve üstü çizgili olarak belirtilmelidir.
14. Ondalık ifadelerde nokta kullanılmalıdır.
15. Tür isimleri ve anatomik terimler gibi Latince ifadeler *italik* karakterle yazılmalıdır. Tüm ölçü birimleri SI (*Systeme Internationale*)'e göre verilmelidir.
16. Tablolar kaynaklar kısmından sonra, her bir tablo ayrı sayfada olacak şekilde verilmelidir. Tablo başlıklarının yalnızca ilk harfleri büyük olmalıdır. Tablo başlıkları tablonun üzerinde bulunmalı ve **Tablo 1.** şeklinde numaralandırılmalıdır. Tablolarda iç ve yan kılavuz çizgiler kullanılmamalıdır. Tanımlayıcı bilgi ve açıklamalar tabloların altına yerleştirilmelidir.
17. Her resim, grafik ve çizim; şekil olarak kabul edilip **Şekil 1.** gibi yazılmalı, her biri ayrı sayfada olacak şekilde verilmelidir. Tanımlayıcı bilgi ve açıklamalar şekil ismi ile birlikte şeklin altına yerleştirilmelidir. Resimler 300dpi çözünürlükte olmalıdır.
18. Kaynaklar metin içinde cümle sonunda belirtilmelidir. Yazar soy isimleri ve tarihi yazı içinde her kaynağa ait yayın yılı yazar isminden hemen sonra parantez içinde belirtilmelidir. Kaynak iki isimli ise isimler belirtilmeli (örn; Kaldhone ve Nayak, 2008). Kaynakta yazar sayısı ikiden fazla ise sorumlu yazar "ve ark." şeklinde belirtilmelidir (örn, Kaldhone ve ark., 2008). Eğer kaynak cümlenin başında kullanılıyorsa yazar isimlerinden sonra parantez içinde yayın yılı belirtilmelidir.
19. Kaynaklar yazılırken alfabetik sıraya konulmalı, kaynaklar bölümünde 0.5 cm içeri doğru asılı halde yazılmalıdır. Noktalama işaretlerine örneklerde gösterildiği şekilde dikkat edilmelidir. Dergi kısaltmaları *Index Medicus* ile uyum içerisinde olmalıdır. **Orijinal araştırma makaleleri, derlemeler ve olgu sunumları sırasıyla 30, 45 ve 15'ten fazla kaynak içermemelidir.**
    Kaynaklar;

19.1. Kaynak süreli yayın ise;
Örnek: Kaldhone P, Nayak R, Lynne AM, Dvaid DE, McDermott PF. Characterisation of *Salmonella enterica* serovar Heidelberg from Turkey-associated sources. Appl Environ Microbiol 2008; 74(16): 5038-46.

19.2. Kaynak editörlü kitaptan bir bölüm ise;
Örnek: Hornbeck P. Assay for antibody production. Colign JE. Kruisbeek AM. Marguiles DH. eds. In: Current Protocols in Immunology. New York: Greene Publishing Associates, 1991; pp. 105-32.

19.3. Kaynak kitap ise;
Örnek: Fleiss JL. Statistical Methods for Rates and Proportions. Second Edition. New York: John Wiley and Sons,1981; p.103.

19.4. Kaynak editörlü kitap ise;
Örnek: Balows A, Mousier WJ, Herramafl KL, eds. Manual of Clinical Microbiology. Fifth Edition. Washington DC: IRL Press, 1990; p. 37.

19.5. Kaynak kongre bildirisi ise;
Örnek: Entrala E, Mascarp C. New structural findings in *Cryptosporidium parvum* oocysts. Eighth International Congress of Parasitology (ICOPA VIII). October, 10-14, 1994; İzmir-Türkiye.

19.6. Kaynak tez ise;
Örnek: Erdem V. Köpek göz hastalıklarında klinik oftalmoskopik ve ultrasonografik bulguların değerlendirilmesi, Doktora tezi, Ankara Üniv Sağ Bil Ens, Ankara 2003; s. 1-2.

19.7. Kaynak internette bulunan bir web sitesi ise;
Örnek: TUIK. Hayvancılık İstatistikleri. http://www.tuik.gov.tr/hayvancilik.app/hayvancilik.zul; Accessed Date: 14.03.2010.

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